Cattle helminth infections in England and Wales: An investigation into prevalence, risk factors, attitudes and impacts

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¡Música!

Melancólico alimento

para los que vivimos de amor.

Julio Cortázar; Rayuela

Abstract

Worldwide, there is an increasing demand for food, especially meat and milk. Alongside concerns around sustainability and other issues such as animal welfare, high expectations are put on livestock systems for an increased production and efficiency in order to meet such a demand. Helminth infections are ubiquitous on cattle farms and represent a growing concern for the industry around the world. In the UK, *Ostertagia ostertagi* and *Fasciola hepatica* are recognised as parasites of major importance in terms of their economic impact on cattle production and animal welfare. Rumen fluke is another strong candidate in the list of helminths which represent challenges for the sector in the UK. However, because helminth infections are mainly subclinical, their control is often very difficult. In this context, farmers generally adopt blanket treatment in young-stock to prevent or regain production losses due to these infections. This results in increasing problems of helminth resistance to available drugs, making such a practice unsustainable. Motivated by these concerns, several guidelines for best-practice on cattle helminth control have been published in the past few years. Nonetheless, farmers' have been reluctant to adopt the recommendations put forward by these documents.

Cattle helminths infections are influenced by the interplay of a wide range of factors. These include not only interactions between different species of parasites, but also climate conditions, management practices, availability of resources, and farmers' attitudes, for which the role of comprehensive and reliable epidemiological information is key. An alternative to the use of cattle anthelmintic drugs is to avoid contamination of pasture to prevent the exposure of most susceptible cattle. The choice of diagnostic tools and the design of the studies are determinant for capturing the complexity of factors influencing helminth infections and control. However, basic epidemiological information on helminth infections in cattle in England and Wales is currently lacking, especially for *O. ostertagi, F. hepatica* and rumen fluke. Second, the relationship between economic losses and helminth infections remains to be clarified, particularly in the case of poly-infections and first lactation heifers. Third, previous studies informing potential alternatives (e.g. grazing management), suffer from limitations in terms of their scope and the adequacy of their recommendations. Finally, although being as relevant as epidemiological information, understanding what are the factors driving farmers' decisions on cattle helminth control is a topic still poorly addressed in the literature.

To address the issues above, this project was based on a mix-methods research (quantitative and qualitative methods) and a multidisciplinary framework that incorporates both veterinary epidemiology and sociology. The research analyses the cases of dairy and beef cattle in England and Wales by using longitudinal and cross-sectional studies, respectively. For dairy cattle, 43 farms (1,500 heifers) were studied. Data was collected and analysed in relation to the prevalence of *O. ostertagi* and *F. hepatica*; current practices in helminth control; demographics and management risk factors of young-stock helminth infections; impacts on milk production, reproduction and health performances in heifers; and farmers' attitudes. As for beef cattle, data was collected for both single and poly-infections in 974 cattle (at slaughter), to support the analysis of the prevalence of *O. ostertagi, F. hepatica* and rumen fluke; demographic risk factors; and impacts on prime beef carcase performance. The main findings of this thesis are summarised below:

Prevalence: the ubiquity of *O. ostertagi* and the significant presence of *F. hepatica* infections are confirmed; rumen fluke infections, most probably by *C. daubneyi*, can be considered as well-established in the UK; poly-infections by the three parasites are very common within the sample analysed;

Risk factors: different types of grazing management practices can help with reducing dairy heifer exposure to *O. ostertagi* on pasture at specific times during their first years of grazing;

Impact: the three parasites were significantly associated with low carcase performance in prime beef cattle; heifer exposure to *O. ostertagi* was significantly associated with lower milk production, reproduction and health performances;

Farmers' practices and attitudes: dairy farmers tend to overuse anthelmintic drugs on young-stock; they actively search and access information on cattle helminth infections and critically assess it in terms of management and business sustainability; farmers' epistemology and contextual challenges should be taken into account while developing guidelines for helminth control.

This thesis makes several contributions to veterinary and sociological studies of cattle helminth control. The different studies conducted shed light on a series of overlooked epidemiological and behavioural aspects that are critical for helminth control in the UK. Importantly, the thesis contributes to a better understanding of the complexity that is inherent to cattle helminth

control. By considering both the epidemiology of the infections and broader societal and cultural factors, it offers a comprehensive analysis and a pioneer representation of how the system of cattle helminth control might operate in the UK. The results of this research are extremely valuable to veterinarians, farmers, experts, and policy-makers who all wish to develop and implement sustainable control of helminth infections in cattle.

Acknowledgments

The quote of A. Rimbaud, ‘l’éclosion de ma pensée’, expresses very well the long journey I have been through while discovering, confronting, and resolving obstacles standing in my way during these three years of my PhD. Here I wish to quote an excerpt from *Cyrano de Bergerac* of Edmond Rostand (Acte II - Scène VIII), whose words have always guided me somehow, reminding me how conviction, thoroughness and passion always overcome challenges. I dedicate this text to my parents. I would like to thank everyone who has provided me with space, guidance and resources to make this work happen, all who made time to participate in my research, my supervisory and examination team, everyone at the Dairy Herd Health research group and the School of Medicine and Veterinary Science of the University of Nottingham, the administrative staff, everyone at the QMMS and, most importantly, the various farmers I have engaged with, and all my supportive friends and my family.

Et que faudrait-il faire?
Chercher un protecteur puissant, prendre un patron,
Et comme un lierre obscur qui circonvient un tronc
Et s'en fait un tuteur en lui léchant l'écorce,
Grimper par ruse au lieu de s'élever par force?
Non, merci! (…)
Non, merci! non, merci! Non, merci! Mais... chanter,
Rêver, rire, passer, être seul, être libre,
Avoir l'œil qui regarde bien, la voix qui vibre,
Mettre, quand il vous plaît, son feutre de travers,
Pour un oui, pour un non, se battre, - ou faire un vers!
Travailler sans souci de gloire ou de fortune,
À tel voyage, auquel on pense, dans la lune!
N'écrire jamais rien qui de soi ne sortît,
Et modeste d'ailleurs, se dire: mon petit,
Sois satisfait des fleurs, des fruits, même des feuilles,
Si c'est dans ton jardin à toi que tu les cueilles! (…)

Declarations

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Supervisory team

The primary supervisor for this project was Associate Professor Jasmeet Kaler, School of Veterinary Medicine and Science, University of Nottingham.

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Examination team

Professor Jonathan Huxley School of Veterinary Medicine and Science, University of Nottingham acted as an internal examiner. The thesis was examined externally by Professor Javier Guitian, Department of Production and Population Health, Royal Veterinary College of London.

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Abbreviations

AHDB	Agriculture and Horticulture Development Board
AMTRA	Animal Medicines Training Regulatory Authority
BTM	Bulk Tank Milk
BVA	British Veterinary Association
CAP	Common Agricultural Policies
CCC	Concordance Correlation Coefficients
CCW	Cold Carcase Weight
CI	Confidence Interval
COWS	Control Of Worms Sustainability
DIM	Days In Milk
GIN	Gastro-Intestinal Nematode
DEFRA	Department for Environment, Food and Rural Affairs
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FECRT	Faecal Egg Count Reduction Testing
Ig	Immunoglobulin
ODR	Optical Density Ratios
OD	Optical Density
OR	Odds Ratio
PP	Percent Positivity
PWC	Pre-Weaned Calves
MCMC	Markov Chain Monte Carlo

QMMS	Quality Milk and Management System
RIGLS	Reweighted Iterative Generalised Least Squares
SCC	Somatic Cell Counts
SCOPS	Sustainable Control Of Parasites in Sheep
SE	Standard Error
SQP	Suitably Qualified People
UK	United Kingdom

Chapter 1.
General introduction

The global food production and consumption system is currently experiencing major structural changes and pressures. Recent projections suggest that the world population will increase to over nine billion people within the next forty years (FAO, 2009). Some group of people will become wealthier and most of the world's population will live in urban areas, increasing their demand for livestock products, such as meat and milk (Rushton and Bruce, 2016). Such a trend can create new trade opportunities, especially for cattle farmers (FAO, 2009).

At the same time, there is increasing evidence for global warming and the negative effects of production intensification, which has triggered public debate and has motivated the creation of new legislation. This is particularly the case in Europe, with the recent reform of the Common Agricultural Policies (CAP) (Commission, 2013b). New policies aiming to protect both the environment and consumers, especially in relation to food products quality and safety, were therefore introduced. Consumers' concerns about drug residues and animal welfare have also been responsible for increasing the pressures towards more "ethical", organic and animal-friendly approach to food production (Gasbarre et al., 2001a; Waller, 2006; Commission, 2013a).

To ensure the sustainability of their businesses, cattle farmers have become compelled to improve the efficiency of their production and also to minimise the negative effects of their system intensification, whilst considering competition with other forms of land use, natural resources, biodiversity and infectious and zoonotic diseases (Herrero and Thornton, 2013).

Helminth infections are ubiquitous on cattle farms and represent one of the main concerns for the cattle industry around the world; this is argument is supported by increasing evidence of cattle anthelmintic resistance and control failure (Waller, 2006). Cattle are indeed infected by a diversity of helminths on pasture, which are known to have a negative impact on their productivity (e.g. feed intake, growth rate, reproduction, milk yield and carcase composition) and welfare (Charlier et al., 2014). Moreover, cattle infected with helminths are known to produce more greenhouse gases (Sargison, 2014). However, because helminth infections are mainly subclinical, their control is often difficult (Charlier et al., 2014). Recent trends in

livestock farming towards a greener farming with higher access to pasture, are making the control of helminth infections in cattle farms even more difficult (Hovi et al., 2003; Robinson and Dalton, 2009; Van Dijk et al., 2010). This is especially the case in the UK (Rushton and Bruce, 2016).

This thesis uses a multidisciplinary approach that incorporates veterinary epidemiology and sociology to improve the understanding of helminth control in cattle in England and Wales. The studies included in this project focus on three major helminths in dairy and beef cattle, namely *Ostertagia ostertagi*, *Fasciola hepatica* and rumen fluke. It is worth noting that, to date, the rumen fluke species infecting livestock in the UK are not completely identified (Skuce and Zadoks, 2013).

1. The structure of the cattle industry in England and Wales

Infectious disease control strategies do not work the same for all farmers. Moreover, farming systems are subject to inevitable changes (e.g. disease outbreak and economic crisis), which often cannot be predicted (Tisdell et al., 1999; Shigayeva and Coker, 2015). Therefore, it is necessary to understand the complexity of cattle farming systems and the networks on which they rely (e.g. veterinarians, fellow farmers, and industry), as well as the challenges and pressures faced by cattle owners. Understanding the contextual elements that play a role in cattle farming can help transforming, adapting and innovating both the theory and practices of cattle helminth control. This, in turn, contributes to ensuring its sustainability in England and Wales (Charlier et al., 2015; Shigayeva and Coker, 2015).

1.1. Cattle farming in England and Wales

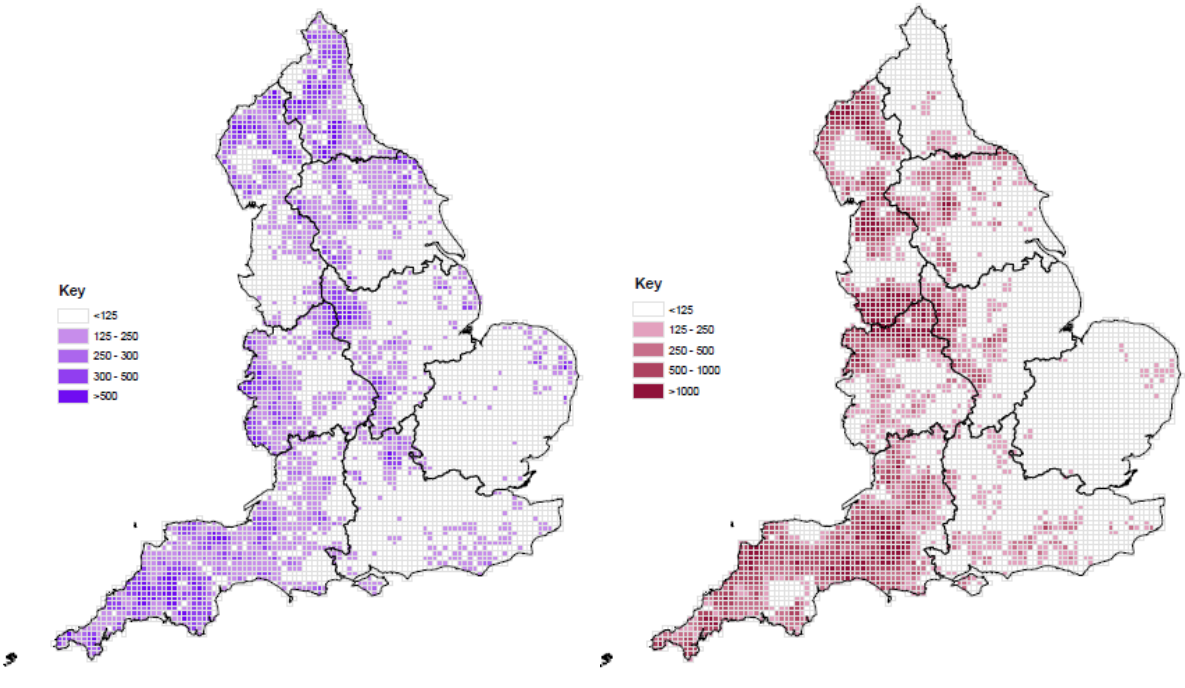
1.1.1. Structure of cattle farming in England and Wales

Beef production takes place in a wide variety of systems in England and Wales. Farmers have specialised their enterprise in breeding and/or rearing and produce certain types of animals for meat, with different breeds, management approaches and quality of lands.

Suckler herds (i.e. beef) are often considered as low-cost and mainly rely on permanent grazing with limited winter housing. These herds use a wider range of pasture types than dairy (Figure 1-1), which often allows farmers to add value to lands that are not suitable for other agricultural activity, because of their topography or soil quality (Hopkins, 2008; EBLEX, 2012).

Dairy herds, on the contrary, are predominantly raised in the southwest, north midlands and northwest (Figure 1-1), where grass-growing conditions are better and allow farmers to graze cows outdoors from spring to autumn (AHDB, 2013). The different calving patterns of the dairy herd (respectively spring-, autumn-block or all year-round calving) are important factors for farmers to consider with regards to their profitability and farm management implications. A survey conducted in 1987 highlighted that the majority of the British dairy herds were calving from August to December, but patterns are changing and a trend towards autumn-early winter calving has been observed more recently (DairyCo, 2005).

Figure 1-1: Geographical distribution of beef (left) and dairy (right) cattle herds in England (number of cattle by 5km^2 grid squares) (adapted from (DEFRA, 2010))



1.1.2. Evolution of cattle farming in England and Wales

Historically, beef supply has mainly relied on dairy herds. However, the decline of dairy herds since the mid-nineties due to increasing pressures on farmers (i.e. poor milk return and environmental regulations) reversed this trend. Over the past few years, this balance has changed again with the ban on cow slaughter following the Bovine Spongiform Encephalopathia (BSE) crisis (1996-2006). Between 1990 and 2011, total beef breeding fell by 30% in England, bringing the number of cattle within this sector to its lowest level in 80 years (EBLEX, 2012).

Given the length of the beef production cycle and farmers despondency caused by legislation restrictions (e.g. CAP), it was projected that beef production in the UK could continue to decline. In 2014, the number of beef cattle holdings in England and Wales (about 59% of the UK beef cattle holdings) was 35,103 (4.8% less than 2011) and the number of beef cows was 878,000 (5.8% less than 2012) (AHDB, 2016d).

Since the mid-nineties, the overall number of UK dairy farms fell by about 45% - to less than 20,000 - and the number of dairy cows by about 20% - to about 2 million (2005 data). In the period between 2011-2014, 75% of the UK dairy cow holdings were in England and Wales (AHDB, 2016d). In 2014, 15,919 dairy cattle holdings were located in England and Wales (21,184 in the entire UK); about 11% less than in 2011 (AHDB, 2016d). However, simultaneously, the average size of UK dairy herds (estimated at 79 cows in England in 2011 compared to 89 in 2014) and the average milk yield per cow increased (from 5,000 L/cow in the eighties to 7,912 L/cow in 2015/16). In 2015, the total UK dairy population had reached 1.9 million, which was 3.6% higher than in 2011 (Hopkins, 2008; AHDB, 2016c, d).

1.1.3. Current economic situation of the cattle industry in England and Wales

In the mid-nineties, the UK was for a short period (i.e. between two and three years) a net exporter of beef products. However, following the BSE crisis, the country ended its self-sufficiency in beef supply, which fell from 109% in 1995 to less than 80% of the demand in 2012 (EBLEX, 2012). Today, the gross value of the output of the beef sector represents just over 12% of that of UK agriculture as a whole (£2.3 billion) and around 0.06% of the total gross value added of the UK economy (with agriculture counting for 0.5%). Nevertheless, as a subsector, meat, and particularly beef, represents a significant component of the British

industry as a whole. The net value added to the UK economy by the English red meat sector has been evaluated at nearly £1.7 billion, with more than 55,000 people directly employed in beef farming in England. However, today's prices paid to producers have been criticised for being too low and therefore not providing a return on the investment made (EBLEX, 2012).

The dairy industry represents the single largest agricultural sector in the UK, accounting for around 17% of the UK country's agricultural production in terms of value (DEFRA, 2012a). In 2014, the UK was ranked the eleventh largest milk producer in the world, accounting for 2.3% of the world production. In 2016, the country remained the third largest producer in Europe, behind Germany and France, with nearly 13 million litres of milk delivered to dairies in the EU-28 (AHDB, 2015b). Fresh milk sales represent in the UK more than 45% of the total milk produced, of which over 25% is used for making cheese. Although the UK is self-sufficient in fresh milk, the country is net-importer, by about one-third, of both butter and cheese (Hopkins, 2008).

To some extent, the future of beef and dairy farming will depend on farm gate prices. Substantial variations in farm gate prices were observed over the last two decades in the UK and continue today to put pressure on cattle farmers by either discouraging them, forcing them out of business or changing their mentalities towards even more profit maximisation. These fluctuations are due to different issues, such as health crises (e.g. BSE, foot and mouth disease, and bluetongue), the implementation of new regulations (e.g. CAP reform, milk quota, welfare, and farm health planning), public opinion (e.g. meat eating habits, concerns about climate change, toxicity issues, and animal welfare) and market competitiveness (new demands, money currency, and cyclicity of prices) (Verbeke and Viaene, 2000; DEFRA, 2004; EBLEX, 2012; Patton et al., 2013; Bergmann et al., 2015). Regarding the latter, recent political developments, such as the removal of the UK from the European Union, will have a significant impact on meat and dairy prices in the near future due to a series of changes in subsidies, labour and trade exchange (AHDB, 2016b, a; van Berkum et al., 2016).

UK cattle businesses are also constantly threatened by endemic, new or re-emergent diseases such as mastitis, lameness, bovine tuberculosis, Johne's disease and bovine virus diarrhoea (BVD) (Bennett and Ijpelaar, 2005; DEFRA, 2012b). A recent survey in England reported that 86% of the farmers carried animal health practices on the farm because of animal welfare

concerns and that about 80% considered animal health practices as the main factor to prevent economic loss (DEFRA, 2013).

2. Major cattle helminths in England and Wales

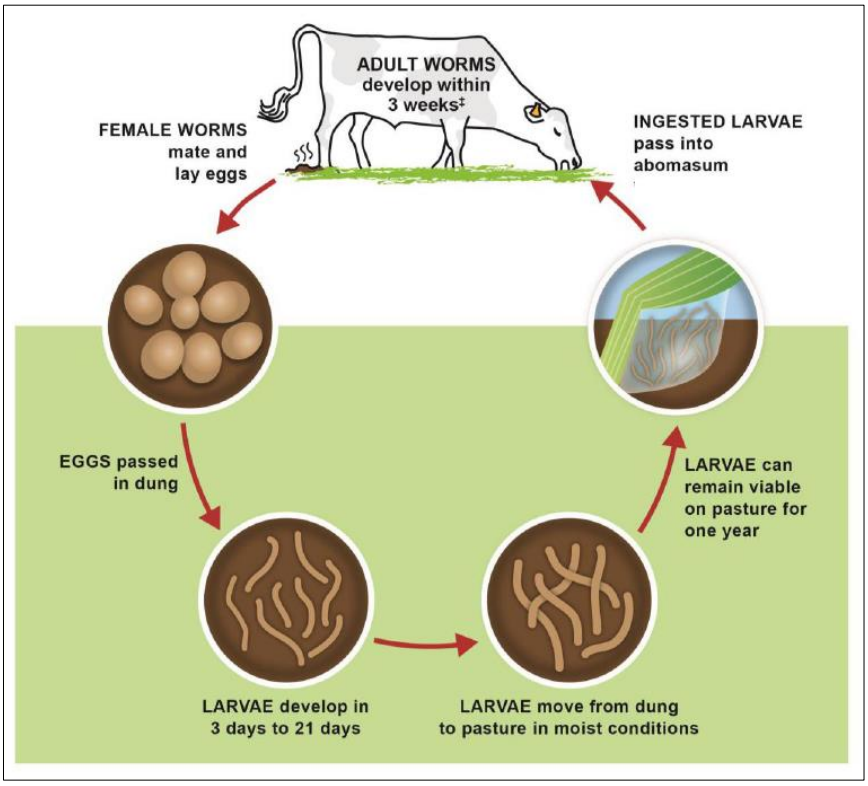
In temperate areas, such as England and Wales, the gastro-intestinal nematode (GIN), *Ostertagia ostertagi (O. ostertagi),* and the liver trematode, *Fasciola hepatica (F. hepatica),* are two helminth parasites of major economic importance in cattle (Van Dijk et al., 2010). Recently, an increased number of rumen fluke cases have been reported in cattle in Western Europe, which raised concerns about the potential negative impact this parasite could also have in the region (Gordon et al., 2013; Zintl et al., 2014). Given their importance in the UK and in Europe, these three helminths were selected as the focus of this thesis, with particular attention given to the case of *O. ostertagi*. The following sections offer an overview of the characteristics of each of these parasites.

2.1. *Ostertagia ostertagi*

2.1.1. Lifecycle

The GIN *O. ostertagi,* also known as 'brown stomach worm', has a simple direct lifecycle, characterised by a free-living stage on pasture and a parasitic stage in cattle abomasum (Figure 1-2).

Figure 1-2: Lifecycle of the cattle gastro-intestinal nematode, *Ostertagia ostertagi* (Source:(COWS, 2010))



The adults of *O. ostertagi*, found in the abomasum, pass the eggs in cattle faeces. Under optimal conditions of temperature and moisture, the eggs hatch into a first-stage larvae, L1. After a first moult, a second-stage larvae emerge, L2, which, in turn, develop and moult to third-stage and infective larvae, L3. It is only then and given environmental conditions are appropriate (i.e. temperature and oxygenation), that L3 (protected by the outer sheath of L2) migrate laterally from cattle dung onto herbage (i.e. translocation phase) (Myers and Taylor, 1989). Increased faecal moisture in the rainy season, which usually coincides with the second half of the grazing season in England and Wales, facilitates and stimulates the translocation of L3. Overall, L3 translocation remains very slow: in temperate conditions, 50% of the L3 population is expected to migrate from dung pat to pasture in 80 days. In addition, L3 migrates from soil to herbage, which is also influenced by rainfall (Smith and Grenfell, 1985). L3 live longer than L1 and L2. Studies report that the average life span of L3 larvae in cattle dung pat is 35 days, whereas in pasture it increases to 113 days (Smith and Grenfell, 1985). It has also been suggested, that under severe winter conditions, L3 can survive for up to one year on pasture (Myers and Taylor, 1989). Once ingested, L3 enter the gastric glands of cattle abomasum and moult to fourth-stage

larvae, L4, which in turn grow and moult to become adults that will break out of the glands and start egg production/shedding. In the case of uninterrupted development, egg production starts around three weeks after ingestion of L3 (Myers and Taylor, 1989) and the adults will survive for a relatively short period of 25-50 days (Coles, 2002). During cold winters, drought summers and housing periods (i.e. during periods of extreme temperatures and light decline), which are unfavourable conditions to the survival of the free-living stages of the larvae, L4 cease their development after the third moult. This cessation occurs at a point where it ensures little production of a host immune response and little host tissue damages (Smith and Grenfell, 1985). This could last between 4 and 7 months (Myers and Taylor, 1989). The restart of larvae maturation can take place gradually or in a burst, when weather conditions improve (Myers and Taylor, 1989).

2.1.2. Pathophysiology, pathology and disease

The presence of *O. ostertagi* larvae in cattle gastric glands is responsible for several histological changes. The mucosal surface of the abomasum becomes red, swollen and present umbilicated white nodules, which are the result of the larval growth (L4). When adults emerge from the gland, secondary nodules are created, which, in the case of severe infection, can coalesce and cause thickening and hyperplasia of the mucosa. This appearance of 'morocco leather' is pathognomonic from ostertagiasis and can be used as a marker of *O. ostertagi* infections. This has been used previously in abattoir surveys (Larraillet et al., 2012). At that stage, inflammation is maximal (Raynaud and Bouchet, 1976; Myers and Taylor, 1989).

Macroscopic gross-lesions induced by *O. ostertagi* are a result of severe cellular alterations, which impair the digestive properties of the abomasum. The number of chief cells (i.e. major source of pepsinogen) and parietal cells (i.e. responsible for cell differentiation, proliferation and acid secretion) drastically decreases, whereas the number of mucus-secreting cells increases, resulting in hyperplasia. The subsequent elevation of gastric pH prevents the activation of pepsinogen into proteolytic pepsin and damages abomasal digestion. In addition, this can cause bacterial overgrowth, which might contribute to an increase in greenhouse gas' emissions (Sargison, 2014). Elevated pH also induces hyper-gastrinemia, which not only promotes hyperplasia but also disrupts gastro-intestinal motility, causing a loss of appetite in cattle (Fox et al., 2002; Rinaldi and Geldhof, 2012). Moreover, gland distension, a consequence of hyperplasia, can damage intercellular junctions (i.e. between mucosal epithelial cells and

endothelial cells), resulting in loss of water, electrolytes and proteins (Myers and Taylor, 1989; Rinaldi and Geldhof, 2012).

Generally, in cattle, clinical manifestations of ostertagiasis can be divided in type I and type II. In temperate areas, such as England and Wales, type I is the typical form in young susceptible cattle, infected from July to October with a continuous development of the larvae. The severity of the infection varies from a mild loss of appetite, with a decrease in calf growth rate, to profuse diarrhoea, rapid loss of weight, submandibular oedema, anaemia and eventually death. On the other hand, type II mainly occurs in yearlings or older cattle, either during the early spring, when the weather conditions improve or during particular situations, such as parturition or stress, where the immunity of the animal decreases. In this case, the disease is driven by the resumption of the development of inhibited-L4 larvae and presents the same clinical signs as the first type, except for the mortality rate that often remains higher without appropriate treatment (Myers and Taylor, 1989).

However, due to the extensive use of broad-spectrum anthelmintic treatments, cattle clinical forms of ostertagiasis have become rare, making the control of the disease even more difficult (Vercruysse and Claerebout, 2001; Charlier et al., 2014).

2.1.3. Immune response

As opposed to other helminth infections, cattle immunity against *O. ostertagi* develops slowly (i.e. it takes around two years) and is relatively low (Gasbarre, 1997). Several studies demonstrated that, rather than the level of infection, it is the duration of infection that allows for the development of *O. ostertagi* immunity in cattle (Vercruysse and Claerebout, 1997). Studies reported that several months after a primary infection to *O. ostertagi*, the host immune response leads to morphological changes in the parasite, larva hypobiosis and egg output reduction. This contributes to an increase in herd immunity on-farm (Gasbarre et al., 2001a). The fact that, even after several seasons of grazing, older cattle carcases continue to be infested with *O. ostertagi*, also suggests that there is no protective immunity against this parasite in cattle (Gasbarre, 1997; Larraillet et al., 2012).

Cattle infections due to *O. ostertagi* cause both a cellular and humoral immune response (i.e. mixed Th1/Th2) (Rinaldi and Geldhof, 2012). After a series of infections, several types of antibodies are released in the serum of the cattle. These are, IgM (for agglutination and

cytolytic reactions), IgA (for epithelial surfaces), IgE (for cellular hypersensitivity reactions) and finally IgG, predominantly IgG1 (for complement activation). IgG antibodies are the most prevalent type of antibodies after *O. ostertagi* exposure and are often used as a marker for *O. ostertagi* infection in cattle (Berghen et al., 1993; Charlier et al., 2014). After experimental infection, serum kinetic of IgG1 in cattle peaks at day 35 post-infection and a memory response can be identified from 65 to 77 days post-challenge infection. In naïve animals, under natural conditions, IgG1 levels typically peak after two months of exposure to infected pasture. Although the immune system of cattle responds to infection with relatively low serum antibody concentrations, IgG1 levels can significantly increase with different levels of worm burden (Berghen et al., 1993; Klesius, 1993; Gasbarre et al., 2001a). In addition, experimental studies suggest that a decrease in IgG in serum occurs between day 45 and 50 post-infection, in the absence of subsequent challenge (Berghen et al., 1993).

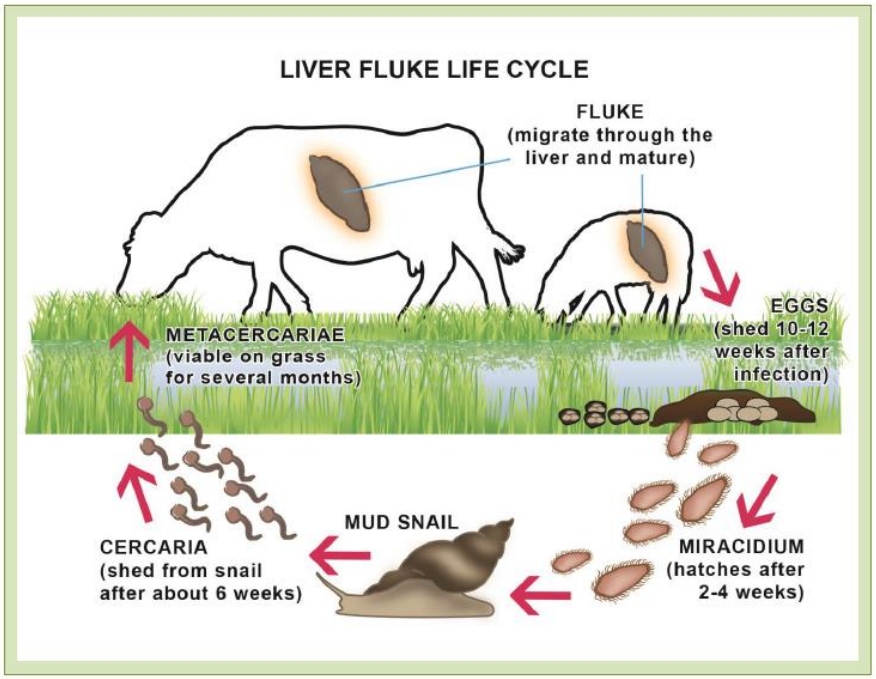
The slow development of cattle protective immunity against *O. ostertagi* suggests that the parasite develops strategies to evade or suppress host immune responses (Gasbarre, 1997). Excretory-secretory mediators produced by the parasite modulate the host immunity, although this mechanism is not completely understood. Concerns have been raised about the implications of immune suppression to cattle health, for example, in terms of disease susceptibility and vaccine failure. However, to date, there is a lack of information available on the matter (Gasbarre, 1997; Gasbarre et al., 2001a; Rinaldi and Geldhof, 2012; Qu et al., 2014).

2.2. *Fasciola hepatica*

2.2.1. Lifecycle

Compared to *O. ostertagi*, *F. hepatica* has a more complex lifecycle (i.e. indirect) that involves a fresh water snail as an intermediate host (Figure 1-3). *F. hepatica* commonly infects sheep, which *O. ostertagi* rarely do, although both cattle and sheep can host *Ostertagia spp.* Such differences in helminth lifecycles are important to consider at the time of implementing control strategies on-farm.

Figure 1-3: Lifecycle of the cattle liver fluke, *Fasciola hepatica* (Source: (COWS, 2010))



Although several species of snails can be intermediate hosts of *F. hepatica*, *Galba truncatula* is considered as the main one (Jones et al., 2015). Eggs pass in cattle faeces and, hatch under optimal light and temperature conditions, releasing the short-lived miracidium (i.e. life-span between 8 and 10h). It is suggested that, at 26±1°C, eggs need between 12 to 15 days to reveal the mature miracidiae (Hussein et al., 2010; Williams, 2014). Other studies have reported that increased temperatures speed up egg development and that, above 10°C, this can take 2 to 4 weeks to happen (Williams, 2014). Eggs are resistant to extreme temperatures and can survive for several months on pasture, especially during the winter, when development stops. Once hatched, miracidiae migrate to infect the intermediate host (i.e. snails) and continue their maturation. These snails typically live in pH neutral, stagnant water, that are close to sources of calcium for the building of their shell (i.e. edge of ponds, hoof prints). About six weeks after infecting the snail, a great number of cercariae are released over a week on pasture (i.e. up to several thousands) (Williams, 2014). The cercariae encyst on the vegetation, forming infective metacercariae. After ingestion by cattle, the metacercariae hatch, releasing juvenile flukes that migrate through the gut wall into the liver. It takes about 10-12 weeks for juvenile flukes to reach the bile ducts, mature and start passing eggs in cattle faeces (Williams, 2014). The adult fluke can remain for 1-2 years in cattle liver. During the winter, snails hibernate and the development of *F. hepatica* stops until the following season. In England and Wales, the

development of *F. hepatica* typically occurs between May and October and cattle are infected between August and October (Williams, 2014).

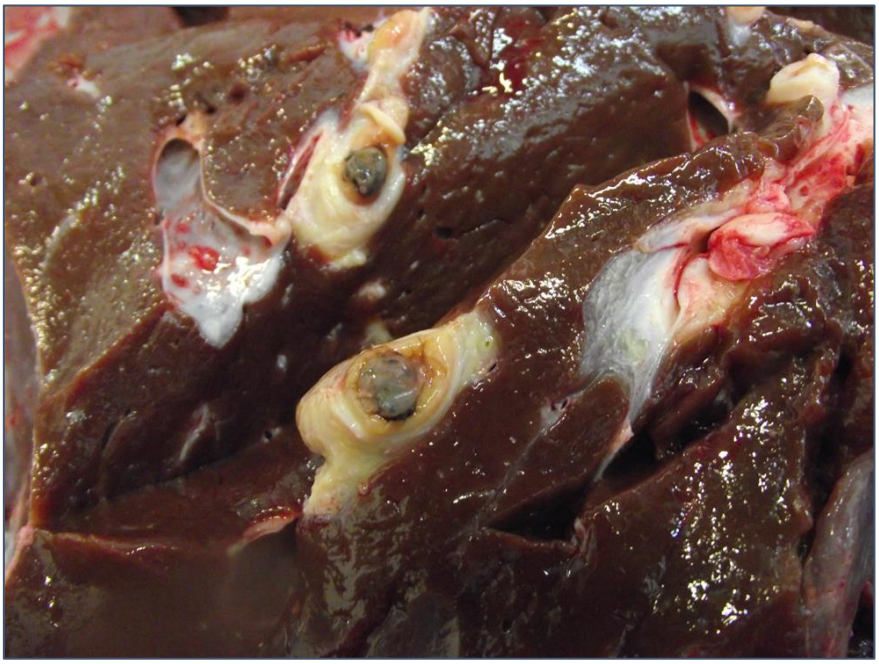
2.2.2. Pathophysiology, pathology and disease

Acute or subacute diseases due to *F. hepatica* occur in the case of large ingestion of metacercariae and are characterised by distended and painful abdomen, anaemia and death. This is however rarely seen in cattle.

By contrast, chronic fascioliasis is more frequent and is due to the presence of a large amount of fluke adults in the bile ducts after ingestion of metacercariae in the autumn. This form of the disease is mainly seen in non-treated animals during the winter season and leads to chronic weight loss and diarrhoea. To date, most infections due to *F. hepatica* in cattle, in England and Wales, are subclinical and are associated with gradual and small ingestion of infective metacercariae on pasture. Although in this case cattle do not manifest clinical signs, their production performances are substantially affected (e.g. weight gain, milk production, and fertility) (Crowther, 2001; Williams, 2014).

Livers collected in subclinical chronically infected cattle present pathognomonic cholangiohepatitis lesions with extensive fibrosis and calcification of the bile ducts ('pipe stem' appearance) (Figure 1-4). As for *O. ostertagi*, the high-specificity of the lesions due to *F. hepatica*, makes them a useful diagnostic marker in abattoir studies on the epidemiology of *F. hepatica* infection in cattle (Sanchez-Vazquez and Lewis, 2013).

Figure 1-4: Liver showing typical cholangiohepatitis lesions ('pipe stem' appearance) (source: A. Forbes)



2.2.3. Immune response

To date, there is no evidence of the development of protective immunity and resistance against *F. hepatica* in cattle and previous observations suggest that animals can be repeatedly infected by the parasite (Clery et al., 1996; Williams, 2014).

As for *O. ostertagi*, the dominant immunoglobulin isotype that is produced by cattle after *F. hepatica* infection is IgG1. After single infection to *F. hepatica*, primary infected calves show an increase of IgG1 in serum 3-4 weeks after the infection (Phiri et al., 2006), whereas in chronically infected animals, this increase is identified 2 weeks after the infection (Clery et al., 1996). A return to baseline levels have also been reported in chronically infected cattle in case of no re-infection by week 6 post-infection (Clery et al., 1996).

Recent studies suggest that *F. hepatica* is responsible for cattle immune suppression. This effect contributes to the establishment of the chronic state of the disease in cattle and allows the adult parasite to persist in the liver (Mendes et al., 2013).

2.3. Rumen fluke

The lifecycle of rumen fluke is very similar to *F. hepatica* and involves a fresh water snail as an intermediate host. Evidence suggests that both rumen and liver flukes commonly infect cattle and sheep and share the same intermediate host, *Galba truncatula* (Jones et al., 2015). However, there is still much uncertainties in the published literature as to the identity of this parasite intermediate host(s) and lifecycle characteristics (Zintl et al, 2014).

Microscopically, the egg of rumen fluke resembles that of *F. hepatica*, which may lead to erroneous diagnoses of liver fluke infection and/or treatment failure (Gordon et al., 2013). This might be one of the reason why, although rumen fluke infections have been described in UK livestock as far back as the1950s, rumen flukes were not recorded by the Animal Health and Veterinary Laboratories Agency prior to 2010 (Gordon et al., 2013; Tilling, 2013). Since 2011, there have been incidental reports of cattle rumen fluke infections in England and Wales (Tilling, 2013). However, there is still a considerable confusion over the classification of the rumen flukes and an uncertainty as to the identity of the parasite species present in the UK (Zintl et al., 2014). Recent research suggest that the most common rumen fluke present in Scotland and Ireland is *Calicophoron daubneyi* and not *Paramphistomum cervi,* as originally thought (Gordon et al., 2013; Zintl et al., 2014). By contrast, in England and Wales, such information is currently lacking. .

As for *O. ostertagi* and *F. hepatica,* infections due to rumen fluke are mainly subclinical. While adult rumen fluke is considered benign and well-tolerated by cattle, it is believed that the juvenile forms of the parasite that are responsible for the emergence of clinical signs of the disease (e.g. diarrhoea and weight loss). However, recent evidence suggests that adult rumen fluke can also induce substantial pathological changes in cattle (Fuertes et al., 2015). The level of uncertainty present in the available literature highlights a need to further explore the pathogenicity of rumen flukes in cattle and evaluate the likely significance of rumen fluke for the economy of the livestock industry in the UK (Zintl et al., 2014).

Finally, although determinant to consider at the time of implementing control strategies on-farm, there is still scant data on host immune response to rumen flukes in cattle. The only evidence is that, as for other helminths, *C. daubneyi* produces excretory-secretory antigens and induces a humoral response with an increase of IgG in cattle serum (Diaz et al., 2006).

3. Current prevalence of *Ostertagia ostertagi*, *Fasciola hepatica* and rumen fluke infections in cattle in England and Wales

Recent research on cattle helminth prevalence in England and Wales has mainly focused on the herd prevalence of *F. hepatica* infection in dairy cattle (Salimi-Bejestani et al., 2005a; McCann et al., 2010b; Howell et al., 2015). In fact, information on the prevalence of helminth infections in beef cattle, and of *O. ostertagi* and rumen fluke infections are still very much needed in these two regions, especially if the use of anthelmintic blanket treatments are to be avoided in cattle and replaced by selective targeted treatment.

As evidence of such a lack of information, there have been no abattoir surveys conducted on cattle helminths (i.e. *O. ostertagi* and *F. hepatica*) in England and Wales since the eighties (Froyd, 1975; Burrows et al., 1980; Hong et al., 1981). Moreover, the only recent study estimating the prevalence of *O. ostertagi* infection in adult dairy cows in England relied on faecal egg count (Fox et al., 2007), which is considered as an inadequate tool to be used in adults (Charlier et al., 2014) (see below, section 3.1.1., for explanation).

In the light of the increasing concerns in relation to helminth infections and recent evidence of their expansion in England and Wales (Crowther, 2001; Pritchard et al., 2005b; Skuce et al., 2013), there is an urgent need to evaluate and update cattle helminth infections in the region.

4. Diagnostic and monitoring tools available for cattle helminths

The accurate diagnosis of infections is a key determinant of helminth control in cattle farms. Recent developments in diagnostic methods provide new opportunities for improving the understanding of helminth epidemiology and, as a consequence, the control of parasite infections in cattle farm (Roeber et al., 2013). In this context, the choice of the tools used for diagnostic is critical and often depends on specificity and sensitivity of the approach, besides requirement in terms of time and financial resources.

4.1. Faecal markers

4.1.1. Faecal eggs

Faecal Egg Count (FEC) is a tool commonly used for the diagnosis of cattle helminth infections, given it is a relatively inexpensive, fast and easy tool to use in most diagnostic settings. The technique can be applied to achieve different objectives, such as (1) to estimate the intensity of the infection in cattle; (2) to estimate contamination levels in the environment (e.g. pasture burden); (3) to evaluate the efficacy of anthelmintic treatments; and (4) to support decision-making on cattle treatment (Roeber et al., 2013).

Many methods for FEC have been developed, of which 'McMaster' is the most used. Over time, the protocols on which these methods are based have been modified and adapted in laboratories (e.g. dilution and counting technique). This led to important changes in terms of method sensitivity and created challenges for comparing results from different research institutes (Roeber et al., 2013). Also, there is indication that factors inherent to the study protocol (e.g. water content, faeces, and preservation) can influence the results (Levecke et al., 2012).

There are limitations to the interpretation of FEC. While this technique provides information on mature parasites during the patent phase of the infection, it does not work for juveniles. Additionally, in the case of *O. ostertagi* (i.e. gonochoric) it limits observations to the female population. Also, several factors can influence the intensity of egg excretion by the host, especially the host immunity and the age of the parasite, which in turn has an impact on FEC results (Roeber et al., 2013). It is suggested that *O. ostertagi* FEC is correlated to worm burden at the start of the infection (i.e. two months after the first turn-out of calves). However, such a correlation decreases with time due to an increase of host resilience to the parasite (Charlier et al., 2014). In the case of *F. hepatica*, the low shedding of eggs in the chronic stage of the disease and the morphological similarities that exist with rumen fluke eggs can make the counting difficult and the results less reliable (Salimi-Bejestani et al., 2005b; Gordon et al., 2013).

As previously reported in the literature, FEC should therefore not be used to assess: (1) adult cattle levels of infection (i.e. animals older than two years of age); (2) levels of pathological damages induced by the parasite and (3) therapeutic thresholds in adults (Vercruysse and

Claerebout, 2001; Charlier et al., 2014). Moreover, the frequent use of FEC in the detection of cattle anthelmintic resistance (Stafford and Coles, 1999; Gordon et al., 2013) is also questionable, given the detection limit of some flotation techniques (Roeber et al., 2013).

4.1.2. Copro-antigen

Copro-antigen enzyme-linked-immunosorbent assays (ELISA) are based on the identification of parasites excretory-secretory antigens contained in the faeces. This technique has recently been developed to improve the ability to quantify, at an early stage, parasite burden, in particular *O. ostertagi* and *F. hepatica*. To date, however, this approach is only available commercially for *F. hepatica* (Mezo et al., 2004) since attempts at using it for *O. ostertagi* have failed (in fact, only a weak correlation was observed between excretory-secretory antigens levels in the faeces and the number of *O. ostertagi* in the animals) (Agneessens et al., 2001).

The shared antigens between parasites and the risk of cross-reactivity that follows represent an important challenge for the specificity of this technique (Roeber et al., 2013). Moreover, the variability of excretory-secretory antigens that exist across stages of the parasite lifecycle complicates the development and sensitivity of this technique (Rinaldi and Geldhof, 2012). It has also been suggested that faecal material complicates the purification of the antigens (Roeber et al., 2013).

Overall, these considerations indicate that the use of copro-antigen is still a limited approach to rely on in longitudinal studies in the field.

4.1.3. Copro-deoxyribonucleic acid (DNA)

The development of copro-DNA techniques has provided new insights in the diagnosis of cattle helminth infections. Such tools have been developed for both *O. ostertagi* and *F. hepatica* in cattle (Ai et al., 2011; Hoglund et al., 2013). Their major advantages are that they are able to: (1) specifically identify parasites independently from their stage of maturation; (2) detect the infection at a very low level and (3) inform on parasites genetics variabilities, which is particularly important for the detection of anthelmintic resistance (Roeber et al., 2013). Nevertheless, such an approach remains time-consuming and is considered expensive, which limits its use for epidemiological studies (Roeber et al., 2013).

4.2. Sero-markers (non-antibody)

Measurements of serum pepsinogen or gastrin levels have been widely recommended to be used as a proxy for *O. ostertagi* infection in cattle (Berghen et al., 1993; Charlier et al., 2014). However, besides being laborious, these methods lack of standardisation and, most importantly, are unspecific (Scott et al., 1995; Vercruysse and Claerebout, 2001). In fact, a wide variety of parasitic and non-parasitic diseases can induce high levels of serum pepsinogen or gastrin (Roeber et al., 2013). Moreover, levels of pepsinogen can remain high even after several years of previous exposure, limiting their use in adults (Berghen et al., 1993). Finally, both pepsinogen and gastrin depend on the pathophysiological changes induced by the parasite and therefore on the parasitic stage and level of infection. It has been suggested that the level of serum pepsinogen could be very low even in presence of high burden of *O. ostertagi* inhibited larvae (Berghen et al., 1993).

4.3. Antibody levels

The development and success of antibody ELISA techniques for the diagnosis of animal infections have rapidly increased over the last decades. This approach has been widely applied in the detection of cattle helminthiasis.

The high diversity of parasite antigens and the frequent lack of information related to specific antigens responsible for cattle immune response means that the use of such a tool is often difficult. However, for the detection of anti- *O. ostertagi* and anti- *F. hepatica* antibodies, ELISA has been shown to be a very reliable technique in cattle (Keus et al., 1981; Salimi-Bejestani et al., 2005b). Nevertheless, it is worth noting that such an approach (1) cannot differentiate between past and current infection and, therefore, represents a marker of cattle exposure to helminth rather than a tool to measure levels of infection, which can limit the evaluation of the use of anthelmintic drugs (Roeber et al., 2013); (2) permits cross-reactivity between helminth antigens, especially with other GIN and *F. hepatica,* in the case of *O. ostertagi* ELISA (Keus et al., 1981; Klesius, 1988; Bennema et al., 2009); and (3) is more reliable to apply in animals older than two years old for *O. ostertagi*, given that the host acquired immunity against this parasite develops rather slowly.

Milk has so far been the preferred choice over serum for helminths antibody levels estimations in dairy cattle, given it is straightforward and safe to sample animals (Kloosterman et al., 1993). For the same reasons, bulk tank milk (BTM) has predominantly been used in longitudinal studies in dairy cattle (Sanchez and Dohoo, 2002; Salimi-Bejestani et al., 2005a; Charlier et al., 2013). However, BTM are pooled samples of lactating cows, which makes interpretation of antibody levels often difficult considering variations in numbers of lactating cows, relative sero-positivity, Days In Milk (DIM) and milk yield (Sekiya et al., 2013). Recent evidence suggests that because adult cow individual levels of *O. ostertagi* antibody highly vary within a farm, the use of individual milk samples can be more informative than BTM samples (Charlier et al., 2007a; Blanco-Penedo et al., 2012).

Some limitations of testing individual animals however need to be considered. Individual milk antibody levels against *O. ostertagi* in adult cows are indeed influenced by several individual parameters, such as somatic cell counts (SCC), DIM, lactation number and milk yield (Kloosterman et al., 1993; Dohoo et al., 1997; Sanchez et al., 2004b). Evidence suggests that DIM effect on antibody levels is absent between 30 and 200 DIM in adult cows (Sanchez et al., 2004b). Overall, this suggests that there is a need to collect information about these parameters at the time of the milk sampling or, in the case of the DIM, to restrict the period during which the individual milk is sampled to adjust the ELISA results before interpretation. The use of individual milk from heifers can also be a way to control for the effect of 'lactation number' on *O. ostertagi* milk antibody levels. However to date, research focused in heifers has been very limited and it is not known whether confounding effects of DIM, SCC and milk yield on milk antibodies exist in heifers.

4.4. Gross lesions

Post-mortem examinations are commonly used to determine the levels and profiles of cattle helminth infections in abattoir surveys, especially in the cases of *O. ostertagi*, *F. hepatica* and rumen fluke (Borgsteede and vd Burg, 1982; Vercruysse et al., 1986; Borgsteede et al., 2000; Gonzalez-Warleta et al., 2013; MacGillivray et al., 2013; Toolan et al., 2015).

While these diagnoses can be very time-consuming and fastidious, they have the advantage of being highly specific and of determining the intensity of the infection; something that other

methods do not offer (Sanchez-Vazquez and Lewis, 2013). On the other hand, the main disadvantage of post-mortem diagnoses is that they have the potential to underestimate parasite burden. This is due to (1) the proportion of the total volume or the number of aliquots examined; (2) the length of the intestinal section examined; (3) the difficulty to access the parasites (i.e. juvenile forms); and (4) the migration of the parasites after the death of the animal (Levieux and Ollier, 1999; Rapsch et al., 2006; Roeber et al., 2013).

More recently, research has focused on the interest of gross-lesion examinations as a tool to determine the impact of helminth infections on cattle carcase performance. The use of post-mortem gross-lesion examination has the potential to overcome the limitations of other approaches (e.g. FEC and milk ELISA), allowing specific estimations of the levels, profiles and effects of different helminths infra-communities on slaughter cattle (i.e. presence and lesion) (Larraillet et al., 2012; Sanchez-Vazquez and Lewis, 2013) (Figures 1-4). However, although a wide range of different parasites can be found on pasture, most of the research available has focused on single helminth infections and there are no studies on the effects of different helminth infra-communities on host productivity (Murphy et al., 2006).

5. Economic, welfare and human health aspects of cattle helminth infections in the UK

It is well-acknowledged that helminth infections are responsible for considerable economic losses in the cattle industry. The assessment of the economic impact of helminth infections is highly dependent on (1) the type of information required (e.g. production losses associated with the disease, preventive and curative measures undertaken, animal welfare, and human health implications of the disease); (2) the resources needed in order to obtain such observation (e.g. research costs) and (3) the change in disease prevalence according to different environmental conditions (Tisdell et al., 1999; Bennett and Ijpelaar, 2005). Moreover, the subclinical nature of cattle helminth infections makes it difficult to establish and estimate a causal relationship between infections and financial loss (Hawkins, 1993).

Although there have been some attempts to estimate the economic impacts of cattle rumen fluke in the UK, associations between infections and loss in cattle production, reproduction and health performances are not confirmed (Sargison et al., 2016). The following sections review

the research on factors associated with the economic costs of cattle infections due to *O. ostertagi* and *F. hepatica* in the UK.

5.1. Factors associated with the economic costs of *Ostertagia ostertagi* and *Fasciola hepatica* infections in cattle in the UK

5.1.1. Growth and weight

- *Growth rate*

Calves entering their first grazing season are more susceptible to helminth infections. Therefore, most studies have so far focused on this age group to estimate the potential benefits of anthelmintic treatments on weight gains (Purvis et al., 1994; Purvis and Whittier, 1996; Shaw et al., 1998; Forbes et al., 2002; Loyacano et al., 2002). Most of these studies have reported that calves treated against GIN had significantly higher weight gains and body condition score than untreated animals. For example, Shaw et al. (1998) reported that calf weight gain increased with the decrease of GIN infections severity (i.e. weight gain of 530 g/day for "sub-clinical" control groups compared to 375 g/day for "clinical" control groups). Moreover, the beneficial effects of anthelmintic treatments on weight gain were significantly higher for GIN "sub-clinical" groups (690 g/day) than for GIN "clinical" groups (600g/day) (Shaw et al., 1998). By contrast, current literature suggests that calves treated against *F. hepatica* have significantly higher weight gains and body condition score than untreated animals only if simultaneously treated against GIN. In this case, the beneficial effect observed is significantly higher than that obtained if animals are solely treated against GIN (Loyacano et al., 2002). This suggests that the effects of GIN and *F. hepatica* on calves performance are through different mechanisms and that the effect of the two forms of parasitism may be additive (Loyacano et al., 2002).

Biological markers of helminth infections (i.e. antibody levels and larval egg output) have also been significantly associated with the decrease in calf growth performance after housing (Ploeger et al., 1990a; Ploeger et al., 1990c). In a recent study losses in heifer average daily weight gain related to *O. ostertagi* exposure at the end of the first grazing season (i.e. highest level of serum antibodies) were estimated to be as high as 39kg. However, this significant

association between weight loss and *O. ostertagi* infection was only clear for heifers previously subjected to medium or high parasite exposure on pasture (i.e. characterised by grazing management practices under risk of helminth exposure) (Merlin et al., 2016).

Such negative impacts of GIN on growth performance were also reported for second-grazing dairy heifers (Ploeger et al., 1990b) and adult dairy cows (Forbes et al., 2004).

- *Carcase composition*

Live body weight does not accurately reflect - or fully reflect - the cost of helminth infections on cattle weight growth without considering carcase composition (Hawkins, 1993). Cattle helminths, in particular, *O. ostertagi* and *F. hepatica*, are known to disrupt cattle digestion and protein absorptions and induce the redistribution of nutrients from muscle, liver and skin to intestines (Hawkins, 1993). Several studies have reported that the treatment of calves with anthelmintic drugs significantly increased carcase yield, carcase total muscle weight, specific retail cut (i.e. rib joint) and fat (Entrocasso et al., 1986; Bell et al., 1990; Suarez et al., 1991; Hawkins, 1993). Impacts of *O. ostertagi* and *F. hepatica* infections on carcase weight, conformation, fat and liver condemnation were also reported at slaughter for both young and adults beef cattle, although results remain equivocal in the case of *F. hepatica* (Charlier et al., 2009; Larraillet et al., 2012; MacGillivray et al., 2013; Sanchez-Vazquez and Lewis, 2013). Moreover, recent estimations suggested that 86% of UK condemned livers at slaughter present active lesions of *F. hepatica* (MacGillivray et al., 2013).

The importance of carcase weight loss associated with *O. ostertagi* infection in adult cows depends on the severity of the infection (Larraillet et al., 2012). However, it is often hard to accurately measure the levels of infection severity in cattle and most of the available diagnostic techniques, such as FEC and immunological detection, are limited (Charlier et al., 2014). By contrast, post-mortem examination is considered as a more reliable and promising tool (Larraillet et al., 2012; Sanchez-Vazquez and Lewis, 2013). In their study, Larraillet et al (2012) reported an inverse correlation between the number of abomasum lesions and cows carcase weight: cows with less than 100 lesions in the abomasum had an average carcase weight that was 10 kg higher than the average carcase weight of cows with more than 100 lesions. The results remain, however, limited since they did not take into account the effect of confounders (e.g. breed, season, age, concurrent helminth infections).

5.1.2. Reproduction

Considering the relations that exist between post-weaning growth rate, puberty and first lactation yield in heifers (Ferrell, 1982), research has also focused on the potential effects of helminth infections in heifers reproduction performance at first calving. Several intervention studies concluded that the treatment against nematodes (1) advances the onset of puberty (Purvis and Whittier, 1996; Mejia et al., 1999); (2) increases first-service conception rate (Purvis and Whittier, 1996); (3) increases heifer pelvic area (Mejia et al., 1999) and (4) increases calves weight at weaning (Loyacano et al., 2002). Nevertheless, these studies often used broad-spectrum anthelmintic treatments that are not specific markers of helminth infections (e.g. Ivermectin and Oxfendazole).

Other reports have suggested that there is a significant positive association between *O. ostertagi* antibody levels in BTM and the age of heifers at first calving (Delafosse, 2013). However, by using BTM markers (i.e. antibodies), such a result is difficult to interpret (see above, section 4.3.). Moreover, similar studies have reported contradictive observations (Charlier et al., 2007c; Howell et al., 2015), which prevent firm conclusion being drawn on this association.

To date, the use of individual milk markers to establish an association between helminth exposure and failure in cattle reproduction has mainly focused on adult cows. Whereas some studies have reported a significant association between high *O. ostertagi* burden and low reproductive performance (i.e. higher calving to conception interval and higher number of services per conception) (Walsh et al., 1995; Stromberg et al., 1997; Sanchez et al., 2002a), others did not (Sithole et al., 2006; Derouen et al., 2009). Because this discrepancy in results could be related to the influence of several individual characteristics on milk antibody levels (in relation to cow physiology and immunity), the use of individual milk from heifers could facilitate these types of analysis (see above, section 4.3.).

5.1.3. Milk production

Little is known about the possible mechanisms by which helminths, especially *O. ostertagi* and *F. hepatica*, might reduce milk production. Common hypotheses include the effect of parasitism on host neuronal and hormonal activities (Hawkins, 1993), and the substantial nutritional costs caused by the host immune responses (Greer, 2008). In order to understand

the association between helminth infections and milk production, different approaches have so far been developed.

Traditionally, research on the topic used experimental inoculation of nematode larvae in adult cows. Results from this research suggested that nematode infections can reduce milk production in adult cow by 1 to 3 kg per day (Bliss and Todd, 1977). However, due to their small sample sizes, most of these experiments can be considered as inconclusive (Barger and Gibbs, 1981; Kloosterman et al., 1985). Moreover, it is difficult to extrapolate from experimental observations to real farm conditions, which limits the use of such an approach.

Instead of looking at nematode infections, other approaches have investigated the effects of nematode anthelmintic treatments on milk yield. Results of clinical trials have often been equivocal and difficult to compare, mainly because of a lack of standardisation between studies, whether it is in relation to the drug used, the time of treatment (i.e. dry-off, calving, mid-lactation, and strategic treatment), the treatment coverage (i.e. individual *versus* herd), the parity of the cow or the measure and the timeframe of the milk record (e.g. daily weight, day 305) (Sanchez et al., 2004a). In order to overcome these challenges and understand the effect of anthelmintic treatment on milk yield, Sanchez et al. (2004a) conducted a meta-analysis of studies published between 1972 and 2002. Their results suggest that, on average, anthelmintic treatment of naturally infected lactating adult cows induces an increase in milk production of around 0.35kg/cow/day (Sanchez et al., 2004a). However, despite controlling for confounders, Sanchez et al. (2004a) also reported variability across studies, suggesting that different levels of helminth exposure might be one of the reasons behind it. In addition, evidence suggests that anthelmintic drugs could directly stimulate milk production (Purvis and Whittier, 1996). As a consequence, such an approach might also not be the most appropriate to explore an association between *O. ostertagi* exposure and milk production.

The use of antibody markers in milk has recently increased in research given that it is an easy, cost-effective and reliable method to measure parasite burdens in adult cows (Sanchez et al., 2004b). Recent studies have reported interesting results suggesting a negative association between milk antibody levels against *O. ostertagi* and *F. hepatica* and milk yield (Guitian et al., 1999; Sanchez and Dohoo, 2002; Charlier et al., 2005b; Charlier et al., 2007c; Almeria et al., 2009; Mezo et al., 2011; Howell et al., 2015). However, these observations relied on BTM indicators, which limits their interpretations (see above, section 4.3.) (Charlier et al., 2007a;

Sekiya et al., 2013). In the case of individual milk, the few reports made on the association between antibody levels against helminths (mostly *O. ostertagi*) and milk yield (either in the presence of absence of anthelmintic treatments) remain equivocal (Sanchez et al., 2004b; Sanchez et al., 2005; Charlier et al., 2010b; Ravinet et al., 2014; Verschave et al., 2014). In fact, it is not known to which extent the level of milk yield acts as a dilution factor for antibody concentrations in adult cows (Sanchez et al., 2004b). Whereas Sanchez et al. (2004b) suggested that individual milk ODR should be adjusted in order to compare ODR values obtained from high and low producing cows, Charlier et al. (2010b) suggested that individual milk ODR is not significantly affected by milk yield. Since all these studies focused on adult cows, the use of first lactation heifers could be useful in limiting the presence of confounders in terms of previous helminth exposure and level of milk production (Sanchez et al., 2004b).

The effect of helminth infection on milk solid contents, in particular protein and fat, has rarely been explored. The very few studies investigating the issue have reported a significant negative association between antibody levels of *F. hepatica* and *O. ostertagi* and milk solid contents (Charlier et al., 2005b; Charlier et al., 2007c). However, evidence suggests that these associations are likely to be due to the confounding effect of milk yield (Kloosterman et al., 1993)

5.1.4. Disease susceptibility and mortality

Increasing evidence suggests that infection due to *O. ostertagi* and *F. hepatica* can lead to suppression of non-specific cellular and humoral immunity (Hawkins, 1993; Williams, 2014). Several experimental studies reported a decrease in antibody levels induced by *O. ostertagi* after injection in cattle of *Brucella abortus* vaccine, Infectious Bovine Rhinotracheitis (IBR) vaccine, clostridial vaccine and Keyhole Limpet Hemocyanin (KLH) (i.e. a potent immunological adjuvant) (Hawkins, 1993). Various reports also suggest that GIN priming infection promotes the subsequent establishment of other pathogens in Buffalos (i.e. coccidia) and cattle (i.e. lungworm) (Kloosterman et al., 1989; Gorsich et al., 2014). Besides, *F. hepatica* immunomodulation properties are well understood in cattle and are recognised as a cause for an increase in cattle susceptibility to intracellular pathogens, especially *Bordetella pertussis*, *Salmonella dublin* and *Mycobacterium bovis* (Aitken et al., 1978; Claridge et al., 2012). More recently, dairy herds with higher BTM antibody levels against *O. ostertagi* or positive to fascioliasis had a higher level of calves mortality (Delafosse, 2013). However, stronger field

evidence of dairy cattle disease susceptibility induced by helminth infections are still lacking, especially for *O. ostertagi* (Gasbarre, 1997).

5.1.5. Animal welfare

It has been suggested that helminth infections can affect four of the five fundamental principles of animal welfare, which include guaranteeing the absence of thirst, hunger and malnutrition (i.e. Freedom 1); pain, injury and disease (i.e. Freedom 2); distress (i.e. Freedom 3); and abnormal behaviour (i.e. Freedom 5) (Mellor, 2016).

Depending on the burden of infestation, helminth infections can lead to a decrease in cattle feed intake (i.e. Freedom 1), both in the presence and absence of clinical disease (Hawkins, 1993). Several studies have shown that nematodes and rumen fluke infections affect young and adult cattle grazing behaviour (e.g. idling time and grazing jaw movements) (i.e. Freedom 3 and Freedom 5) and performance (e.g. mean meal duration, diet selection, live weight, body condition score, and faecal consistency) (i.e. Freedom 1) (Forbes et al., 2000; Forbes et al., 2004, 2007; Malrait et al., 2005). In addition, tissue damage caused by helminth infections, changes in intestinal motility and subsequent flow of feed, besides the release of toxic substances in the animal's body, may result in cattle pain (i.e. Freedom 2) and overall malabsorption and fluid loss (i.e. Freedom 1) (Hawkins, 1993). In Great Britain, *F. hepatica* was rated just behind lameness, Bovine Virus Diarrhoea (BVD) and Bovine Infectious Keratoconjunctivitis (IBK) in relation to its implications to animal welfare (Bennett and Ijpelaar, 2005).

5.1.6. Human health

In general, *F. hepatica* has always been more of a concern in animals than in humans. However, since the eighties, the number of cases of human fascioliasis, particularly due to *F. hepatica*, significantly increased, especially in Europe and the UK (Esteba et al., 1998; Mas-Coma et al., 1999). The attention paid to this disease in humans is still little in the UK and its occurrence is considered to be mainly related to imported cases (Bennett and Ijpelaar, 2005; Chand et al., 2009). However, given the evidence in terms of cattle reservoir, disease profile (i.e. sub-clinical), poor diagnostic tools and absence of disease notification, the impact of *F. hepatica* infection in human health is likely to be underestimated (Mas-Coma et al., 1999).

5.1.7. Current research needs

The previous sections have highlighted diverse factors that play a role in economic losses related to cattle helminth infections and emphasised the importance of controlling such diseases. As discussed above, the currently available literature has many limitations. These are mainly related to the choice of the diagnostic markers in experiments (i.e. BTM antibody levels) and the characteristics of the studies population (i.e. adult dairy cows). Based on these considerations, it is suggested that individual milk markers in heifers are used in order to strengthen associations and improve the quality of research on the topic. Moreover, this could shed some light on the effects of helminth infections on a population of animals that is considered as the future of a dairy herd and assist an early control of the disease.

Finally, a recurrent limitation of current research on cattle helminths includes the considerable difficulty in attributing economic losses to different species of helminths (e.g. *O. ostertagi*, *F. hepatica*) (Viney and Graham, 2013). While studies tend to provide insights into the general effect of helminth infections in cattle production (Charlier et al., 2009), evidence suggests that each parasite interacts differently with the host and amongst themselves, therefore leading to different impacts on productivity (Loyacano et al., 2002). In a recent study using targeted specific anthelmintic treatment, impacts of *F. hepatica* on beef heifer body weight gain were only present in the case of co-infection with GIN (Loyacano et al., 2002). Moreover, the resulting effect of this co-infection was higher than the one obtained if GIN was alone. Therefore, there is a need to better characterise the association between helminth infections and cattle productivity, taking into account different profiles of infections and co-infections (Murphy et al., 2006). This could support the development of more adequate strategies to be implemented on cattle farm in relation to the cost-effectiveness of specific forms of helminth control (Ploeger et al., 1990c; Murphy et al., 2006; Viney and Graham, 2013). In order to do that, the use of highly specific diagnostic markers, such as gross-lesions examination, would be determinant.

5.2. Current estimates on the economic costs of *Ostertagia ostertagi* and *Fasciola hepatica* infections in cattle in the UK

Current literature indicates that the evaluation of the magnitude of economic losses due to helminth infections in cattle has often been difficult, resulting in rather imprecise estimates (Tisdell et al., 1999). In the absence of active surveillance in cattle, the exact number of animal deaths caused by helminth infections is based on estimations rather than actual measurement. These estimates indicate that, in the UK, at least one hundred animals die every year due to helminth infections (Van Dijk et al., 2010), which would represent a minimum annual cost of approximately £20,000 considering a loss of £200 per dead animal (Tisdell et al., 1999). In reality, most of the direct economic costs associated with helminth infections in cattle are a consequence of subacute and chronic diseases (see above, sections 2.1.2., 2.2.2. and 2.3.), which are difficult to estimate accurately (Tisdell et al., 1999). In the mid-eighties (i.e. when parasitic gastroenteritis was completely uncontrolled), the direct annual cost associated with infections due to *O. ostertagi* was estimated at £45 million (Tisdell et al., 1999). This value accounted for both death and weight loss in calves (Bain and Urquhart, 1986) but did not consider economic losses related to impaired reproduction, drop in milk yield and lower carcase classifications; hence these figures are likely to be largely underestimated. Recent research shows that the annual cost on individual cow milk production and reproduction performance due to helminth infections is on average (25^{th}-75^{th} percentile) of €46 (29–58) for GIN and €6 (0-19) for *F. hepatica* (Charlier et al., 2012). In addition, evidence suggests that carcases with *F. hepatica* have lower price than those carcases free of fluke (estimated coefficient -£1.5) (Sanchez-Vazquez and Lewis, 2013). Current estimations for the UK set the annual direct costs (i.e. production loss and cost of control) due to *F. hepatica* chronic infections in cattle at £40 million (Bennett and Ijpelaar, 2005). Nevertheless, these estimations do not include potential costs related to human health, which, to date, remain very abstruse in the UK.

Overall, economic assessments of helminth infections in cattle are complicated by (1) the complexity of the diseases epidemiology (e.g. environmental conditions, host nutritive state, and poly-parasitism) (Hawkins, 1993; Zinsstag et al., 1997; Loyacano et al., 2002); (2) underestimates of cost; (3) absence of widely accepted and standardised methods for welfare cost measurement (Bennett and Ijpelaar, 2005); and (4) understanding of stock-owners attitudes and behaviour (e.g. anthelmintic treatments, cattle management, and slaughter)

(Tisdell et al., 1999). Although a challenging task, accounting for these aspects, which are both technical and social nature, is crucial for a more comprehensive and reliable evaluation of the overall economic impact of cattle helminth infections in the UK (Tisdell et al., 1999).

6. Cattle helminth infections control in England and Wales

The control of cattle helminth infections have traditionally relied on the use of anthelmintic drugs, whether because of their efficacy, their relatively low cost, or because they are considered to be fairly easy to use in comparison to other control alternatives. However, evidence of failures in helminth control due to anthelmintic resistance have recently increased in the UK, motivating discussions on the need to redesign control strategies against helminths in cattle (Waller, 2006; Taylor, 2012). Alongside this, the fast development of "green" farming in Europe have been urging the need for other alternatives than drugs for cattle helminth control since their use are very restricted in these types of farming (Waller, 2006). The following sections offer an overview of the different methods and strategies available for cattle helminth control in the UK.

6.1. Anthelmintic drugs

Conventional approaches to cattle helminth control are usually based on the use of anthelmintic drugs by farmers. Moreover, these treatments are mainly used in young-stock (in dairy herds, the young-stock represent all the animals that have not yet entered in the milking herd) (COWS, 2010). Anthelmintic drugs can be considered as easy to use, efficient, safe, relatively inexpensive and often present a wide spectrum of activity (Waller, 2006). A wide range of anthelmintic drugs have been developed for cattle and are today used in the UK (Table 1-1), with Macrocyclic lactones being the most commonly used anthelmintic drugs in cattle in the country (Vercruysse and Rew, 2002).

Table 1-1: List of cattle anthelmintic drugs currently available in the UK categorised by chemical groups with details on the number of available products, the spectrum activity, the form of application, the trace of elements and the withdrawal period (adapted from (COWS, 2010))

Chemical name (N products)	Spectrum of activity	Form	Trace of elements (N products)	Withdrawal period (meat)	Withdrawal period (milk)
Group 1: Benzimidazoles ("White")					
Albendazole (12)	Roundworm Lungworm Tapeworm Fluke (adult)	Oral drench	Co, Se (10)	14 days	60 hours
Fenbendazole (7)	Roundworm Lungworm Tapeworm (not all)	Oral drench/ in-feed or pre-mix		12, 14 or 200 days	120, 132 hours or X
Oxfendazole (4)	Roundworm Lungworm Tapeworm	Pulse release bolus/ bolus		9, 19 days or 6, 8 months	84 hours or X
Group 2: Levamisole ("yellow")					
Levamisole (7)	Roundworm Lungworm	Oral drench/ injection S/C or Pour-on	Co, Se (1)	14, 20 or 28 days	X
Levamisole + Triclabendazole (1)	Roundworm Lungworm Fluke	Oral drench		56 days	X
Levamisole + Oxyclozanide (1)	Roundworm Lungworm Fluke	Oral drench		5 days	X
Group 3: Macrocyclic lactones ("clear")					
Ivermectin (24)	Roundworm Lungworm Mites, Warbles, Lice	Injection S/C or Pour-on		15, 28, 31, 35 or 49 days	60 days or X
Ivermectin + Clorsulon (5)	Roundworm Lungworm Fluke Mites, Warbles, Lice	Injection S/C		66 days	60 days
Ivermectin + Closantel (3)	Roundworm Lungworm Fluke Mites, Warbles (not all), Lice	Injection S/C or Pour-on		28 or 49 days	X
Doramectin (3)	Roundworm Lungworm	Injection S/C or Pour-on		35 or 56 days	60 days

	Mites, Warbles, Lice, Hornflies (not all)			
Eprinomectin (2)	Roundworm Lungworm	Pour-on	10 or 15 days	0 days
	Mites, Warbles, Lice			
Moxidectin (5)	Roundworm Lungworm	Ear injection/ Injection S/C or Pour-on	14, 65 or 208 days	6, 60 or 80 days
	Mites, Warbles, Lice, Hornflies (not all)			
Moxidectin + Triclabendazole (1)	Roundworm Lungworm Fluke	Pour-on	143 days	X
Flukicides				
Nitroxynil (1)	8 weeks onwards	Injection S/C	60 days	X
Oxyclozanide (1)	10 weeks onwards	Oral drench	28 days	72 hours
Closantel (1)	7 weeks onwards	Injection S/C	77 days	X
Triclabendazole (6)	2 weeks onwards	Oral drench	56 days	45 or 48 days (dry) + 2 days (milk) or X

***Key: X=not for use in cattle producing milk for human consumption**

The majority of livestock farmers have become dependent on anthelmintic drugs in the UK. However, despite the widespread use of anthelmintic drugs to treat animals, suboptimal productivity due to helminths is now commonplace. Improper and/or overutilisation of anthelmintic drugs has already resulted in increasing problems of anthelmintic resistance in sheep and goats and today cattle are facing similar challenges (Sutherland and Leathwick, 2011; Taylor, 2012). Growing evidence of cattle anthelmintic resistance has been reported in the UK, especially in relation to Albendazole, Macrocyclic lactones and Triclabendazole (Stafford and Coles, 1999; Sargison et al., 2009; Coles et al., 2010; Sargison et al., 2010). To date, cattle anthelmintic resistance remains, however, in the UK less obvious than in other parts of the world (Coles et al., 2010; Taylor, 2012; Williams, 2014).

Recent studies suggest that the differentiation between 'true' anthelmintic resistance in cattle and what are rather treatment failures is often not clear (Taylor, 2012). Treatments can fail for many reasons, including improper dosing, drug activity, form of application and timing, which are known to be important confounders of FEC reduction testing (FECRT) results (El-Abdellati et al., 2010). This way, comprehensive and detailed information on farmer deworming practices and grazing management, in addition to reliable FECRT, are needed to understand

the current situation of cattle anthelmintic resistance in the UK (Coles, 2002; Taylor, 2012). However, only very few recent surveys were conducted on the use of cattle anthelmintic drugs by farmers in the UK, especially in the dairy sector. In addition, no information is currently available on how cattle farmers make decisions about anthelmintic use (Stafford and Coles, 1999; Barton et al., 2006; Heasman et al., 2012).

6.2. Grazing management practices

Public concerns around agriculture, including citizens' pledges for a decrease on the use of chemicals and the promotion of organic farming, have recently pushed farmers towards non-chemical forms of helminth control in cattle (Waller, 2006). In conventional systems (i.e. where anthelmintic use is not restricted), recent changes in cattle helminths epidemiology have also led to the emergence of new factors that can influence the efficacy and the resistance of anthelmintic treatments. Among them, are the host-parasite co-adaptations, climate change and the inappropriate timing of drug application. In this context, the sole use of anthelmintic drugs for helminth control in cattle seems unsustainable (Sargison, 2014).

The development of intensive (e.g. high stocking rate and sharing of pasture between young-stock and adult cattle) and organic cattle farming have created a favourable environment for the development of cattle helminths and increased cattle exposure to infective larvae (Sargison, 2014). One common way of controlling/preventing cattle helminth infections on pasture is to set up strategies by which the contamination of pasture will be diminished and the exposure of naïve/most susceptible cattle avoided (Morley and Donald, 1980; Hoste and Torres-Acosta, 2011). A considerable amount of research has been dedicated to investigate the risk factors behind cattle helminth infections associated with grazing management, with some studies focussing on the UK (Forbes et al., 2008; Bennema et al., 2010). Among these risk factors, researchers have identified the length of the grazing season, the proportion of grass in animals' diet, the time spent on pasture, the month of turn-out/housing, the stocking rate, the mowing of pasture, the spread of manure, the use of boggy grazing land, the set-stocking and the presence of co-grazing/mixed herd (Bairden et al., 1995; Guitian et al., 1999; Sanchez and Dohoo, 2002; Charlier et al., 2005a; Almeria et al., 2009; Bennema et al., 2010; Charlier et al., 2010a; Bennema et al., 2011; Vanderstichel et al., 2012). In their literature review, Rahmann and Seip

(2007) identified three types of grazing management strategies, namely a "preventive", "evasive" and "diluting" (Table 1-2).

Table 1-2: Overview of the different grazing management strategies against cattle helminth infections (adapted from (Rahmann and Seip, 2007))

Preventive strategies	Evasive strategies	Diluting strategies
Turning out parasite free animals on clean pastures	*Worm challenge is evaded by moving animals from contaminated to clean pasture*	*Worm challenge is relieved by diluting pasture infectivity*
• Delayed turn-out • Changing pastures between season • Grass reseeds • Cultivation of annual forage crops • Silage/hay aftermath • Alternation of different host species	• Moving to safe pastures within the same season • Alternate grazing of different species • Silage/hay aftermath • Grass reseeds • Cultivation of annual forage crops	• Avoid stocking rates close to carrying capacity of plant production • Reduction of the general stocking rate • Mixed grazing with other host species • Mixed grazing with other age groups

Current research on grazing management strategies, however, suffers from many limitations. Investigations have predominantly used BTM antibody levels as a marker of infection, which is likely to be confounded (see above, section 4.3.) and does not consider the importance of individual factors in cattle response to management practices (i.e. immunity and physiology). Moreover, this approach prevents the exploration of the effectiveness of different time-sequential managements (i.e. differentiation between first-, second- and third-grazing animals). As a consequence, the use of individual information in terms of diagnostic markers and management practices could be considered as a more appropriate approach in these cases.

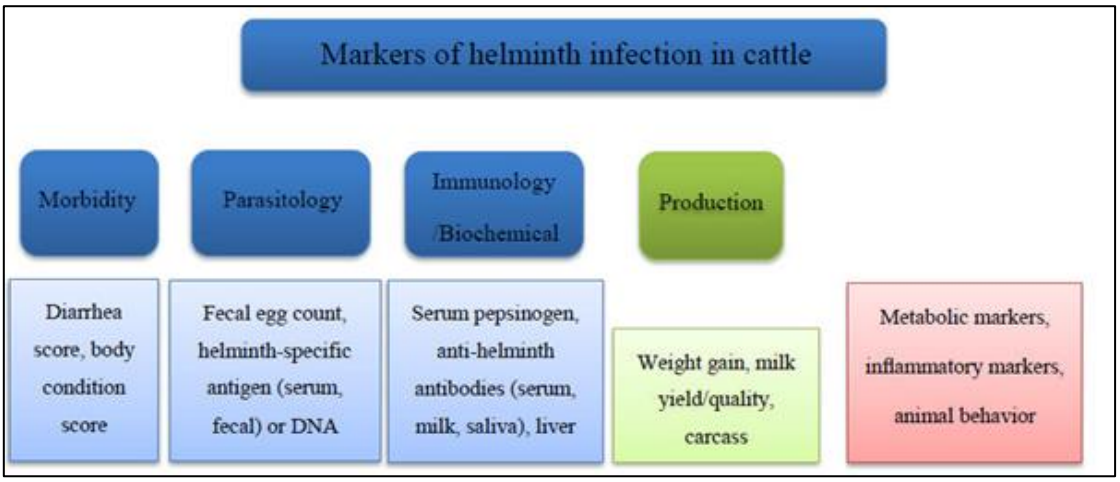
The difficulty in obtaining detailed and accurate information on cattle grazing management often limit researchers in their exploration of management factors that play a role in cattle helminth exposure and control. Evidence suggests that part of the reason is the predominant use of close-ended postal questionnaire, which do not offer opportunities to describe complex systems of grazing (Bennema et al., 2010). Moreover, the use of overly simplified management descriptors may demotivate farmers, hindering their engagement and decreasing their efforts

in replying to the questions. The use of face-to-face, in-depth interviews can overcome some of these challenges and be a more suitable approach to studies interested in understanding the complexity behind cattle management practices (Chapter 2., section 5.2.). Moreover, considering the difficulty in capturing data on complex cattle management, focussing on first lactating heifers whose exposure on pasture has been more restricted in time can deliver more accurate results.

6.3. Drivers behind cattle helminth control

Given that helminth infections are sub-clinical, attitudes of farmers and other professionals have mainly been to blanket treat animals in order to prevent or restore the losses caused by helminth infections (Morley and Donald, 1980). Recent research has focused on the identification of diagnostic markers that could help identifying animals that would benefit from anthelmintic treatments and, ultimately, reduce the use of blanket treatments (Figure 1-5) (Kenyon and Jackson, 2012; Charlier et al., 2014).

Figure 1-5: Diagnostic markers available for assessing cattle helminth infections and their effects on the host (adapted from (Charlier et al., 2014))



***Key: Blue, biological markers; Green, production markers; Red, potential useful markers**

However, farmers' decision-making processes are influenced by fundamental elements of the whole-farm system, such as labour, finance, land and skills, which may be compatible with or compete against cattle helminth control (Morley and Donald, 1980). Recommended

management practices for helminth prevention and control may be therefore adapted or rejected by farmers following their evaluation of their suitability within the overall farming system (Sargison, 2014). This might explain why a recent survey in the UK reported that most cattle farmers were not currently following the guidelines for sustainable worm control in cattle (i.e. COWS) (Heasman et al., 2012). This suggests that, in order to be efficient and sustainable, cattle helminth control in England and Wales should take into account the different factors that influence farmers' decision-making in the management of their production systems (Morley and Donald, 1980; Charlier et al., 2015).

7. Project research questions

In light of what has been discussed in the previous sections, this thesis aims to:

1. Evaluate the value of individual milk antibody levels as a marker of *O. ostertagi* infection in heifers (i.e. relevance of the marker and requirements for interpretation);
2. Estimate the prevalence of single and poly-infections caused by *O. ostertagi*, *F. hepatica* and rumen fluke in dairy and beef cattle in England and Wales, with a particular focus on young-stock;
3. Identify demographics and management factors associated with individual milk antibody levels against *O. ostertagi* in heifers;
4. Identify production, reproduction and health factors associated with individual milk antibody levels against *O. ostertagi* in heifers;
5. Identify performances associated with *Ostertagia spp.*, rumen fluke and liver fluke single- and poly-infections in prime beef carcases;
6. Explore the knowledges (i.e. the diversity and the changing nature of their knowledge), the practices and the values of dairy cattle farmers towards helminth infections in England and Wales.

8. Research approaches and designs

Science has radically changed over the years and continue to change according to technological and socio-cultural shifts. Scientific approaches adopted to address specific research questions are shaped by the views of those responsible for designing and conducting the research. Therefore, scientists' preferences and concerns guide the way questions are framed and determine how they should be studied (Kuhn, 2012). The definition of what is 'acceptable' or 'valid' knowledge and the extent to which knowledge can be gained, are part of the process. Researchers' views rely on multiple paradigms and theories that provide a framework within which research questions can be solved and results interpreted. These norms orientate with more or less challenges (e.g. the level of abstractness of concepts) the way data should be collected and analysed (Dohoo et al., 2009; Bryman, 2016). The following sections detail and discuss the different approaches and designs that were selected to address the research questions of this thesis.

8.1. Research approaches

Quantitative and qualitative approaches represent two different strategies of knowledge building in scientific research. The values behind each of them and the ways in which information is handled are not the same. Although both can be guided by deductive and inductive approaches, quantitative research is generally more interested in testing hypotheses that derive from original 'theories' about a topic of interest (i.e. deducting approach), while qualitative research usually attempts at building theories from specific observations (i.e. inductive approach) (Silverman, 2014). The main objectives of quantitative research are to (1) measure patterns or concepts; (2) identify associations or causality for the explanation of patterns/concepts; (3) generalise results beyond the particular context of the study; and (4) replicate the study findings; hence demonstrating the objectivity of the research (Bryman, 2016). Although sampling can be done via several methods, one important feature of quantitative research is that study samples are required to be as representative as possible of the entire population (Dohoo et al., 2009). In qualitative research, scientists are interested in describing trends in opinions and beliefs of particular subjects and groups. Here they pay special attention to meaning, while aiming to identify new concepts and theories. Both context

and process are important in qualitative studies and samples are often much smaller than in quantitative approach (Bryman, 2016).

The selection of an adequate research strategy, study design and methods for collecting and analysing data is bound to (1) the research questions being investigated; (2) the nature of the topic/population studied; and (3) the current extent of knowledge on a given topic (Bryman, 2016). Considering the research objectives outlined in section 7, a mixed strategy based on both quantitative and qualitative studies was therefore chosen for this thesis. Mixed-methods research represents an integrative approach for collection, analysis and interpretation of quantitative and qualitative data. The combination of two methods strengthen the understanding of the research questions overpassing the possible limitations of either approach taken on its own (Bryman, 2016). There are different mixed-methods designs where quantitative and qualitative data can be collected and integrated for analysis. In the particular context of this thesis, the quantitative and qualitative data were collected during the same phase of the research process and the results combined in an overall interpretation. The mixed-methods approach allowed, on the one hand, (1) to understand the epidemiology of cattle helminth infections in general (i.e. factors associated and impacts on productivity) and in particular (i.e. prevalence and anthelmintic practices in England and Wales); and, on the other, (2) to explore knowledges, attitudes and practices of dairy cattle farmers towards cattle helminth infections in England and Wales. Joint quantitative and qualitative observations were seen as an opportunity to analyse and confirm information on current farmers' practices and rationales behind them.

8.2. Research designs: objectives and limitations

8.2.1. Cross-sectional studies

Cross-sectional studies are very useful to assess disease prevalence and to identify predictors of multiple outcomes at a single point in time. They are relatively easy to conduct and have the advantage of not requiring investigators' intervention or additional resources, resulting in lower cost. These studies, however, cannot be used to demonstrate any causal inferences: they report associations that raise assumptions, which could be further confirmed via other observational studies (Mann, 2003).

8.2.2. Longitudinal studies

Longitudinal studies are observational studies that are frequently used for determining the natural history of a condition (Mann, 2003). They represent a class of cohort studies where the study population, instead of presenting two groups of 'exposed'/'non-exposed', includes one group of subjects (i.e. single cohort) thought to be heterogeneous enough in terms of exposures of interest. The subjects that will not develop the outcomes of interest during the study hence represent the internal controls (Dohoo et al., 2009). One advantage of such an approach is that several outcomes can be investigated in the same study (Mann, 2003). Longitudinal studies can be conducted prospectively (i.e. exposure follow-up starts when the study starts) or retrospectively (i.e. exposure follow-up ends before the study starts). Both approaches can lead to some bias and confounding, which need to be anticipated in the study design and taken into account in both data analysis and interpretation of associations.

8.3. Reliability and validity of the research

Reliability and validity are important criteria of quality assessment in quantitative research. Reliability is related to the consistency of research measures. It can be assessed by evaluating the stability of the measures across time (i.e. test-retest reliability). However, the distinction between lack of measures' stability and real measures' changes over time is not always easy. It is also possible to test the reliability of the indicator used for the measure (i.e. internal-reliability) and, in the case of method subjectivity, to test the inter-operators reliability (e.g. inter-laboratories reliability). On the other hand, validity refers to the 'truth' of the study findings for the study subjects (i.e. internal validity) or to the generalizability of the findings to the entire population (i.e. external validity) (Bryman, 2016). Multiple biases (e.g. selection bias, confounding, detection bias, and withdrawals) can affect the internal validity of a study, preventing generalizability of quantitative research outputs. Therefore, it is necessary to take them into account while designing the study and analysing the data (Cochrane, 2016). By contrast, there has been much debate on the relevance of such criteria to assess the quality of qualitative research (Noyes et al., 2011). While there is still no consensus on the topic, some argue that the rule of thumb to resolve the 'of quality' assessment here is to use the same basic strategy used to ensure rigour in quantitative studies (and in scientific research in general), that

is to be self-conscious of research design, data collection, interpretation and communication; of course, always ensuring transparency (Mays and Pope, 2000).

9. Chapters outline of the manuscript

This thesis is divided into two parts: (1) Part I, a study in dairy farms and (2) Part II, an abattoir survey. This manuscript has eight chapters (Figure 1-6), which are described below:

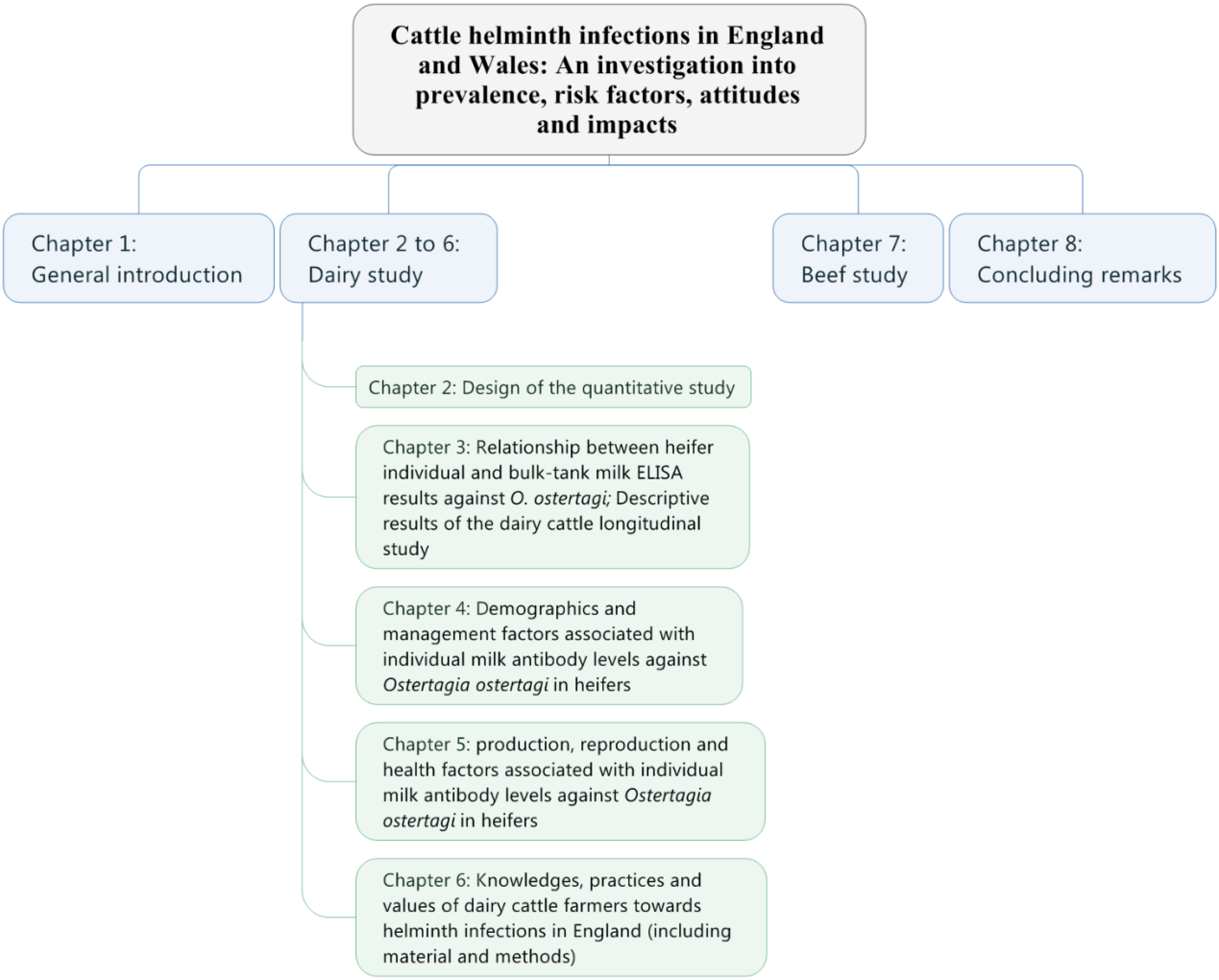
In the current chapter, **Chapter 1**, a general introduction to the available literature on cattle helminth infections is presented. It identifies current knowledge and gaps and raises new research questions for the particular context of England and Wales. The chapter also discusses the research approaches and designs available to address the different research questions.

The first part of the PhD project covers Chapter 2 to Chapter 6 with the first four chapters focussing on the dairy quantitative study and the fifth on the dairy qualitative study. **Chapter 2** presents the design of the dairy cattle longitudinal study; **Chapter 3** explores the association between heifer individual and bulk-tank milk ELISA results against *O. ostertagi*, and presents the descriptive results of the dairy cattle longitudinal study; **Chapter 4** focuses on the demographics and management factors associated with individual milk antibody levels against *O. ostertagi* in heifers; **Chapter 5** focuses on the production, reproduction and health parameters associated with individual milk antibody levels against *O. ostertagi* in heifers; **Chapter 6** explores the knowledges (i.e. considering the diversity and the changing nature of knowledge), the practices and the values of dairy cattle farmers towards helminth infections in England.

Chapter 7 presents the abattoir study; that is the prevalence of *Ostertagia spp.*, rumen fluke and liver fluke single- and poly-infections in cattle in England and Wales and the performances associated with these infections in prime beef carcases.

Finally, **Chapter 8** provides concluding remarks of the entire project, reflecting more broadly on cattle helminth control in the UK. This chapter expands the empirical relevance of the project by exploring other complementary levels and scales that also need to be taken into account so cattle helminth control can be improved in the UK.

Figure 1-6: Chapters outline of the manuscript



Part I. Dairy study

Chapter 2.
Design of the dairy cattle longitudinal study (quantitative study)

1. Introduction

Epidemiologists seek to understand patterns of disease development and impacts on populations under field conditions, in order to improve the quality of life and safety of animals, farmers and consumers. In this regard, main challenges are to design studies and select samples that are representative of targeted populations and to quantify, within their natural environment, associations between predictors and outcomes of interest, while minimising bias in data collection and analysis. Observational studies, in particular longitudinal studies, are often used to identify factors of disease exposure and to evaluate their associations with different outcomes of interest, such as health, welfare, production and reproduction performances (Dohoo et al., 2009). To address the issue, a wide range of sampling strategies and data collection tools (i.e. questionnaires, interviews, and routine recording) are available. Their selection depends on researcher's specific research questions and objectives.

This chapter describes and discusses the design of the dairy longitudinal study used to collect data for this thesis. The materials and methods used for the qualitative component of this study are described separately, in chapter 6.

2. Determination of dairy heifer sample size

The determination of dairy heifer sample size involved both statistical and non-statistical considerations (e.g. time, budget, and farm recording). These were aligned to the study objectives of identifying the association between individual milk antibody levels against *O. ostertagi* in heifers and other collected variables related to demographics, management and production. Given that the levels of heifers' exposure to helminths were not known beforehand, the study sample had to include a substantial level of individual heterogeneity (Dohoo et al., 2009). Since evidence suggests that there is a large variation of individual milk antibody levels against *O. ostertagi* within-farm (Charlier et al., 2007a), the sampling aimed to sample more heifers per farm across the seasons than farms. In order to statistically identify significant associations between outcomes and predictors, where these existed, estimation of heifer sample size was based on current reports of *O. ostertagi* impacts on cow milk production and included (1) the expected variance in heifer milk production; (2) the desired level of confidence to ensure that the sample observations are close to the real population value; and (3) the power of the study to detect real effects. No estimate of likely drop outs and withdrawals were taken into consideration in the heifer sample size determination since the study used a convenience sample of dairy farms, all members of the Quality Milk Management Services recording scheme (Somerset, England), highly compliant to data recording. The estimation is described by the equation (1) (Dohoo et al., 2009):

$$n = 2\left[\frac{(Z_\alpha - Z_\beta)^2 \sigma^2}{(\mu_1 - \mu_2)^2}\right] \quad (1)$$

Where n was the heifer sample size per group (i.e. animals treated and untreated against helminths); Z_α=1.96 and was the value required for a 95% Confidence Interval (CI) of the study estimates (Type 1 (α) error=0.05); Z_β =-0.84 and was the value required for a study power of 80%; σ was the estimated standard deviation of the parameter of interest in the population (i.e. standard deviation of the daily milk yield in heifers, estimated at 3.4kg/day (Charlier et al., 2007b)); μ_i was the estimated mean of the cow daily milk production per group *i* (i.e. difference estimated at 0.35kg/cow/day (Sanchez et al., 2004a)). The study sample size was estimated at 1,479 heifers, which was rounded up at 1,500 heifers.

3. Selection of dairy farms

One hundred and twenty-seven dairy farmers (N=127), both conventional and organic, were contacted by email and/or postal letters (Appendix 1). All studied farms were clients of the Quality Milk Management Services (QMMS) Ltd., based in Somerset (England). This facilitated the individual milk sampling (i.e. routine sampling), the testing (i.e. laboratory facilities) and the access to individual cattle records. Farmers were invited to participate in the study and informed that the aims were to investigate associations between cattle management practices, levels of milk antibodies against *O. ostertagi* and other parameters of cattle production (i.e. milk production, reproduction and health). To increase the consistency and reliability of the study, only main managers responsible for the dairy herd were invited to participate. Those who agreed to participate in the study were asked to (1) fill in a questionnaire on general demographics, housing, vaccination and previous history of helminth infections in the farm; (2) allow for a farm visit by the researcher; (3) be interviewed about cattle grazing management and helminth control practices (interviews to be audio-recorded and anonymised); (4) collect heifer individual milk samples to be tested against *O. ostertagi* during a one-year period; (5) have a three-monthly telephone contact for following-up on cattle grazing management; (6) collect two BTM samples for the testing of *O. ostertagi* and *F. hepatica*; and (7) agree to let the PhD student access herd recording data, kept in QMMS information management system (TotalVet recording programme, Sum-It computer systems Ltd., Oxfordshire), for the entire period of the study.

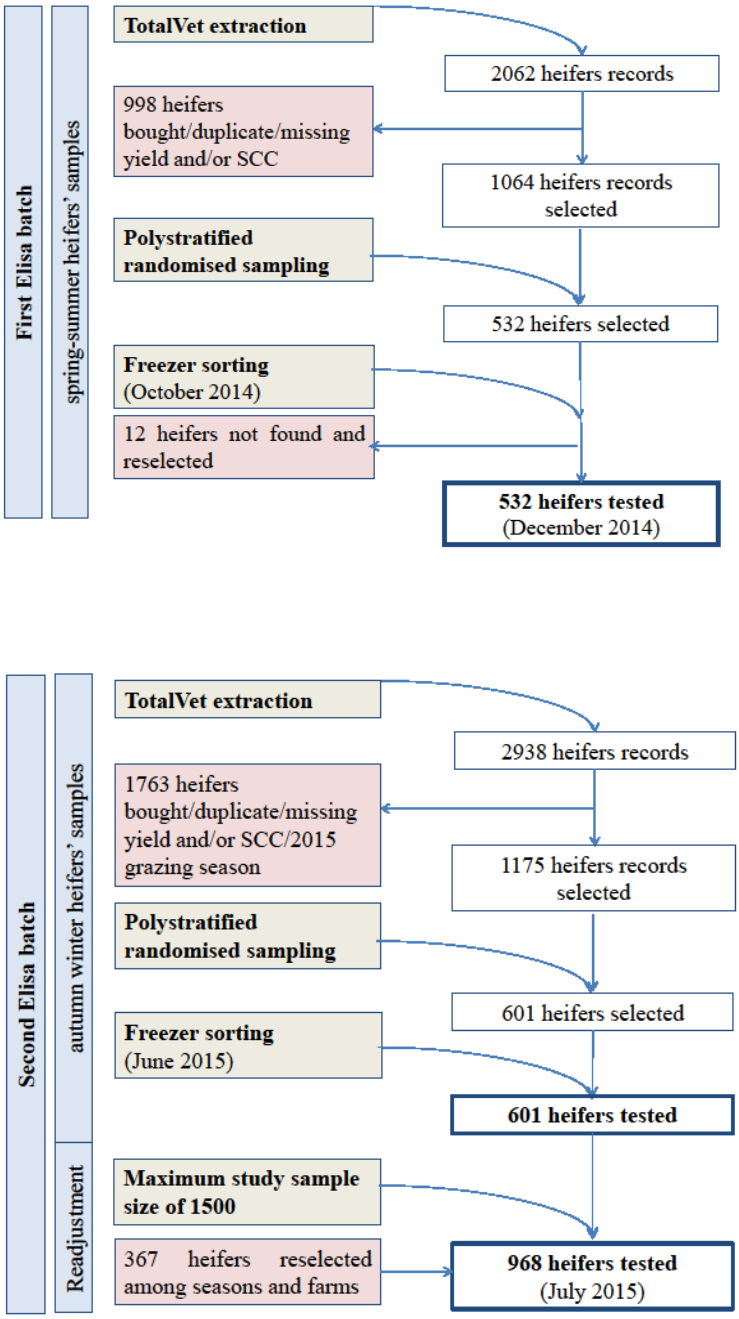
A total of 59 (46.5%) dairy farmers agreed to take part in the study. These were contacted by telephone to determine whether they met a further pre-defined inclusion criteria for the study. These included to (1) calve all-year-round or at least during two different seasons in a year; (2) rear heifers at home; (3) record cow milk production at least bi-monthly in the TotalVet recording programme, along with fat, protein and SCC. A total of 43 farmers (72.9%) met these criteria and were finally selected for the study. The median [25^{th} percentile (p25)-75^{th} percentile (p75)] number of heifers reared per farm was 46 (30-80). Farmers received a letter with a proposed date and time for a farm visit and a copy of the questionnaire (Appendix 2). The selected farms were visited by the researcher between the months of April and May of 2014.

4. Selection of heifer milk samples

Heifer milk samples were obtained from samples routinely taken by QMMS from the beginning of March 2014 to the end of March 2015 and stored in freezers. Only one sample per heifer was randomly selected among the stored samples. It was estimated that around 35 heifers (1,500 heifers/43 farms) had to be sampled and tested against *O. ostertagi* in each farm. To be included in the study, stored milk samples had to comply with several criteria. These were (1) to originate from home reared heifers; (2) to be collected between 30 and 90 DIM avoiding confounding of antibody levels by factors of milk production (Sanchez et al., 2004b); (3) to include recording of milk yield, fat, protein and SCC; and (4) to originate from heifers that would not have started a new grazing season in 2015. If several milk samples were eligible for one heifer, the one with the lowest DIM was included in the study to reduce the complexity of previous exposure to *O. ostertagi* on pasture.

To select the 1,500 heifers responding to the above criteria, all the records of heifers kept by QMMS between March 2014 and March 2015 were extracted from TotalVet recording programme. Two strata were then considered and related to the risk factors of heifer exposure to *O. ostertagi* on pasture: stratum 1, the season (i.e. spring - between April and June; summer - between July and September; autumn - between October and December; and winter - between January and March), and stratum 2, the farm. This approach ensured that the main risk factors of heifer exposure to *O. ostertagi* were represented in the study sample. In total, 9 heifer samples (1,500 heifers/4 seasons/43 farms) were randomly selected per farm and per season. When a farm did not have the 9 samples in a given season, numbers were adjusted for the following season. To achieve the calculated study sample size of 1,500 heifers, additional samples were proportionally randomly selected over the year from the remaining of QMMS stored samples (see Figure 2-1 for a scheme of the different steps taken in the sampling process). The selection of heifer milk samples in QMMS laboratories took place at two different time points: (1) in October 2014 (batch 1), for the selection of spring-summer samples; and (2) in July 2015 (batch 2), for the selection of autumn-winter samples and the readjustment of the study sample size (Figure 2-1).

Figure 2-1: Illustration of the stratified random sampling used for the selection of the 1,500 heifer individual milk samples to be tested against *Ostertagia ostertagi*



5. Demographics, management, productivity parameters and antibody levels against *O. ostertagi* in selected heifers

Tables 2-1 and 2-2 summarise the different tools used in this study, along with their purpose and time of application. The reasons for these choices are covered in the following sections.

Table 2-1: Methodology and timeframe of the dairy longitudinal study data collection

Tool	Time of use	Study year	Data coverage	Type of data
Postal questionnaire	March-14	1	2010-2013	Retrospective
Face-to-face interview	April to May-14	1	2010-2013	Retrospective (Qualitative (Chapter 6.))
Telephone interview	July-14 to May-15	1 & 2	2014-2015	Retrospective; Prospective
QMMS routine recording (TotalVet)	March-14 to April-16	1 & 2	2010-2016	Retrospective; Prospective
Milk sampling	March-14	1 & 2	2014-2015	Cross-sectional

Table 2-2: Retrospective and prospective data collected for the 1,500 heifers included in the dairy longitudinal study

Type of information	Tool	Type of data
Demographics	Postal questionnaire; TotalVet*	Variables on farm, farmer and heifers (e.g. age, education, system, herd size, breed)
Pre-weaned calves	Postal questionnaire; TotalVet	Variables on preweaned calves management (e.g. birth, grouping, feeding, vaccination)
Housing	Postal questionnaire; telephone interview	Variables on cattle housing (e.g. grouping, feeding, dates)
Vaccination	Postal questionnaire; telephone interview; TotalVet	Variables on weaned calves, heifers and cows vaccination
Infection history	Postal questionnaire; telephone interview; TotalVet	Variables on previous helminth infections
Grazing	Face-to-face and telephone interviews; TotalVet	Variables on cattle grazing management (e.g. dates, groups, pasture, stocking rate, cut, manure, fertiliser, movement, feed, treatments)
Productivity	TotalVet	Variables on milk production, reproduction and health
Exposure to *O. ostertagi* and *F. hepatica*	ELISA tests	Variables on bulk tank milk (*O. ostertagi* and *F. hepatica*) and individual antibody levels

***TotalVet=QMMS recording programme**

5.1. Postal questionnaire

Postal questionnaires were sent to the 43 farms in March 2014. Quantitative information was gathered on farm, farmer and cattle demographics, pre-weaned calves management, housing, vaccination, and previous history of cattle helminth infections (Appendix 3). The period of interest comprised the years 2010 to 2013. Questions were asked for the year 2013 and, in the case of any change from the previous years, farmers were asked to specify the change. In order to be effective and enhance response rate, the questionnaire did not exceed three pages and included short, close-ended questions. This decision was based on the type of data to be collected and on the intention to make it straightforward for farmers to answer the questions

accurately (Dohoo et al., 2009). The questionnaire also included an introduction explaining the study rationale, its relevance and the way data would be used. Questions were grouped into sections according to subjects (e.g. demographics, housing, and vaccination) and chronology (e.g. preweaned calves, weaned calves, and bulling heifers). In addition, farmers were asked to complete the questionnaire prior to the farm visit so it could be double-checked by the PhD student and finalised with the farmer during the visit. The questionnaire was pilot-tested prior to its distribution on three colleagues of the dairy herd health research group at the School of Medicine and Veterinary Science, University of Nottingham. The pilot was aimed to identify questions that could be confusing, misleading or overly sensitive (e.g. financial estimates). Minor amendments were made after the pilot testing.

5.2. Interviews

To achieve the objectives of the research, accurate and detailed information on the grazing management history of the sampled heifers was needed. The majority of British dairy cattle systems are grass-based with rotational grazing, which implies constant movement of groups of cattle across fields. Animals are mainly managed by groups of age and their management can vary depending on weather conditions, grass availability, age, and stage of lactation (AHDB, 2013). In order to capture the complexity of each farm grazing management and related heifer exposure to *O. ostertagi* on pasture, face-to-face and telephone interviews appeared to be the most suitable approaches.

5.2.1. Face-to-face interviews

Forty-three (N=43) face-to-face semi-structured interviews were conducted during the farm visits between April and May 2014. Before starting the interview, the PhD student reminded farmers of the objectives of the study and the farm visit. The study was approved by the ethics committee of the School of Veterinary Medicine and Science, University of Nottingham. To comply with research ethics requirements, farmers were asked to sign an informed consent form (Appendix 4).

The interviews were audio-recorded and followed a pre-designed and pilot-tested interview schedule (Appendix 5). Open-ended questions were asked to farmers on their grazing

management practices from 2011 to 2013. The interview schedule was divided into three different sections that referred to animals' age: (1) section 1, calves (i.e. defined as animals from weaned to bulling age); (2) section 2, bulling heifers (i.e. defined as animals from bulling age to in-calf); and (3) section 3, in-calf heifers (i.e. defined as animals from in-calf to not-yet-in-calf). The definition of these terms was developed beforehand and discussed with farmers in order to avoid any misunderstanding. For each of the sections (1-3), grazing management questions were split into three time periods: period 1, from the time of animal turn-out to the 1st of June; period 2, from the 1st of June to the 1st of August, and period 3, from the 1st of August to the time of animal housing. Times of turn-out and housing were also confirmed beforehand by farmers. The questions considered the years 2011 to 2013 for the case of calves and 2012 to 2013 for the cases of bulling and in-calf heifers. Given the complexity of some of the rotational grazing management systems, information was checked against detailed maps of the grazing field (Figure 2-2).

Figure 2-2: Examples of detailed maps of cattle grazing fields in two of the study farms

5.2.2. Telephone interviews

At the end of the face-to-face interview, farmers were asked to record, for the current season, the same grazing management information (i.e. prospective data). They were informed that they would be contacted again by telephone, for a three-monthly follow-up, to answer similar questions. Regular reminders were sent to farmers during this period (Appendix 6).

5.3. Quality Milk Management System routine recording

The parameters of heifer milk production, reproduction and health were extracted from the TotalVet recording programme. In order to collect for each heifer one-year of prospective data on production, reproduction and health, data covered the period between March 2014 (i.e. start of the study sample collection) and April 2016 (i.e. at least one year after the end of the study sample collection). The extracted variables included data on: milk production, protein, fat, lactose, SCC, length of the first lactation, breeding, pregnancy diagnosis, calving event(s), current status on the farm (i.e. alive, dead, culled and sold), mastitis, lameness, and Johne's antibody levels. Information was collected, along with the date and DIM at the time of the record. Information on heifer milk production at day 305 had been calculated by QMMS beforehand, using the 'test-interval' method that is the method of reference recommended by the International Committee for Animal Recording (ICAR) (ICAR, 2016). The full description of data items is presented in Table 2-3. The number of days for which information on production, reproduction and health could be collected varied between heifers and depended both on heifer lifespan in the farm (e.g. dead, sold and culled) and farmer assiduousness to record.

Table 2-3: Production, reproduction and health parameters extracted from the TotalVet recording programme for the 1,500 heifers included in the dairy longitudinal study

Category	Description
Milk production	
L1_Record	Number of the first lactation milk recordings
L1_ Length	Length of the first lactation (days)
Yield1	Yield at the first milk recording (kg)
305Yield	305 day milk yield (kg)
305Fat	305 day fat percentage (%)
305Protein	305 day protein percentage (%)
305Lactose	305 day lactose percentage (%)
305Fat_Yield	305 day fat percentage (kg)
305Protein_Yield	305 day protein percentage (kg)
305Lactose_Yield	305 day lactose percentage (kg)
LactYield	Milk yield from first calving to last record day (kg)
LactFat	Fat percentage from first calving to last record day (%)
LactProtein	Protein percentage from first calving to last record day (%)
LactLactose	Lactose percentage from first calving to last record day (%)
Fat_Yield	Fat from first calving to last record day (kg)
Protein_Yield	Protein from first calving to last record day (kg)
Lactose_Yield	Lactose from first calving to last record day (kg)
Somatic cell count	
SCC1	SCC at first milk recording (x1,000c/mL)
L1_SCC>200k	Number of first lactation milk recordings with SCC > 200,000 c/mL
Breeding	
L1_ServiceAge	Age at first service in the first lactation
L1_Serves	Number of services in the first lactation
L1_PD	Number of pregnancy diagnosis in the first lactation
L1_CalvingAge	Age at first calving
L2_CalvingAge	Age at second calving
Calving_Int	Calving interval between first and second calving (days)
Health information	
Status	Current status on farm (i.e. alive, dead, culled, sold)
Mastitis	Number of mastitis case in the first lactation
Lameness	Number of lameness case in the first lactation
L1_JDTitre	Last Johne's disease titre in the first lactation
L1_JDTest	Last Johne's disease result in the first lactation (i.e. currently negative, uncertain, positive)
JDTitre	Last Johne's disease titre
JDTest	Last Johne's disease result (i.e. currently negative, uncertain, positive)

5.4. Enzyme-linked immunosorbent assay (ELISA)

5.4.1. Bulk tank milk samples

In order to investigate the relationship between heifer individual and BTM antibody levels against *O. ostertagi*, two BTM samples were collected from each farm. In addition to *O. ostertagi*, BTM samples were also tested against *F. hepatica*. The latter aimed to control for some extent of test cross-reactivity between helminths (Bennema et al., 2009). Samples pots with preservative bronopol were sent to farmers in June and October 2014 along with a cotton wool wad, a zip lock bag, a self-addressed prepaid envelope, a cover letter and instructions for sampling (Appendix 6). The number of BTM pots sent to farmers depended on the number of herd tanks. Farmers were asked to take milk sample(s) after a complete day of milking and to record the approximate number of litres present in each tank after sampling. Farmers were contacted by telephone within twelve working days if no sample had been received and, in the case of loss or damage, sample pots were resent. BTM samples arrived at ambient temperature within the next 48h after collection on farms. At arrival in the laboratory, BTM samples were frozen and stored at -20°C (±2°C) until further testing.

5.4.2. ELISA tests against Ostertagia ostertagi and Fasciola hepatica

Individual and BTM samples were defrosted, defatted by centrifugation (2000 x g, 2 min) and their supernatant collected for the detection of *O. ostertagi* (i.e. individual and BTM) and *F. hepatica* antibodies (i.e. BTM). Samples were tested undiluted and not in duplicate, as this is not reported to affect test results (Sanchez et al., 2002c). If farms presented several tank samples in a season, proportional volumes of each were collated before testing. ELISA tests were carried out at the QMMS laboratory, according to kits manufacturer's instructions. Each helminth ELISA kit relied on the same batch of antigens, which, due to antigens complexity, reduced the chances of affecting test results (Sanchez et al., 2002c). Both individual and bulk tank milk tests were conducted by the same QMMS technicians.

The *F. hepatica* test used the Pourquier® ELISA *Fasciola hepatica* serum and milk verification test (IDEXX, Montpellier, France), which is based on an "f2" antigen purified from *F. hepatica* extracts. Results were given after assessment of the corrected Optical Density (OD) of the sample at 450 nm and calculation of the percentage of the positive control:

$$\text{Percent positivity} = \frac{100 \text{ X corrected OD450 value of the sample}}{\text{Mean corrected OD450 value of the positive control}}$$

O. ostertagi test used the Svanovir® kit sourced from Svanova Ltd. (Sweden). This test is an indirect ELISA based on crude saline-extracts of *O. ostertagi* adult worm as antigens (Keus et al., 1981; Sanchez et al., 2002c). Results were expressed as an Optical Density Ratio (ODR) of the sample to guarantee test repeatability (Sanchez et al., 2002c), after the measure of both sample and controls OD at 405 nm:

$$\text{ODR} = \frac{\text{OD milk sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}}$$

6. Data coding and editing

Computer data entry was done using Microsoft Excel and Access (2013). The data provided for analysis on heifer production, reproduction, health and exposure to parasites (i.e. ELISA results) were coded and formatted in Excel by QMMS. Laboratory technicians were blinded of the ELISA results when the results of production were extracted from TotalVet. The data on management that were captured by questionnaires and interviews were extracted, coded and entered in databases, paying attention to the consistency of coding and missing values. The related process of data validation is covered below.

Due to the nature and the complexity of the grazing management information, a systematic process of data entry was performed: (1) the date of birth of the heifer estimated the year, the month and the age of the heifer at first turn-out; (2) each heifer was affiliated to a group for each grazing season, according to their age at the start of the grazing season; and (3) this was used to infer on their specific grazing management for the given season. This process was used for each grazing season of heifers, from first turn-out to sampling time. Each interview recording was carefully evaluated by the PhD student to identify animals that had moved from a group to another, during the grazing season. If heifers were born prior to 2010 (i.e. out from the frame of the data collection) or were never turned out (i.e. without any exposure to *O. ostertagi* on pasture), they were excluded from the study. Iterative and triangulation processes between sources (i.e. interviews, farmers, and TotalVet) ensured that interview data were (1) complete and unique: farmers were contacted again in case of missing, ambiguous or duplicate

data; (2) reliable: data comparisons between face-to-face and telephone interviews were performed to check for the consistency of the information provided by farmers and, in the case of identified differences, further explanation was requested by the PhD student; and (3) valid: data conversions were undertaken when different units were used by different farmers (e.g. hectares *versus* acres), and a cross comparison was done with TotalVet records to check farmers' declaration on the time and sequence of events (e.g. Huskvac vaccination and age at turn-out and insemination of bulling heifers).

At the end, several databases were built in relation to the different research questions.

7. Study participants and variables

7.1. Farmers recruitment

Out of the 127 dairy farmers, who were invited to participate in the study, 43 (33.9%) took part in this study. The 43 farms were distributed over thirteen counties of England. The majority of the farms were clustered around south-west counties, including counties of: Somerset (N=19; 44.2%); Wiltshire (N=9; 20.9%); Devon (N=3; 7.0%); Cornwall (N=2; 4.7%); Dorset (N=1; 2.3%); and Gloucestershire (N=1; 2.3%). Other counties were Lancashire (N=2; 4.7%); Leicestershire (N=2; 4.7%); Cumbria (N=2; 2.3%); East Sussex (N=1; 2.3%); Shropshire (N=1; 2.3%); and Staffordshire (N=1; 2.3%).

7.2. Farmers participation and withdraw from the study

Forty-three farmers (N=43) were interviewed during farm visits. Two farmers withdrew from the study shortly after the farm visit, during the spring-summer 2014: one due to a family bereavement and one due to an unwillingness to further participate. Three farmers stopped routinely recording information on their herd productivity during the summer-autumn 2014 because of lack of time and/or financial constraints but continued to contribute to telephone interviews on grazing management. Finally, one farmer sold his herd to move abroad in summer 2015. Table 2-4 summarises the different levels of farmers participation according to the type of data collected.

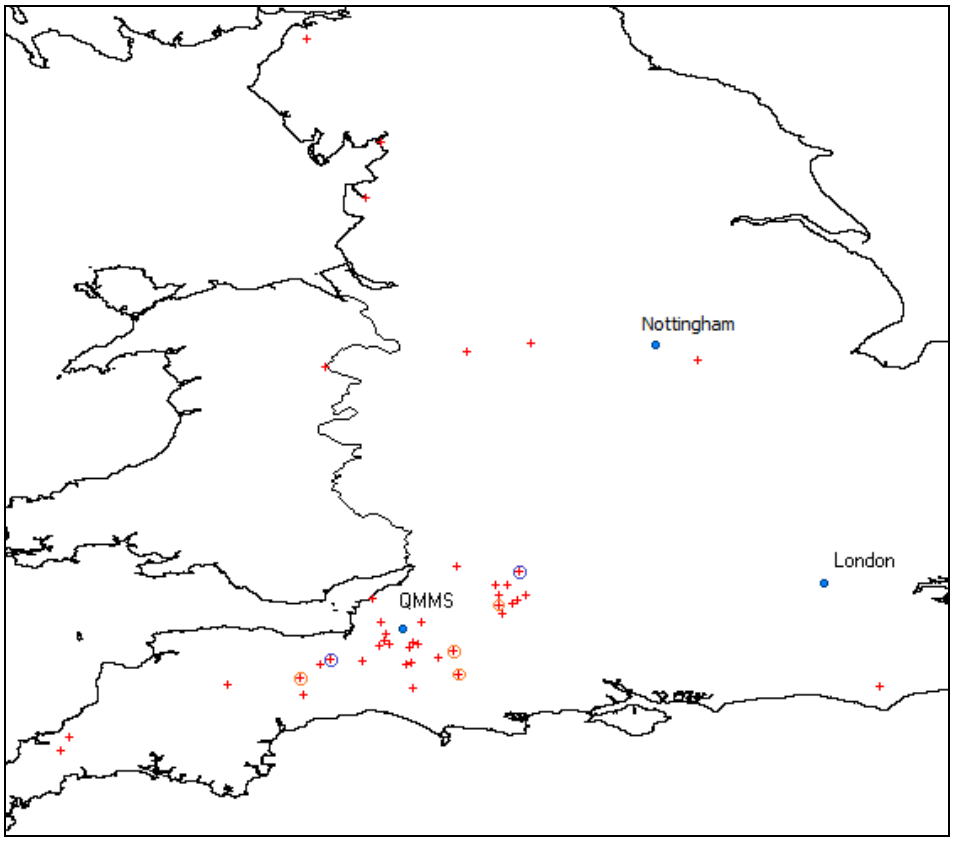
Table 2-4: Total number of dairy farmers included in the dairy longitudinal study within each phase of the data collection process

Tool	Time of use	Data coverage	Farmer participation (%)
Postal questionnaire	March-14	2010-2013	43 (100)
Face-to-face interview	April to May-14	2010-2013	43* (100)
Telephone interview	July-14 to May-15	2014-2015	41 (95.3)
TotalVet	Entire study	2010-2016	37 (86.0)
Milk sampling	March-14	2014-2015	41 (95.3)

***Out of 43 farms, two farms belonged to the same farmer resulting in 42 farms included in some parts of the descriptive analysis of the quantitative study and in the qualitative study (Chapter 6.)**

As a consequence, the number of dairy farms included in the different quantitative analyses varied according to the type of research questions: (1) 41 farms were used to explore the relationship between heifer individual and BTM ELISA results against *O. ostertagi* (Chapter 3.); (2) 42 farms were included in some parts of the descriptive analysis of the dairy cattle longitudinal study (Chapter 3.); (3) 41 farms were included in the investigation of the demographics and management factors associated with individual milk antibody levels against *O. ostertagi* in heifers (Chapter 4.); (4) between 37 and 41 farms were included in the investigation of the production, reproduction and health parameters associated with individual milk antibody levels against *O. ostertagi* in heifers (Chapter 5.). Figure 2-3 represents the geographical distribution of the farms included in the different analysis conducted in the dairy longitudinal study.

Figure 2-3: Locations of the dairy farms included in the dairy longitudinal study



***Key: Red cross, dairy farms enrolled in the study (N=43); Blue circle, farms withdrawn after the farm visits (N=2); Red circle, farms that stopped to routinely record in TotalVet programme (N=4)**

7.3. Data collection, coding and editing

7.3.1. Questionnaires, interviews and routine recording

Around three farms were visited per day, four days a week, from the 1st of April 2014 to the 29th of May 2014. Most postal questionnaires were completed and ready for collection during the farm visit, although 7 (16.3%) had to be completed with farmers on the day of the visit. Farm demographics and management practices (except grazing) did not change significantly after 2010 for all farms. Interviews on grazing management lasted on average 47 minutes (min-max: 20min-1h47). Forty-one (N=41; 95.3%) farms participated in the telephone interviews between July 2014 and May 2015. Farmers were called three times: between July and August 2014, between October and December 2014 and between April and May 2015. Two farms (4.9%) failed to respond to telephone calls and management questions were gathered by emails.

All demographics and management data collected by questionnaires and interviews were coded, edited, checked and entered in Excel and Access databases by September 2014 and May 2015, respectively. In total, the database included 568 variables.

Thirty-seven farms (N=37; 86.0%) had, from March 2014 to April 2016, routinely recorded their cattle milk production, reproduction and health in TotalVet. Records were done at least bimonthly. After extraction, the database included 110 variables.

7.3.2. Antibody levels against Ostertagia ostertagi and Fasciola hepatica

- *Bulk tank milk samples*

Forty-one farms (N=41) sent BTM samples. In four farms, sampling pots had to be sent again, due to the loss of the pot or milk clotting, and in two farms, the volumes of the tanks had to be asked again. The majority of the farms (N=31; 75.6%) had one tank; the rest had two. The majority of the sampling pots that were sent in June were received by QMMS between June (N=21; 51.2%) and July (N=18; 43.9%); others included the months of August (N=1; 2.3%) and September (N=1; 2.3%). In the case of the October sampling, the majority of the pots were received between October and December; i.e. October (N=9; 22.0%); November (N=20; 48.8%); and December (N=8; 19.5%); others were received in January (N=3; 7.3%) and February (N=1; 2.3%). Depending on when BTM samples were received by QMMS, BTM milk samples were stored at -20°C (±2°C) for a period of 103 to 248 days prior to testing.

- *Heifer individual milk samples*

Fourteen heifers (N=14; 1%) were born prior to 2010 and 32 (2%) heifers had never been turned out on to pasture and thus were excluded from the study. This resulted in 1,454 (97%) usable heifer samples. The number of usable heifer milk samples was not far off from the estimated sample size requested for the study (N=1,479) (see above, section 2.). The distribution of heifer milk samples was: N=350 (24%) in the spring; N=357 (24%) in the summer; N=373 (26%) in the autumn and N=374 (26%) in the winter. The median (p25-p75) number of heifers sampled per farm was 34 (25-45). Depending on when heifer samples were received by QMMS, individual milk samples were stored at -20°C (±2°C) for a period of 126 to 253 days prior to testing.

8. Advantages and limitations of the research approach

8.1. Study design and representativeness

The study design aimed to collect data to identify associations between several heifer exposures (e.g. risk factors and treatment) and outcomes (e.g. milk antibody levels against *O. ostertagi* and parameters of milk production, reproduction and health). The complexity of the factors that naturally affect cattle exposure to helminths on pasture justified the use of an observational longitudinal design in this study (Dohoo et al., 2009). Considering the optimum sample size of 1,500 heifers and the need, in a limited period of time, to visit and interview farmers individually, the study sample size of 43 farms was considered sufficient. Farmers were committed and cooperative throughout the study. Their participation remained high over the two years, which is of significant value in the case of a longitudinal study (Goldstein et al., 2015). Farmers were proactive; especially, while reporting by text messages or emails cases of cattle helminth infections in the farm (e.g. *F. hepatica* after slaughter and faecal egg counts). It is possible that affiliation to QMMS Ltd. influenced such an active participation of farmers. Moreover, since some members of the dairy herd health research group (Veterinary school, University of Nottingham) were veterinarians of the farms, it is also possible that farmers were encouraged to participate. Besides, both research approach and design ensured that farmers were fully engaged throughout the study and that constant contacts between them and the PhD student were maintained (e.g. telephone follow-up and greeting cards), which reduced the possibility of them withdrawing from the study.

Despite the fact that this study used a convenience sample of dairy farms, whose description of systems and helminths prevalence might not be representative of English dairy herds, a purposive selection ensured the representation of different farming systems (i.e. demographics and management practices), seasons and factors of interest in relation to heterogeneous cattle exposure to helminths (i.e. individual milk ELISA results). The study also relied on a stratified random sampling approach for the selection of heifers within farm, which is more likely to be valid. In summary, the underlying biological associations of risk factors reported in this thesis (Chapters 4. and 5.) are likely to be valid and generalisable to the population of English dairy heifers.

8.2. Data collection and bias

This is the first dairy cattle longitudinal study that explored the risk factors and impacts of *O. ostertagi* infection using such a wide spectrum of data sources with different formats. Previous research has predominantly relied on closed-ended questionnaires to collect data on herd management (Guitian et al., 1999; Sanchez and Dohoo, 2002; Bennema et al., 2010). The reliability of the data collected in the questionnaires was guaranteed by the review of the responses during the farm visits. Questionnaire data were also re-assessed during the interview with farmers. Moreover, additional data validation was conducted by cross checking the responses obtained from face-to-face and telephone interviews (Dohoo et al., 2009). Given that the data were collected and coded by the same person, the chances of data misinterpretation and miscoding were reduced. While questionnaires can lead to either incomplete, misleading or invalid data, interviews provide participants more contexts for information clarification and precision, which improve response rate and data validity (Oppenheim, 1992).

It is possible that the study internal validity was affected by some bias, especially recall bias in relation to the capture of retrospective data (Hassan, 2005). However, particular attention was paid to reduce such bias, in particular by (1) constantly reminding farmers of the time periods; (2) repeating and confirming answers with farmers; and (3) triangulating the data sources (e.g. interviews and QMMS recording). A special focus on the reliability, validity and limitations of the data related to heifer exposure to *O. ostertagi* (i.e. individual milk ELISA results) will be made in Chapters 3 and 4 and therefore are not discussed here.

9. Conclusions

The quantitative design and methods proposed in this dairy longitudinal study offer a reliable and valid approach to collect data and address research questions that are particularly complex. A longitudinal design was chosen given it is best suited for exposures varying over time and for which sequence of events is important. In order to capture the complexity of each farm grazing management and related heifer exposure to *O. ostertagi* on pasture, a wide range of data sources were used. This multiplicity of data sources guaranteed the validation of the collected data and increased the strength of the measured associations.

The study design also offered opportunities for the PhD student to particularly engage with the participants. In doing so, researchers not only increase the quality of their data for subsequent analysis but also have more opportunities to discuss with farmers. In fact, this contributes to foster farmers' trust and the credibility they have in scientific expertise, as well as allowing scientists to understand farmers' local realities that are crucial for their expertise.

To conclude, the quantitative component of this dairy study provides an example of robust epidemiological study design to investigate the prevalence, risk factors and impacts associated with heifer exposure to *O. ostertagi* in England and to engage farmers in the prevention and control of cattle helminth infections in the region.

Chapter 3.
Helminth infections and control: A descriptive study of dairy farms in England

1. Introduction

The control of helminth infections in conventional cattle farming systems typically relies on the indiscriminate use of anthelmintic drugs by producers in order to maximise profit. Given that helminth infections are mostly subclinical, cattle farmers routinely use blanket treatments (Vercruysse and Claerebout, 2001). In the UK, concerns over cattle anthelmintic resistance have contributed to the development of the COWS guidelines (Control Of Worms Sustainability) by the Agriculture and Horticulture Development Board (AHDB) (COWS, 2010). These have been adapted from the SCOPS guidelines (Sustainable Control Of Parasites in Sheep) produced by the sheep industry. However, recent evidence suggests that the adoption of the COWS guidelines by cattle farmers in England is still unsatisfactory (Heasman et al., 2012). While some information on the practices and attitudes of sheep farmers on helminth control is available in England (Morgan et al., 2012), the case of the dairy cattle industry has been so far largely overlooked.

In order to implement optimal and targeted helminth control strategies on the farm, farmers need to understand and apply basic epidemiological information, such as prevalence at both individual and farm levels (Vercruysse and Claerebout, 2001). In the case of dairy farms, this knowledge is especially applied to heifers, since these are arguably the future of the herd (AHDB, 2015a) and therefore the main focus of farmers' anthelmintic treatments (COWS, 2010). Nonetheless, estimations of the prevalence of cattle helminth infections are rather limited in England, especially for *O. ostertagi*. As evidence of this lack of information, no prevalence studies on *O. ostertagi* in cattle have been conducted since the eighties in the country (Bairden and Armour, 1981; Hong et al., 1981). Besides, the most recent prevalence data available for cattle helminths in the region are limited to the case of *F. hepatica* at farm

level (farm prevalence ranging from 48% to 86% according to the year and the climate) (Salimi-Bejestani et al., 2005a; McCann et al., 2010b; Howell et al., 2015).

Diagnostic tools for the identification of cattle helminth infections and to gather information on related herd status, risk factors and impacts are fundamental to address the problem (Charlier et al., 2014). Many studies have highlighted how the use of milk ODR to estimate cattle exposure to *O. ostertagi* is more accessible and reliable than other diagnostic tools (Berghen et al., 1993; Dohoo et al., 1997; Sekiya et al., 2013). It has also been suggested that the storage of milk samples by freezing does not affect the results if these are stored for a maximum period of 244 days (i.e. approximately 8 months), which facilitates the use of such indicators in observational studies (Sanchez et al., 2002c; Charlier et al., 2005c). Given that individual ODR within herds with either low or high BTM ODR are subject to large variations (Charlier et al., 2007a), recent research has also recommended the use of individual milk ODR rather than BTM ODR to provide insights into the parasite status of a herd (Charlier et al., 2010b; Blanco-Penedo et al., 2012).

However, available literature shows that, to date, BTM ODR remains the most used marker among epidemiological studies, with dairy heifers rarely being the focus of the research compared to adult dairy cows (Blanco-Penedo et al., 2012). In order to contribute to filling this gap and expand the knowledge on diagnostic tools for helminth infections, the aims of the current chapter were to (1) estimate the repeatability of *O. ostertagi* ELISA after a long-period of storage of the study samples at -20°C (±2°C), i.e. longer than 244 days; (2) investigate the relationship between heifer individual and BTM antibody levels against *O. ostertagi*; (3) describe both the characteristics of the farms included in the study (in terms of demographics, management and cattle helminth infections), and the practices adopted by farmers for cattle helminth control; and (4) estimate the prevalence of *O. ostertagi* and *F. hepatica* infections at both the herd and heifer levels.

2. Materials and Methods

Forty-one (N=41) dairy farms and 1,454 heifers were included in this study (Chapter 2., section 7.3.2.).

2.1. Pilot study

A pilot study was conducted to evaluate the effect of storage on the repeatability of the ELISA results, given that heifer samples were planned to be stored up to 482 days (i.e. approximately 16 months) after milk sampling in the participating farm. Eighty-six individual milk samples from adult cows (N=86) that had been stored in QMMS laboratory at -20°C and tested against *O. ostertagi* in 2012 were tested again in March 2014. The test used the same Svanovir® ELISA kit and followed manufacturer's instructions (Chapter 2., section 5.4.2.). Since the two ELISA kits used in 2012 and in 2014 relied on different kit batches (i.e. different *O. ostertagi* antigens), the results were adjusted for QMMS internal control before they were compared. The median storage time of adult cow milk samples was 503 days (p25-p75: 476- 518). Measurements of paired test results were computed using paired t-test (McDonald, 2014) and Lin's Concordance Correlation Coefficient (CCC) (Lin, 1989). This coefficient evaluates the agreement between two continuous measures, accounting for the precision and the accuracy of the data, and determines how far the observed data deviate from the perfect concordance (Lin et al., 2002). The CCC computation was done before and after adjusting for the internal control of QMMS laboratory and the analysis was done in STATA 12.1 (STATA Inc., Texas, USA).

2.2. Association between heifer individual and bulk tank milk ELISA results

The relationship between heifer individual and BTM ODR was investigated taking into account seasons and months of sampling, and numbers of samples per month (see Chapter 2., section 7.3.2. for the month distribution of the BTM samples). Two periods of sampling were defined for both individual and BTM samples: period 1, between June and July; and period 2, between October and December. Pearson correlation coefficients (McDonald, 2014) were calculated between the mean ODR for BTM (i.e. average of the ODR obtained for the two BTM samples in a given farm) and the mean, p25 and p75 ODR for heifer individual milk (i.e. considering all heifer samples in a given farm for the defined period). A P-value$\leq$0.05 was considered significant and related correlations interpreted as strong (above ±0.60), moderate (between ±0.40 and ±0.59) or weak (below ±0.39) (MacDonald, 2014). Four BTM ODR categories were deduced from the approximate quartiles of the study BTM ODR. For each farm, the two BTM samples ODR were assigned to a correspondent BTM category. Then, the distribution of heifer

individual milk ODR was plotted within the four BTM ODR categories, according to the month of sampling.

2.3. Descriptive study

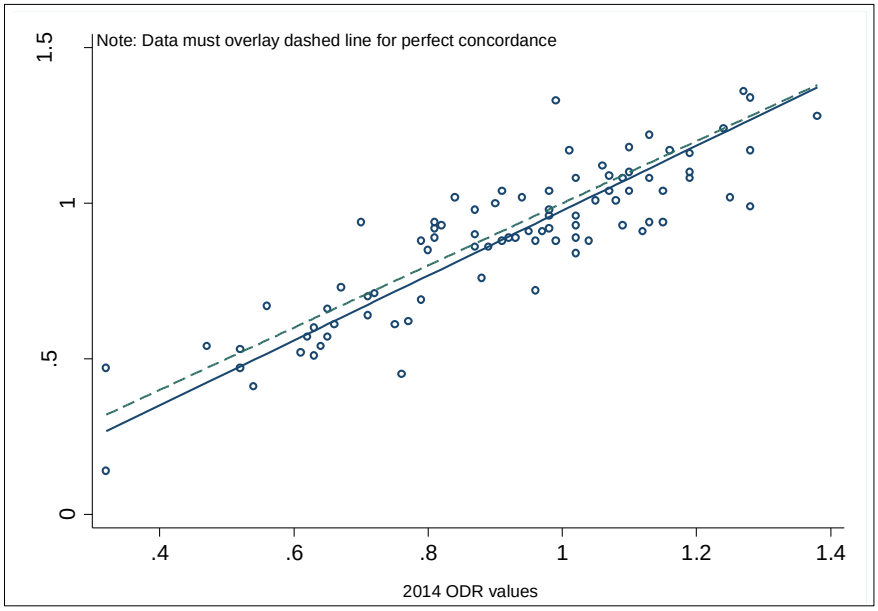
The data used to address the objectives of this quantitative study were collected, coded and edited by the approaches and methods described in the previous chapter (Chapter 2., sections 5. and 6.). Because farm demographics and management practices (except grazing) did not change significantly after 2010 for all farms (Chapter 2., section. 7.3.1.), it was possible to provide a general profile of the dairy farms involved in the study. The characteristics of the heifers (e.g. demographics, grazing, and anthelmintic treatments) were also investigated (N=1,454). BTM and individual milk ELISA results were summarised. Descriptive analysis of results was conducted using STATA 12.1 (STATA Inc., Texas, USA) to summarise the data. Mean (Standard Error (SE)) and median (p25-p75) were calculated for the data. Kruskal-Wallis and Wilcoxon equality tests on unmatched data (McDonald, 2014) were performed to compare the ODR distribution in, respectively, heifer individual milk samples across the four seasons (i.e. spring, summer, autumn and winter) and BTM samples across the two periods of sampling (i.e. period 1, between June and July, and period 2, between October and December). A P-value$\leq$0.05 was considered significant. The herd prevalence of *O. ostertagi* and *F. hepatica* infections was estimated according to published literature. Two *O. ostertagi* herd prevalence estimates were calculated according to the presumed economic loss induced by this infection in cattle (Charlier et al., 2007b; Forbes et al., 2008): (1) prevalence 1, of herds that suffer from moderate economic loss ($0.5 \leq ODR \leq 0.8$) and (2) prevalence 2, of herds that suffer from high economic loss ($ODR > 0.8$). As described in the manufacturer's ELISA test instructions (IDEXX, Montpellier, France), an overall herd prevalence was estimated for *F. hepatica*, with a farm PP of 30 or above defined as a positive farm. A distinction was made between prevalence estimates of lowly ($30 < PP \leq 80$; less than 20% of the herd infestation), medium ($80 < PP < 150$; between 20% and 50% of the herd infestation) and highly ($PP \geq 150$; more than 50% of the herd infestation) exposed herds (IDEXX, Montpellier, France).

3. Results

3.1. Pilot study

There was no significant difference between the 2012 and 2014 ODR means, irrespective of results adjustment for QMMS laboratory internal control (P-value<0.001). The CCC with 95% CI were substantial and ranged from 0.87 (0.82-0.92) (no ODR adjustment) to 0.89 (0.84-0.93) (ODR adjustment). Figure 3-1 presents the data of the duplicate testing without ODR adjustment; the 2012 ODR are plotted against the 2014 ODR for the same cow samples and the dotted line represents perfect agreement between the two readings.

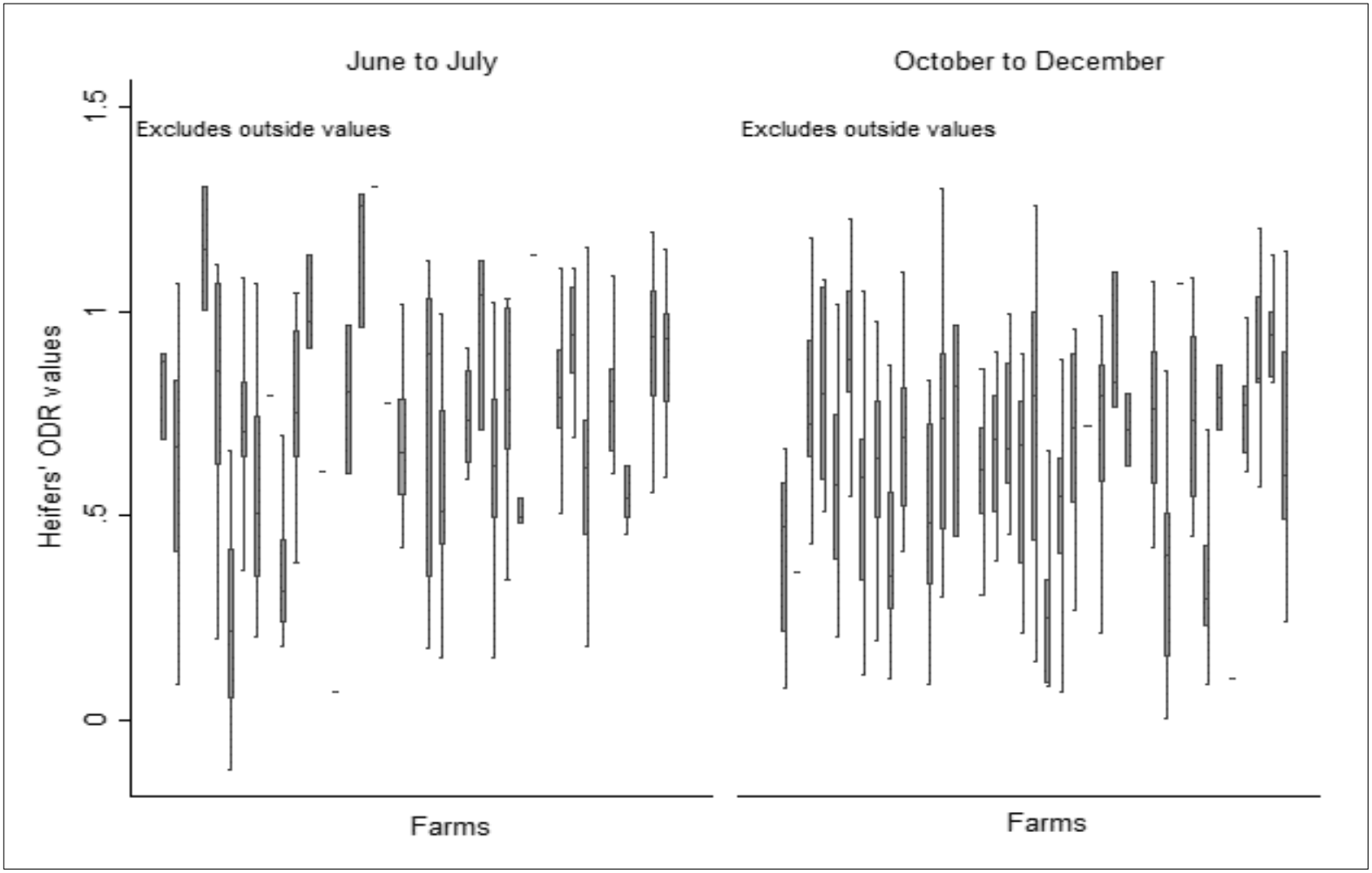
Figure 3-1: Scatter plot of the Lin's Concordance Correlation Coefficient (CCC) for the relationship between the 2012 and 2014 ELISA results against *O. ostertagi* from duplicate testing of 86 milk samples (CCC=0.87; no adjustment for QMMS internal control)



3.2. Relationship between heifer individual and bulk tank milk ELISA results

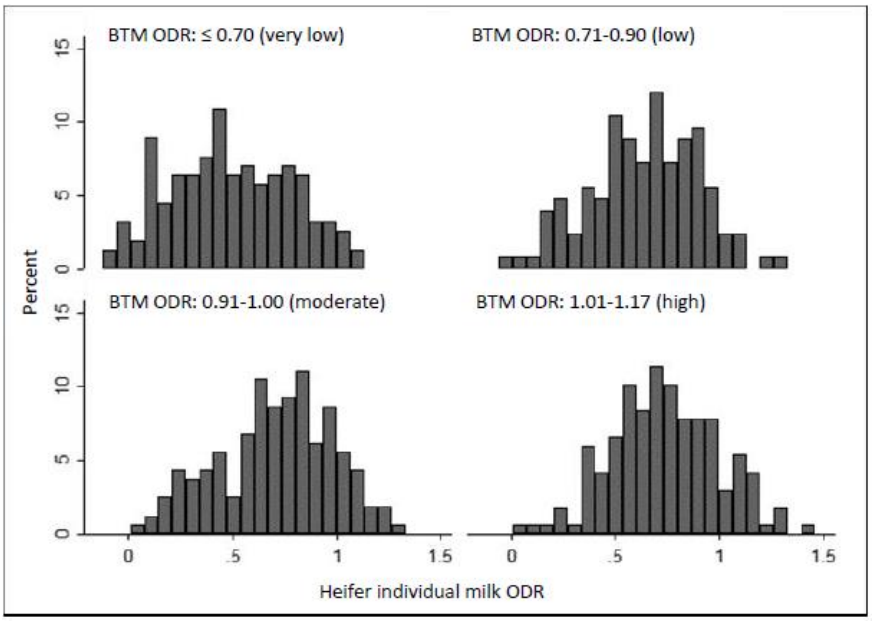
The mean ODR for BTM was significantly correlated (P-value<0.001) with the p25, the mean and the p75 ODR for heifer individual milk in each farm. These correlations were, respectively, strong (0.66 (0.39;0.82)); moderate (0.53 (0.21;0.75)); and moderate (0.47 (0.13;0.71)). The distribution of heifer individual milk ODR presented huge variability among farms for the two periods of milk sampling (Figure 3-2).

Figure 3-2: Box and whisker diagram of heifer individual milk ODR by farm (N=40) and periods of sampling in 2014, displaying minimum ODR, interquartile range (distance between the 25th and 75th percentiles), median and maximum ODR



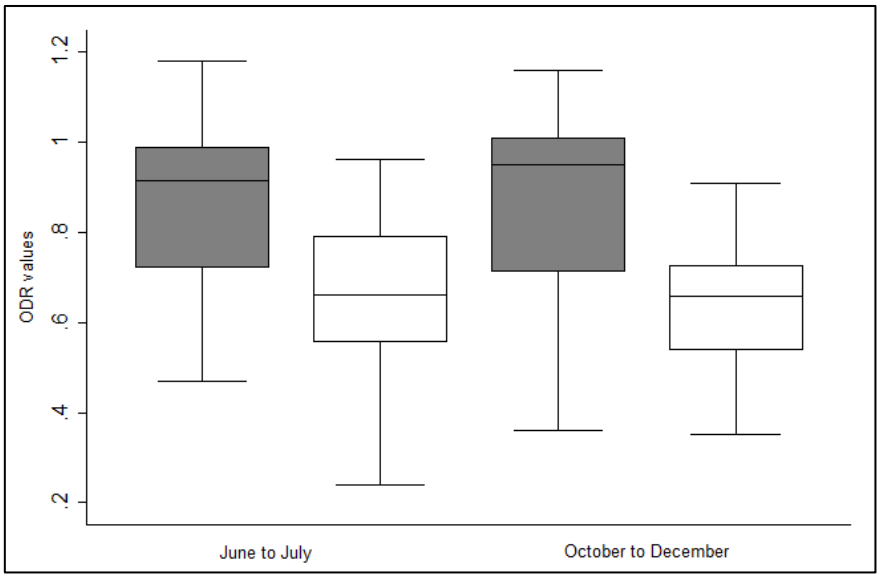
The four quartile-based categories of BTM ODR were: ≤0.70; 0.71-0.90; 0.91-1.00; 1.01-1.17. The distribution of heifer individual milk ODR within each category of BTM ODR is presented in Figure 3-3.

Figure 3-3: Distribution of heifer individual milk ODR by category of bulk tank milk ODR. The distribution shows the range of heifer individual ODR to expected when bulk tank milk ODR range is very low, low, moderate, and high



Considering the different months of individual and BTM sampling, a correlation coefficient between the mean ODR for BTM and the mean ODR for heifer individual milk could be calculated in 14 farms for period 1 (i.e. between June and July) and 24 farms for period 2 (i.e. between October and December). The estimated correlation coefficients were significantly moderate (P-value<0.001) in the two periods of sampling with (1) r=0.51 (-0.024;0.82), for period 1 (i.e. summer); and (2) r=0.54 (0.17;0.77), for period 2 (i.e. autumn) (Figure 3-4).

Figure 3-4: Box and whisker diagram of ODR values against *O. ostertagi* by sample (i.e. heifer individual and bulk tank milk) and period of sampling, with minimum ODR values, 25th and 75th percentiles, median and maximum ODR values. N_{Farms}=14 (June to July) and N_{Farms}=24 (October to December)



***Key: Black, BTM samples; White, Heifer individual milk samples**

3.3. Farm sample

3.3.1. Farmer demographics

Most of the farmers involved in the study were main farm managers responsible for the dairy herd (N=37; 90%) and came from a dairy family (N=37; 90%). The sample included 35 (85%) men and 6 (15%) women, with mean (SE) age at the time of the interview of 46 (2) years old. Farmers mean (SE) age at the start of their dairy activity was 19 (1) years old. Fifty-six per cent (N=23; 56%) of the farmers received an agro-farming education: 46% (N=19) in the university and 10% (N=4) in sporadic short-term trainings.

3.3.2. Farm demographics

- *Geography*

The median (p25-p75) altitude of farms was 53 (19-112) meters above sea level. Farms presented on average one type of soil (p25-p75: 1-2), with a predominance of medium (N=20; 49%) and heavy soils (N=17; 41%). Other soils reported on farms were sandy-silty (N=10;

24%)), clay-limestone (N=6; 15%), peaty (N=4; 10%), clay (N=4; %10) and scrub (N=1; 2%). The median (p25-p75) surface area for cattle grazing on farms was 160 (100-222) hectares.

- *Production system*

The sample included 59% (N=24) of pure-dairy farms and 41% (N=17) of mixed livestock farms, of which 22% (N=9) reared dairy and beef cattle; 12% (N=5) dairy, beef and sheep; and 7% (N=3) dairy and sheep only. The majority of the farms (N=29; 71%) had a conventional system of production; others were integrated 14.5% (N=6) (i.e. integrating environmental considerations into their farming practices) and organic 14.5% (N=6). Seven farms (17%) also reported having other businesses on the farm (e.g. poultry and arable).

The median (p25-p75) age of the dairy farms was 62 (48-102) years old. The median (p25-p75) number of total and fulltime staff was 5 (4-6) and 3 (2-4), respectively. Farm median (p25-p75) number of milking cows and breeds at the time of the farm visit was 150 (101-330) and 1 (1-2), respectively. The majority of the farms (N=37 (90%)) reared Holstein Friesian; the rest being a mixture of dairy pure- and crossbreds (e.g. Holstein Friesian cross, British Friesian cross, and Jersey cross). A total of 37% (N=15) of the farms were closed herds and 17% (N=7) imported cattle from abroad.

- *Calving system*

Twenty-seven (66%) herds calved all-year-round and 14 (34%) during at least two different seasons in a year. Most farms (N=26; 63%) bred heifers through a combination of artificial insemination (AI) and natural breeding, but some used only AI (N=10; 24%) and natural breeding (N=5; 12%). Farms that used a bull for heifer breeding (N=31), mainly purchased the bull (N=25; 81%). On average one bull was present on a farm. Farmers used mainly the following oestrus detection tools: 71% (N= 29) visual observation; 54% (N=22) tail paint; 44% (N=18) electronic heat detection device; 29 % (12/41) mounting activity and only 2% (N=1) vasectomized bull. Twenty-three (66%) farms used pregnancy diagnostic tests. The mean (SE) age targeted for heifers bulling was 15.6 (0.2) months old.

3.3.3. Pre-weaned calves management

The management of pre-weaned calves (PWC) at birth is presented in Table 3-1. Most farmers (N=35; 86%) declared grouping their PWC in groups of 5 (p25-p75:4- 10) at about 7 days of

age (p25-p75:1- 12). On average, PWC received their first starter ration at 7 days, roughage (e.g. hay and straw) at 5 days and water *ad libidum* at 5 days. In total, 76% (N=31) and 20% (N=8) of farmers supplemented their PWC with concentrate and a coccidiostat, respectively. The median (p25-p75) age of calves at weaning was 8 (8- 10) weeks and at first turn-out 6.5 (5.5- 10.1) months.

Table 3-1: Pre-weaned calves management adopted by the 41 farmers included in the dairy longitudinal study

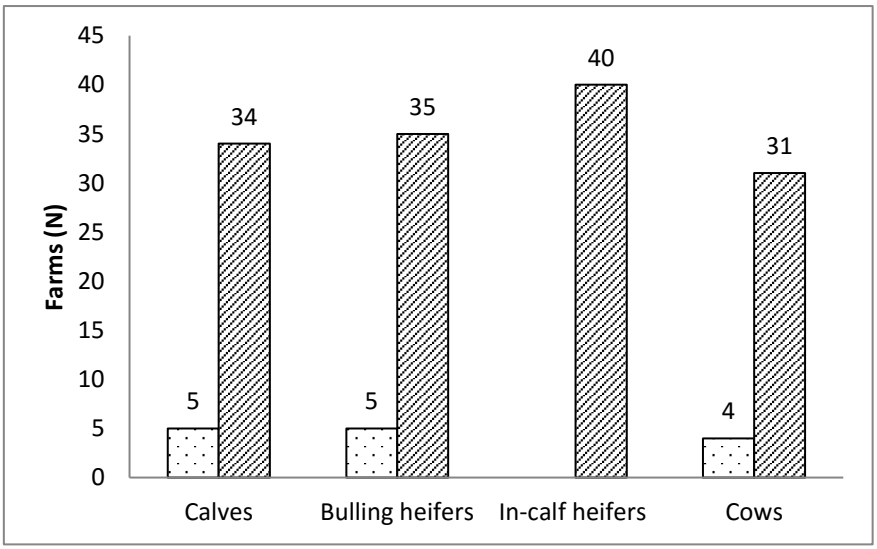
Variable	Number (%)	Median (p25-p75)
Individual pen calving		
No	5 (12)	
Yes	34 (83)	
Not systematic	2 (5)	
Time of separation with the dam (h)		
0	1 (2)	
<12	16 (39)	
12-24	10 (24)	
>24	14 (34)	
Methods of colostrum feeding		2 (1-2)
Oesophageal feeder	25 (61)	
Bottle-bucket	25 (61)	
Unassisted	14 (34)	
Suckling assisted	13 (32)	
Sources of colostrum feeding		1 (1-2)
Own dam	32 (78)	
Pooled including heifers	12 (29)	
Stored	10 (24)	
Pooled excluding heifers	3 (7)	
Commercial	2 (5)	
Volume of colostrum (L)		
First 6 hours		2.5 (2.3-3.0)
First 24 hours		5.0 (5.0-6.3)
Sources of liquid feed		1 (1-2)
Milk replacer	26 (63)	
Fresh cow milk	22 (54)	
Mastitis-AB milk	17 (41)	

3.3.4. Cattle housing

Different profiles of housing (i.e. all-year-round and winter only) existed among farms by cattle category (calves, i.e. from weaning to bulling age; bulling heifers, i.e. from bulling age to

confirmed in-calf; in-calf heifers; and cows) (Figure 3-5). Twenty farms (49%) mixed their calves in the shed with other cattle categories or breed (i.e. beef); 31 (76%) and 38 (93%) did the same with bulling and in-calf heifers, respectively. Most farms (N=25; 61%) supplemented their calves and bulling heifers at housing with concentrate, compared to half (N=26; 39%) their in-calf heifers. A total of 20% (N=8) of the farms also provided coccidiostat to their calves after weaning.

Figure 3-5: Type of housing used for calves (i.e. from weaning to bulling age), bulling heifers (i.e. from bulling age to confirmed in-calf), in-calf heifers and cows in the 41 farms included in the dairy longitudinal study



***Key: Dot, all-year-round; Stripe, in winter only**

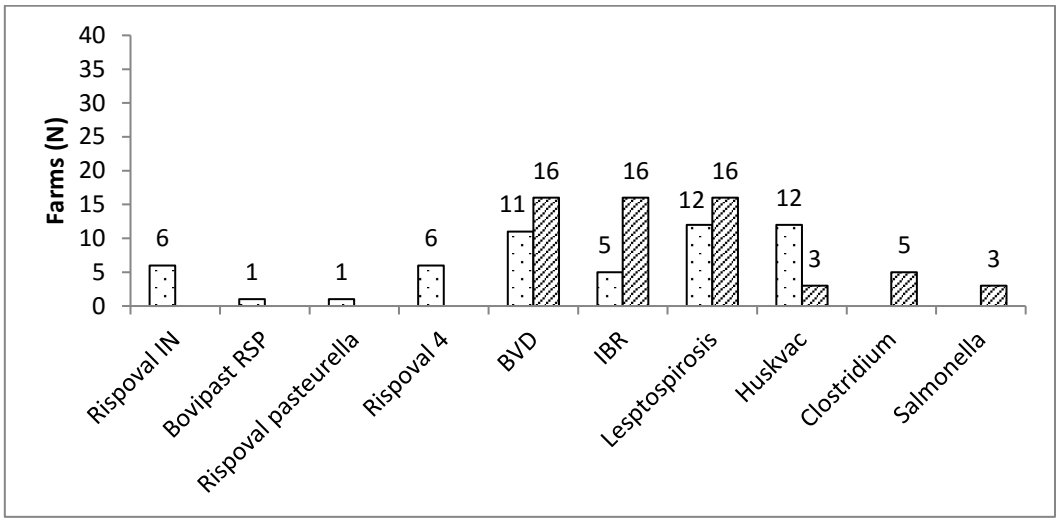
****Farms where housing of animals could 'vary' are not present in the figure**

3.3.5. Infectious diseases

- *Vaccination*

Most farms did not test the quality of their colostrum (i.e. concentration in IgG) (N=30; 73%) but tested their cows against Johne's disease (N=28; 68%). Figure 3-6 presents farms vaccination schedules for calves (i.e. defined as PWC and weaned calves) and adult cattle (i.e. defined as older than 12 months).

Figure 3-6: Type of vaccines used for pre-weaned calves, calves (i.e. from weaning to bulling age), bulling heifers (i.e. from bulling age to confirmed in-calf), in-calf heifers and cows in the 41 farms included in the dairy longitudinal study

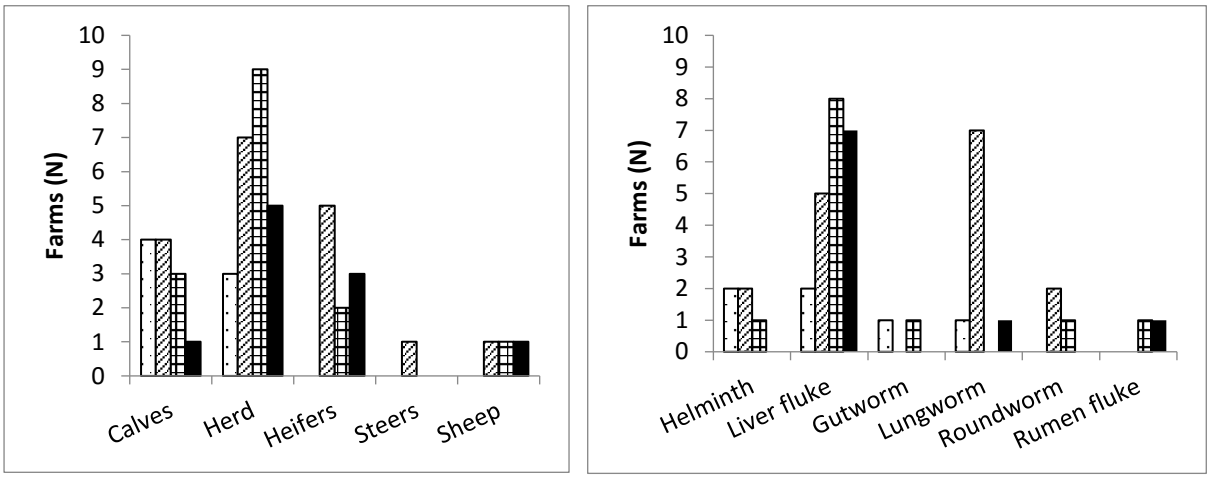


***Key: Dot, pre-weaned/weaned calves; Stripe, bulling heifers/in-calf heifers/cows**

- *Helminth infections*

Half of the farms (N=20; 49%) experienced cases of helminth infections between 2012 and 2013. Of those, 10% (N=2) reported it for 2012; 65% (N=13) for 2013; and 25% (N=5) for 2012 and 2013. Between 2014 and 2015, 37% (N=15) of the farmers had cases of helminth infections in 2014 and 24% (N=10) in 2015 (Figure 3-7). Among the farms that reported cases of helminth infections, 14% (N=1) had the infection diagnosed in 2012 (i.e. by laboratory tests and/or carcases condemnation); 67% (N=12) in 2013; 80% (N=12) in 2014; and 50% (N=5) in 2015.

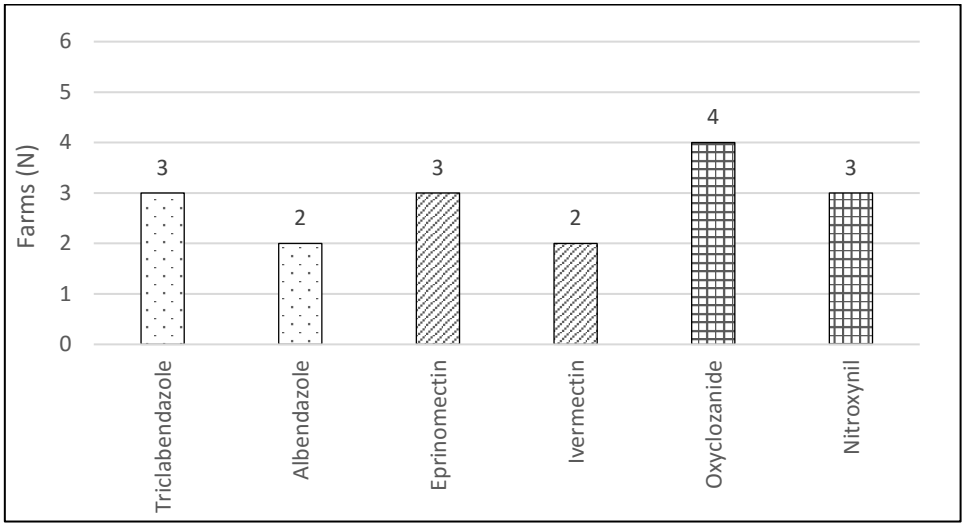
Figure 3-7: Cases of helminth infections reported on-farm between 2012 and 2015 in the 41 farms included in the dairy longitudinal study (left: category of livestock infected; right: type of helminths)



***Key: Dot, 2012; Stripe, 2013; Square, 2014; Plain black, 2015**

Overall, 44% (N=18) of the farmers systematically dewormed their adult cows; the rest did not treat their adult cows against helminths (N=23; 56%). The farms that treated their adult cows did it once a year with only one class of anthelmintic drugs Most farms used drench (N=10; 55%); other forms used were injection (N=6; 33%) and pour-on (N=5; 28%). Figure 3-8 details the different classes of anthelmintic drugs used by farmers in farms systematically deworming their adult cows.

Figure 3-8: Class of anthelmintics used in the study farms systematically treating their adult cows against parasites (N_{Farms}=18)



***Key: Dot, group 1-Benzimidazoles; Stripe, group 3-Macrocyclic Lactones; Squared, group 4-Flukicides**

3.4. Heifer sample

3.4.1. Demographics

Sampled heifers included 14% (N=200) of dairy crossbreds and 86% (N=1,254) of dairy purebreds. The majority of heifers were Holstein Friesian (purebreds (N=1,207; 83%) and crossbreds (N=117; 8%)). Most heifers were born in 2012 (N=1,013; 70%) and 2011 (N=384; 26%); the rest was born in 2013 (N=45; 3%) and 2010 (N=12; 1%). The median (p25-p75) age of heifers at first calving was 27.3 (25.0-30.6) months. According to available records, 10% (139/1,385) of the heifers aborted or had a still birth at first calving; 61% (761/1,249) had female calves and 39% (488/1,249) male calves.

3.4.2. Grazing

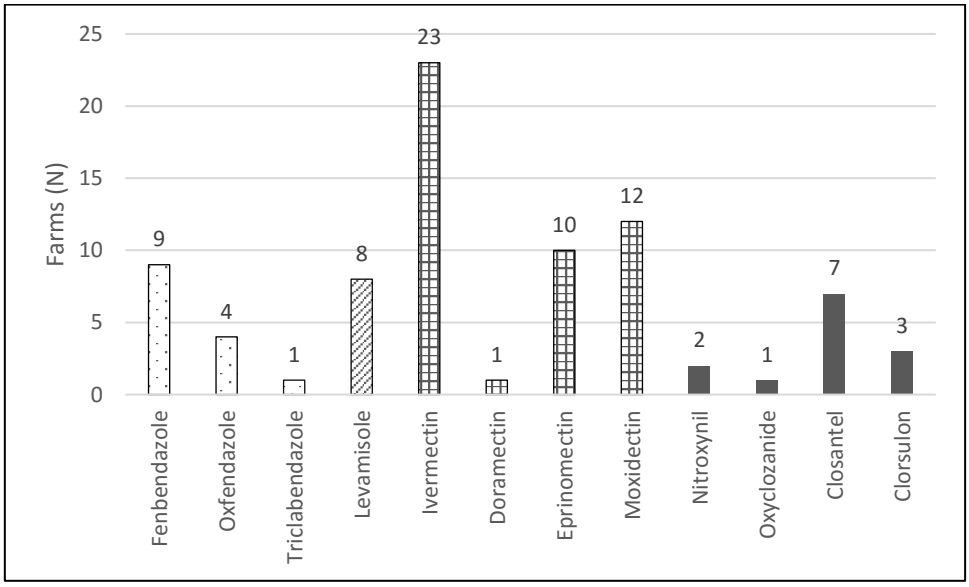
The median (p25-p75) age of heifers at first turn-out was 9.5 (6.9-13.6) months. The majority of heifers had two grazing seasons prior to sampling (N=852; 59%); others had three (N=340; 23%), one (N=251; 17%) or four (N=11; 0.8%). The median (p25-p75) age of heifers at milk sampling was 28.9 (26.6-32.3) months. Given less than 1% of the heifers had a fourth grazing season, only the first three grazing seasons were described; results are displayed in Appendix

7. The in-depth description and analysis of heifer grazing management is the focus of Chapter 4.

3.4.3. Anthelmintic treatments

Most farmers (N=39; 95%) treated their heifers against helminths ($N_{Heifers}$=1,275; accounting for 88% of the total sample size). The median (p25-p75) number of overall anthelmintic treatments, forms and classes applied/used on sampled heifers, from first turn-out to sampling, were 2 (1-4), 1 (1-2) and 2 (1-2), respectively. Farmers predominantly used pour-on (N=27; 69%); other forms of anthelmintics used were injections (N=13; 33%), bolus (N=10; 27%) and drench (N=5; 13%). Figure 3-9 details the different classes of anthelmintic drugs used by farmers in young-stock (i.e. calves, bulling and in-calf heifers). Around 36% (N=14) of the farms treated their heifers during the grazing season and at housing; 18% (N=8) at turn-out, during the grazing season and at housing; 15% (N=6) at turn-out and at housing; 10 % (N=4) at turn-out only, 8% (N=3) during the grazing season or at housing and 5% (N=2) at turn-out and during the grazing season.

Figure 3-9: Class of anthelmintics used by farmers in the heifers included in the dairy longitudinal study (N_{Farms}=39)



*** Key: Dot, group 1-Benzimidazoles; Stripe, group 2-Levamisole; Square, group 3-Macrocyclic Lactones; Plain black, group 4-Flukicides**

3.5. Descriptive epidemiology of *Ostertagia ostertagi* and *Fasciola hepatica*

3.5.1. Farm level

- *Bulk tank milk ELISA results*

Thirty-six (N=36; 88%) farms had sent their two BTM samples during the period between June and July (period 1, summer) and the period between October and December (period 2, autumn). Overall, in these farms, the median (p25-p75) ODR for BTM samples was 0.96 (0.79-1.03) with a median (p25-p75) of 0.96 (0.82-1.03) in summer, and 0.98 (0.76-1.02) in autumn. In the case of *F. hepatica*, the median (p25-p75) PP against *F. hepatica* in BTM samples was 24.66 (3.49-68.91) with a value of 30.02 (3.39-64.32) in the summer, and 20.30 (4.38-89.33) in the autumn. There was no significant difference across seasons (i.e. summer and autumn) in the distribution of the BTM ELISA results against *O. ostertagi* (P-value=0.94) and *F. hepatica* (P-value=0.71).

- *Herd prevalence*

Based on the definitions exposed in section 2.3., the apparent herd prevalence of moderate ($0.5 \leq ODR \leq 0.8$) and high economic losses due to *O. ostertagi* ($ODR > 0.8$) were respectively, 19% and 75% in summer, and 17% and 75% in autumn. In the case of *F. hepatica*, the apparent herd prevalence ($PP > 30$) was 50%, in summer, and 42% in autumn. Apparent herd prevalence estimates for the three levels of *F. hepatica* exposure (i.e. low ($30 < P \leq 80$), moderate ($80 < PP < 150$) and high ($PP \geq 150$)) were, respectively, 33%, 8% and 8%, in summer; and 17%, 6% and 19%, in autumn.

3.5.2. Heifer level

The median (p25-p75) ODR for heifer sample was 0.64 (0.42-0.84). Heifers were on average in their 47 (38-58) DIM at the time of sampling. Table 3-2 summarises the results of heifer individual milk ODR by season of sampling. There was no significant difference across seasons in heifer individual milk ODR (P-value=0.25).

Table 3-2: Average of heifer individual milk ODR by season of sampling (N=1,454)

ODR	Median (p25-p75)	Mean(SE)
Spring	0.63 (0.42-0.89)	0.65 (0.02)
Summer	0.66 (0.43-0.86)	0.64 (0.02)
Autumn	0.64 (0.43-0.83)	0.63 (0.01)
Winter	0.61 (0.41-0.82)	0.61 (0.02)

***SE=standard error; p25-p75=25th-75th percentiles**

The majority of the heifers did not have mastitis (N=1,084; 75%) at the time of the sampling; others had uncertain status (N=143; 10%); recovered (N=87; 6%); were new cases (N=79; 5%); chronic cases (N=48; 3%); and reported infected (N=13; 1%).

4. Discussion

4.1. Repeatability of the ELISA test and effect of storage at -20°C

There was no significant difference between the 2012 and 2014 paired testing results of cow milk samples and observations remained the same irrespectively of results adjustment for laboratory internal control. This confirms previous reports on the high repeatability of the Svanovir® *O. ostertagi* ELISA kit (Sanchez et al., 2002c; Charlier et al., 2005c). It is possible that the use of normalised ODR to express the results of the ELISA test also contributed to these findings (Sanchez et al., 2004b). As previously reported in the literature (Sanchez et al., 2002c; Charlier et al., 2005c), the presence of the preservative bronopol in milk did not affect heifer milk ODR. This is an important point considering that milk samples are often preserved when collected on farms for dairy laboratories. Finally, another key and novel result is that milk samples' freezing up to 575 days (i.e. approximately 17.5 months) did not affect the ODR, which significantly increases the length of similar milk sample storage previously reported in the literature (244 days; i.e. approximately 8 months) (Sanchez et al., 2002c). As a result, the current study suggests that the commercial ELISA kit Svanovir® is a very good candidate for conducting extensive longitudinal studies of *O. ostertagi* infections in cattle.

4.2. Association between heifer individual and bulk tank milk ELISA results

The correlation between the mean ODR for heifer individual and BTM was moderate (i.e. between ± 0.40 and ± 0.59). However, it increased when considering BTM mean ODR and the p25 ODR for heifer individual milk. Such a non-linear relationship between milk antibody levels and ELISA results has already been reported in the literature (Charlier et al., 2010b). Evidence suggests that the ELISA technique has limitations due to the availability of the binding site for the detection of the sample antibodies (Crowther, 2001). In the current study, the mean ODR for BTM samples was significantly higher than the farm mean ODR for heifer individual milk. This is consistent with the moderate correlation obtained between the two markers and could be related to differences between heifers and multiparous cows (present in the tank) in terms of parasite exposure, physiology (e.g. IgG synthesis and IgG leakage from plasma to milk), and anthelmintic treatments (Sanchez et al., 2004a; Charlier et al., 2010b). In fact, evidence suggests that both exposure and immune response to *O. ostertagi* in multiparous cows are higher than in heifers (Klesius, 1988; Charlier et al., 2010b).

There was substantial variability in heifer individual milk ODR within each category of BTM ODR. Considering the economic importance of heifers in a dairy farm (COWS, 2010), farmers may consider the use of individual milk ODR to gain insights into the parasite status of their herd. Similar findings were reported for adult dairy cows (Sanchez et al., 2002b; Charlier et al., 2007a) suggesting that, even in a farm with lower BTM ODR, some animals may benefit from anthelmintic treatments (Sanchez et al., 2002b; Charlier et al., 2007a). The identification of an individual milk ODR threshold, above which response to anthelmintic treatment would be beneficial for production, has been the focus of many studies on adult dairy cows (Sanchez et al., 2002b; Sanchez et al., 2004a; Sanchez et al., 2005; Vanderstichel et al., 2013). Such an approach could be particularly relevant in research looking at heifers to reduce the use of cattle anthelmintic treatments by farmers, given that heifers are the main target of these treatments (COWS, 2010). However, to date, there is a lack of this type of research with a focus on heifers and a need for further investigation on the topic.

4.3. Descriptive epidemiology of *Ostertagia ostertagi* and *Fasciola hepatica*

4.3.1. Levels of Ostertagia ostertagi antibodies in bulk tank milk

The median (p25-p75) BTM ODR was 0.96 (0.79- 1.03), which is higher than that reported in Canada (0.36 to 0.54) (Sanchez and Dohoo, 2002; Sanchez et al., 2002b), in Europe (0.30 to 0.83), and, in particular, in the UK (0.60 to 0.97) (Charlier et al., 2005b; Forbes et al., 2008; Bennema et al., 2010). All of these surveys used the same ELISA test and method (i.e. Svanovir®), as well as herds that had access to pasture and were treated against helminths. In the case of the UK, Forbes et al. (2008) reported a BTM ODR of 0.6 for BTM samples collected across the country at the end of the grazing season, i.e. when cattle antibody levels against *O. ostertagi* are higher (Charlier et al., 2007a). The comparison of this value to that of the current study during the same period of time (BTM_{autumn}=0.98) suggests that the difference between these estimations may be due to other factors than seasonal variations; for instance, herd management and local climate conditions may play a role (Forbes et al., 2008; Charlier et al., 2013). Moreover, since most of the farms included in the current study were located in the south-west of the UK, where the prevalence of *F. hepatica* infection is particularly high (McCann et al., 2010b), it is possible that this resulted in an over-estimation of BTM ODR due to test-cross reactivity.

The current study did not report any significant difference in BTM ODR between the two periods of sampling (i.e. summer and autumn). Likewise, it is worth noting that there was no seasonal variation in herd exposure to *F. hepatica*. Both results disagree with previous similar research related to the expected epidemiological pattern of cattle helminth infections on pasture (Charlier et al., 2005b; Charlier et al., 2007a; Bloemhoff et al., 2015a). Some reports suggested that it can be difficult to describe a seasonal pattern in BTM antibody levels (Sanchez and Dohoo, 2002). In this case, the design of the sampling protocol may be crucial since it determines the capture of the expected rise of cattle antibodies during the summer months (Sanchez and Dohoo, 2002). In the current study, it is possible that the predominance of samples collected during the period between June and July and not August, i.e. when the parasites burden on pasture increases, contributed to this result. Moreover, given that most farms treated their cattle against helminths, it is also possible that this had an effect on antibody levels after cattle exposure to parasites on pasture. In this regard, Charlier et al. (2007a)

reported a seasonal trend in BTM ODR for herds that had not received any anthelmintic treatment for at least two years prior to the start of the survey (Charlier et al., 2007a).

4.3.2. Levels of Ostertagia ostertagi antibodies in heifer individual milk

To date, decision tools for anthelmintic treatments have predominantly relied on cattle individual milk markers (Sanchez et al., 2002b; Sanchez et al., 2005; Charlier et al., 2010b; Vanderstichel et al., 2013; Verschave et al., 2014) and have not assessed individual status of helminth infections in a herd (Charlier et al., 2007a; Blanco-Penedo et al., 2012). Current research has predominantly used BTM markers, since BTM samples are inexpensive and relatively simple to collect (Guitian et al., 1999; Forbes et al., 2008; Bennema et al., 2009; Bennema et al., 2010; Charlier et al., 2013). This is the first study reporting data on the individual status of heifer exposure to *O. ostertagi* in the UK. The median ODR for heifer individual milk was 0.64, which is higher than that previously reported for adult dairy cows in Europe (0.3 to 0.5) (Charlier et al., 2010b; Verschave et al., 2014) and in Canada (0.2 to 0.5) (Sanchez et al., 2002a; Sanchez et al., 2005; Vanderstichel et al., 2013) (taking into account that all studies used the same ELISA test). Given that all studies previously mentioned refer to adult cows, a comparison of ODR is rather difficult. Moreover, a wide range of factors, including animal management, physiology and climate can lead to individual milk ODR variations. These factors are further explored and discussed in Chapter 4.

Similarly to the results of BTM ODR, heifer individual milk ODR did not vary significantly across the seasons. However, since heifer samples were uniformly distributed across the seasons and the months, this result is unlikely to be due to the protocol used for milk sampling (see the stratified random sampling approach, Chapter 2., section 4.). Previous research suggests that individual milk ODR in adult cows significantly vary across the seasons and that the related seasonal pattern is more noticeable in older cows (Sanchez et al., 2002b; Charlier et al., 2007a). Besides, according to a more recent study, the earlier reported significant association between individual milk ODR and individual characteristics in adult cows were not identified in heifers (Blanco-Penedo et al., 2012). In the context of the current study, these findings suggest that heifers might have certain characteristics that could have prevented the capture of any seasonal variation in individual milk ODR. For instance, there is physiological evidence that heifers present lower antibody concentrations in milk than multiparous cows (Levieux and Ollier, 1999). In fact, this may be related to lower synthesis of IgG in young

animals (Sanchez et al., 2004b), which explains the slow development of immunity response against *O. ostertagi* (Klesius, 1988). Moreover, previous research has highlighted that heifers have lower tissue damage than adult cows, which can decrease the leakage of IgG from plasma to milk (Sanchez et al., 2004b). Finally, it is possible that the systematic treatment of heifers against helminths may have prevented the rise of antibody levels in heifer milk at the time of high parasite exposure and, ultimately, the capture of any seasonal variation in heifer individual milk ODR.

4.3.3. Apparent herd prevalence of Ostertagia ostertagi and Fasciola hepatica infections

Based on the methodology of previous similar research, This is the first study reporting an apparent herd prevalence of *O. ostertagi* infection in cattle in England. The majority of the farms included in this study (95%) had high (76%) or moderate (19%) production losses due to *O. ostertagi* infection. In Europe, similar to higher herd prevalence of *O. ostertagi* infection were previously reported, in particular in Belgium (percentage of farms with high production losses estimated between 59% and 70%) (Bennema et al., 2009; Charlier et al., 2013) and in Ireland (percentage of farms with high production losses estimated at 98.9%) (Bloemhoff et al., 2015b). Regional differences are frequently related to differences in herd management and climate conditions (Forbes et al., 2008; Bennema et al., 2010). In Ireland, the predominance of spring-calving systems with cows (1) being turned out earlier in the season, i.e. March; (2) more susceptible to re-emerging infective larvae because of calving in the spring; and (3) grazing for longer time on pasture, might explain the particularly high prevalence of *O. ostertagi* with high production losses reported in this region (Bennema et al., 2010; Bloemhoff et al., 2015b). The apparent herd prevalence of *F. hepatica* infection estimated in the current study was 44%, which is lower to previous estimations in the UK (Salimi-Bejestani et al., 2005a; McCann et al., 2010b; Howell et al., 2015). In these reports, the apparent herd prevalence of *F. hepatica* infection ranged from 48% (in 2005) to 80% (in 2010 and 2015). Different contexts in terms of management and rainfall may have influenced these different observations (McCann et al., 2010a). Moreover, since the current study relied on a convenience sample of farms that were all clients of QMMS laboratory, it is possible that this included some selection bias, impacting on the estimation of the apparent herd prevalence. In fact, the study farms may have reflected a particularly good context of cattle liver fluke control.

An important point to highlight here is the importance of considering ELISA test limitations while interpreting data on disease prevalence that inform disease policies and interventions. It is possible that the different prevalence reported above for dairy cattle fascioliasis in the UK (Salimi-Bejastani et al., 2005a; McCann et al., 2010b and Howell et al., 2015) were due to the different test cut-off values used in the studies. Cut-off values for diagnostic tests, especially for the diagnosis of cattle helminth infections, have often been determined by arbitrary methods without clear rationales (Charlier et al., 2007b; Forbes et al., 2008). Moreover, the use of cut-off values determined with serum, in cattle milk (e.g. with the Pourquier® *F. hepatica* verification test) has already been questioned (Reichel et al., 2005). In the case of *O. ostertagi*, it is also hard to justify the value of a cut-off based on a presumed economic loss induced by infection in cattle since impacts on cattle productivity remain to date still equivocal (Chapter 1., section 5.1.). Moreover, economic losses induced by infection can highly vary according to different environments and production systems. Therefore, the use of one ELISA test cut-off value that would be accurate for all contexts of cattle helminth infections is doubtful. To result in useful and informative prevalence data, there is a need to define cut-off values adapted to different contexts that would maximise the benefit of testing. This includes to integrate the complexity of cattle helminth infections (e.g. economy, environmental factors and medication) and estimate the socio-economic impacts resulting from erroneous diagnosis and prevalence (Ridge and Vizard, 1993). In this context, the development of decision-tree to support decision making on cattle helminth control might be easier and relevant to support targeted treatment decisions of individual animals (Vercruysse and Claerebout, 2001). This way, the control policies adopted for cattle helminth control could be more efficient and sustainable.

4.4. Farmers practices for cattle helminth control in England

4.4.1. Farmers' adoption of current guidelines

In the current study, most farmers turned out their first grazing heifers when they were older than six months (81%). This is a recommended practice to lower the risk of disease and production losses due to helminth infections in cattle (COWS, 2010; Pablos-Tanarro et al., 2013). Farmers also seemed to take into account the progressive development of heifer immunity and build-up of resistance against helminths, since they decreased the frequency of anthelmintic drugs application over time (i.e. from first to third grazing season) (Taylor, 2010).

Moreover, they considered the importance of treating animals at housing to ensure that animals are wintered free of negative effects of helminths (COWS, 2010).

However, farmers overall use of anthelmintic treatments remained systematic and rather excessive, especially on young-stock. As evidence of this, most farmers treated their heifers against helminths (95%) (as opposed to 44% farms for adult cows treatment), whereas only half of them (49%) perceived having a problem in relation to helminth infections. Since heifers are likely to be under constant larval challenge, such a practice is in contradiction with current 'best practice' advices, i.e. closely monitoring the animals in order to treat them only when they need it (i.e. as clinical signs appear) (COWS, 2010). This also suggests that the rationales of farmers behind cattle helminth treatments might be driven by the prevention of production losses in young-stock (COWS, 2010). Contrary to available recommendations (Taylor, 2010), although calves were mostly born and raised indoors, i.e. free of parasite at the time of their first turn-out, 36% (at turn-out) and 42% (throughout the grazing) of the heifers received a treatment shortly after their first turn-out on pasture. It is likely that farmers' inability to adopt a 'clean grazing' system influenced such a practice (COWS, 2010; Taylor, 2010). In fact, 34% and 98% of helminth naïve heifers (i.e. first-grazing heifers) had shared their pastures with cows and older young-stock, respectively. Finally, most farms used macrocyclic lactones, especially Ivermectin (60%), which has a broad spectrum compared to other common anthelmintic drugs such as Levamisole (only used by 10% of the farms). This represents an unnecessary exposure of parasites to anthelmintic drugs, which can increase the risk of drug resistance in cattle (Taylor, 2010). It is possible that, in some farms, contextual and individual factors were responsible for an overuse of certain types of anthelmintic treatments. The convenience of some drugs, such as long-acting compounds that are directly administered at the start of the grazing season, may be one example of these reasons. Importantly, results suggest that farmers are likely to selectively adopt 'best practice' advices and that there is a need to understand farmers' rationales behind cattle helminth control, which ultimately guide their practices. This is the focus of Chapter 6.

4.4.2. Farmers' practices and challenges

Eighteen (N=18; 44%) farmers systematically treated their adult cows against parasites, which is similar to practices previously reported in the UK and Belgium (40%), but different to that reported in Ireland (69%), Germany (8%) and Sweden (3%) (Bennema et al., 2010). Such a

diversity in terms of cattle helminth control practices between countries are likely to be related to different contexts of farming, legislation and culture. For instance, the different channels of drug prescription that exist across Europe might influence the use of anthelmintic treatments in the field (Easton et al., 2016). In Western Europe, many anthelmintic drugs are used indiscriminately by farmers and have become, in the absence of clinical signs, a cheap insurance policy to maximise profit rather than to optimise practices of helminth control (Vercruysse and Claerebout, 2001). As reported in this study, anthelmintic treatments practices are generally focused on young-stock since it represents the future of farmer business (COWS, 2010).

In cattle, more than in sheep, the high diversity of molecules and delivery formulations complicate the understanding of sustainable anthelmintic use (Sutherland and Leathwick, 2011). Therefore, there is a need to gather detailed information on farmers' treatment practices in cattle. Although the use of a convenience sample may pose limits to the generalisation of the current study results to the whole of England, it allowed to capture in-depth information on the different classes and forms of anthelmintic drugs used by English farmers in their animals (as opposed to herd, which is frequently reported in the literature (Steffan and Nansen, 1990; Satrija et al., 1996; Charlier et al., 2010b; O'Shaughnessy et al., 2015)). Overall, farmers used a wide range of forms of anthelmintic drugs and classes. Evidence suggests that farmers are often constrained by labour aspects when treating animals, especially in terms of handling the cattle for the treatment (Taylor, 2010). In this regard, long-acting anthelmintic drugs can offer the possibility to treat animals with a single-dose at turnout without the need for re-application. In this study, bolus and cydectin long-acting were used in 44% of the farms, compared to 70% for pour-on. In fact, although pour-on are known to be less efficient than other forms of application, they are the most convenient and simple form to use (Taylor, 2010; Sutherland and Leathwick, 2011). Forty-four per cent of the farms (N=18; 44%) treated their cattle with flukicides, which agrees with previous reports in the UK for high-yielding herds (46% (Howell et al., 2015)). Interestingly, although Triclabendazole is the most efficient compounds to use against all stages of *F. hepatica*, only 7 farmers (17%) used it on-farm, which might be related to its long milk withdrawal (McCann et al., 2010b).

Improper and/or overuse of anthelmintic drugs have resulted in increasing problems of resistance in sheep (Jackson and Coop, 2000) and there is potential for the cattle industry to face similar challenges (Sutherland and Leathwick, 2011). In the UK, reports of Macrocyclic

lactones resistance in cattle have already been published (Stafford and Coles, 1999; Sargison et al., 2009). In this study, most farms (60%) used macrocyclic lactones, which in its pour-on form is also known to promote the development of helminths resistance (Taylor, 2010). Given that anthelmintic resistance does not work as a direct incentive for farmer behaviour change (Charlier et al., 2015), there is a need to further understand farmers' knowledges and practices in relation to cattle helminth control and their intersection with farmers' values and concerns. To address these aspects, a qualitative study was therefore conducted and is presented in Chapter 6.

5. Conclusions

The current study suggests that the commercially available Svanovir® ELISA test is a very good candidate for conducting extensive longitudinal studies of *O. ostertagi* infection in cattle (up to approximately 17.5 months). The important within-herd variability in heifer individual milk ODR reported in this study suggests that the use of individual milk ODR may provide more insights into the parasite status of the herd. Moreover, this could allow farmers to selectively target the animals that may benefit from anthelmintic treatments (Sanchez et al., 2002b; Charlier et al., 2007a). As a result, such an approach could provide opportunities for farmers to improve their farm net income (focus on heifers) and to ensure the long-term efficacy of cattle anthelmintic drugs.

However, to do so, there is still a need to determine an ELISA cut-off value that would maximize the benefit of helminth testing in heifer individual milk, i.e. the economic and social consequences of both misdiagnosis and disease prevalence. Moreover, if farmers are expected to adopt more sustainable practices against cattle helminth infections in England, experts should seek to provide, where possible, more evidence for efficient alternatives to anthelmintic treatments, especially in the case of young-stock. This is the focus of Chapter 4.

Chapter 4.
Demographics and management factors associated with individual milk antibody levels against *Ostertagia ostertagi* in heifers

1. Introduction

The development of anthelmintic resistance is associated with increasing concerns about the negative economic and welfare impacts this can have on the livestock industry and suggests a need for farmers to adopt more sustainable ways to control cattle helminth infections in the UK (COWS, 2010). As discussed earlier, farmers generally adopt blanket treatment to prevent or regain production losses due to parasite infections in young-stock and ensure the sustainability of their businesses (remembering that, in dairy herds, young-stock represents all the animals that have not yet entered the milking herd) (COWS, 2010).

Cattle exposure to helminths is influenced by a wide range of factors, including climate conditions (e.g. temperature and humidity), specific characteristics of farming systems (e.g. type, production, and management), and availability of resources (e.g. staff, facilities, and land) (Charlier et al., 2015; Wilson et al., 2015). Therefore, if cattle helminth infections are to be better understood and alternatives to anthelmintic treatments identified and recommended to farmers, there is a need to integrate the complexity of cattle helminth exposure on pasture in the analysis. This implies the consideration of different risk periods of exposure (e.g. seasons and age), an accurate account of the history of cattle management, i.e. all the management factors associated with cattle helminth exposure, and the use of reliable markers of helminth exposure.

Although the identification of risk factors associated with cattle exposure to *O. ostertagi* has been the focus of much research on dairy cows, findings still remain limited (Sanchez and Dohoo, 2002; Charlier et al., 2005a; Bennema et al., 2009; Vanderstichel et al., 2012). First, the analyses on which these studies are based are often constrained by the use of close-ended

questionnaires, which restricts the representation of complex systems of management and grazing (Bennema et al., 2010). This is especially the case when these approaches are applied to systems such as the ones present in the UK, where cattle graze in rotation and move constantly across fields (AHDB, 2013). In this context, the use of open-ended face-to-face interviews can be very useful since a dialogue can be established between researchers and farmers, giving the opportunity to the latter to clarify and add detail to the information provided (Oppenheim, 1992). Second, previous research has mainly relied on BTM whose antibody levels are often difficult to interpret because of the pooled nature of the samples (Chapter 1., section 3.3.). Evidence suggests that because cow levels of *O. ostertagi* antibody are highly varied within a farm, the use of individual milk samples might be a better approach. Finally, since the large majority of the available research has focused on adult cows, there is a lack of research focused on heifers and a need for further investigations on the topic.

Therefore, the aim of the current study is to identify the demographics and the management factors associated with heifer exposure to *O. ostertagi* using different sources of data (including face-to-face interviews) and individual milk markers of exposure. This implies the capture of sequential information on heifer management (including grazing) from birth to ELISA testing.

2. Materials and Methods

Forty-one dairy farms and 1,454 heifers were included in this study (Chapter 2., section 7.3.2.).

2.1. Demographics and management data

The predictors used to address the objectives of this observational study were collected, coded and edited by different approaches and methods that were previously described in Chapter 2 (sections 5. and 6.). The different variables related to farm management and grazing practices were described in Chapter 3 (sections 3.3. to 3.5.). These included information on farmers and farms demographics (i.e. geography, the system of production, and the system of calving), pre-weaned calves management, cattle housing and cattle vaccination, as well as information on heifer demographics and grazing history. For this latter, detailed information on heifer grazing management per grazing season was collected (e.g. number and size of pasture, stocking-rate,

mowing and fertilisation of pasture, co-grazing, pasture contamination, and anthelmintic treatments).

2.2. Multilevel linear regression modelling

Since several heifers originated from the same farm, observations could not be considered independent. As a result, a multilevel linear regression model was used to investigate the association between heifer individual milk antibody levels against *O. ostertagi* and collected variables on demographics and management (Dohoo et al., 2009). The model incorporated two hierarchical levels: level 1 (i), the heifer level, level 2 (j), the farm level. The outcome variable was heifer individual milk ODR. All collected variables were firstly tested in a univariable multilevel linear regression model. In the case of categorical variable, categories were built taking into account the number of observations within each category.

The model was developed using a Reweighted Generalised Iterative Least Squares (RIGLS) algorithm in MLwiN 2.30 and took the form of equation (1) (Rasbash et al., 2012):

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_{0j} + e_{ij} \qquad (1)$$

Where: y_{ij} was the outcome, i.e. individual milk ODR of the *i*th heifer from the *j*th herd; β_0 was the intercept; β_1 was the coefficient for the effect of the heifer level predictor x_{ij} on the outcome y_{ij}; β_2 was the coefficient for the effect of the farm level predictor x_j on the outcome y_{ij}; u_{0j} was the level 2 random-effect (farm) and e_{ij} was the bottom level residual (heifer), both assumed to be normally distributed. Associations between heifer individual milk ODR and collected variables were evaluated using a stepwise approach with elimination of non-significant effects (p-value>0.05). All significant main effects at P-value≤0.05 were left in the model. Confounding variables were also retained in the final model (e.g. breed, season, DIM and log (SCC)) (Klesius, 1993; Kloosterman et al., 1993; Sanchez et al., 2004b). Two-way interactions between predictors were tested on the basis of biological plausibility. Model goodness-of-fit was assessed at each hierarchical level by the examination of the normal probability and diagnostic plots of standardised residuals (Dohoo et al., 2009; Rasbash et al., 2012). Pearson, Fisher exact and Spearman correlation coefficients (McDonald, 2014) were estimated between model predictors and other collected variables to investigate correlation

between variables. A P-value≤0.05 was considered significant and related correlations interpreted as strong (above ±0.60), moderate (between ±0.40 and ±0.59) or weak (below ±0.39) (McDonald, 2014).

3. Results

The crude univariable association between heifer individual milk ODR and collected variables are presented in Appendix 8. The final multilevel linear regression model is presented Table 4-1. The baseline mean ODR in heifer individual milk was 0.53 units.

There were no significant differences in heifer individual milk ODR across the seasons. Heifer milk ODR significantly decreased with increasing DIM and milk yield at sampling [Coef. (95% CI): -1E-3 (-2E-3;-4E-5) and -4E-3 (-5E-3;-2E-3), respectively]. In contrast, heifer milk ODR significantly increased with increasing SCC at sampling [Coef. (95% CI): 0.03 (4E-3;0.05)].

Compared to dairy crossbred, dairy purebred heifers had significantly higher ODR [Coef. (95% CI): 0.10 (0.05;0.14)]. Moreover, heifer milk ODR increased when heifers came from larger herds [Coef. (95% CI): 2E-4 (4E-6;4E-4)].

Heifer milk ODR significantly decreased with an increasing number of staff [Coef. (95% CI): -0.01 (-0.02;-2E-3)]. Moreover, heifer milk ODR significantly increased with increasing age at weaning [Coef. (95% CI): 0.02 (0.01;0.03)]. In contrast, heifer milk ODR significantly decreased when farmers tested the quality of their colostrum and when the young-stock could be sent away to another farm for grazing [Coef. (95% CI): -0.11 (-0.20;-0.02)) to -0.10 (-0.18;-0.02) and -0.07 (-0.13;-0.01), respectively].

There was a significant positive association between heifer milk ODR and farm BTM ODR (samples collected between October and February) [Coef. (95% CI): 0.17 (0.04;0.30)]. In contrast, no significant association was observed between heifer milk ODR and farm BTM PP (*F. hepatica*).

Heifers with two or more than two grazing seasons before first calving had significantly higher ODR, compared to heifers with only one grazing season [Coef. (95% CI): 0.15 (0.06;0.23) and 0.17 (0.08;0.30), respectively]. Compared to those turned out older than six months, heifers

first turned-out younger than six months had significantly lower ODR [Coef. (95% CI): -0.05 (-0.09;-0.01)]. Finally, compared to heifers always turned out in the spring, heifers turned out either in the spring/summer or in the spring/autumn had a significant decrease in ODR by -0.08 units (95% CI: -0.12;-0.04) and -0.14 units (95% CI: -0.28;-3E-3), respectively.

There was a significant association between the contamination of heifer pasture and heifer individual milk ODR. Compared to heifers that did not co-grazed with cows, heifers that co-grazed for more than 14 days with either 'dry and milking cows' or 'dry cows only' had significantly higher ODR [Coef. (95% CI): 0.07 (6E-3;0.14) and 0.12 (0.05;0.19), respectively]. Moreover, heifers that grazed on pasture previously contaminated by sheep (i.e. either during the first, second or first two grazing seasons) had a significant increase in ODR by 0.08 units (95% CI: 0.02;0.14), 0.18 units (95% CI: 0.07;0.28) and 0.10 units (95% CI: 0.04;0.16), respectively. Finally, heifers that co-grazed with sheep (at least during their third grazing season) had a significant decrease in ODR by -0.20 units (95% CI: -0.40;-4E-3).

Heifers that had higher minimum stocking rate during their first grazing season had significantly higher ODR [Coef. (95% CI): 0.05 (0.03;0.06)] and heifers that grazed more mowed pastures during their second grazing season had significantly lower ODR [Coef. (95% CI): -1E-3 (-2E-3;-3E-4)].

Heifers that were treated with long-acting anthelmintic treatments at turn-out had significantly lower ODR, compared to non-treated heifers [Coef. (95% CI): -0.15 (-0.23;-0.06)]. Similarly, heifers that were treated, with a combination of pour-on and injection during the grazing season and at housing, had significantly lower ODR, compared to non-treated heifers [Coef. (95% CI): 0.27 (-0.40;-0.15)].

The number of heifer grazing seasons was strongly correlated with the total length of heifer grazing (from first turn-out to sampling) (r=0.74), whereas the size of the herd was moderately correlated with the length of heifer grazing (r=-0.31). The number of staff was moderately correlated with the farm use of BVD (r=0.41), IBR (r=0.44) and Clostridium vaccines (r=0.44). The test of the colostrum quality was moderately correlated with the supplementation of in-calf heifers at housing (r=0.41), the systematic deworming of adult cows (r=0.42) and the use of both Rispoval IN (r=0.45) and Clostridium vaccines (r=0.53). The significant correlations ($r \geq 0.30$) observed between predictors in the final model and other collected variables are displayed in Table 4-2.

Table 4-1: Final multilevel linear regression model of association between heifer individual milk ODR and demographic and management variables as fixed effects ($N_{Heifers}$=1,454; N_{Farms}=41)

Variables		$N_{Heifers}$ (%)	N_{Farms} (%)	β	95% CI[(a)]
Intercept (SE)[(a)]				0.53 (0.06)	
Season at sampling		1,454 (100.0)			
	Spring	350 (24.1)	37 (90.2)	ref	-
	Summer	357 (24.6)	38 (92.7)	-0.03	-0.07;0.01
	Autumn	373 (25.6)	36 (87.8)	-1E-3[(a)]	-5E-3;3E-3
	Winter	374 (25.7)	36 (87.8)	-0.02	-0.06;0.03
DIM (d)		1,454 (100.0)	41 (100)	-1E-3*	-2E-3;-4E-5
Milk yield at sampling (kg)		1,454 (100.0)	41 (100)	-4E-3*	-5E-3;-2E-3
Log (SCC)[(b)] at sampling (x1,000c/mL)		1,451 (99.8)	41 (100)	0.03*	4E-3;0.05
Herd size		1,454 (100.0)	41 (100)	2E-4*	4E-6;4E-4
Dairy breed		1,454 (100.0)			
	Purebred	1,254 (86.2)	38 (92.7)	ref	-
	Crossbred	200 (13.8)	21 (51.2)	-0.10*	-0.14;-0.05
Total grazing season(s) (N)		1,453 (99.9)			
	1	250 (17.2)	14 (34.1)	ref	-
	2	852 (58.6)	37 (90.2)	0.15*	0.06;0.23
	>2	351 (24.1)	30 (73.2)	0.17*	0.08;0.30
Total number of treatment(s)		1,428 (98.2)	40 (97.6)	-4E-3	-0.01;0.02
Treatment protocol		1,392 (95.7)			
	No treatment	164 (11.3)	10 (24.4)	ref	-
	Long-acting dewormer (turn-out)	402 (27.6)	20 (48.8)	-0.15*	-0.23;-0.06
	Drench (turn-out)	8 (0.6)	4 (9.8)	0.03	-0.15;0.22
	Injection (turn-out)	43 (3.0)	4 (9.8)	-0.04	-0.14;0.07
	Pour-on (turn-out)	201 (13.8)	14 (34.1)	-0.04	-0.13;0.04
	Pour-on (grazing)	301 (20.7)	19 (46.3)	-0.08	-0.17;0.01
	Pour-on (housing)	120 (8.3)	10 (24.4)	-0.05	-0.15;0.05
	Drench (grazing and housing)	11 (0.8)	2 (4.9)	-0.03	-0.20;0.14
	Drench and pour-on (housing)	8 (0.6)	1 (2.4)	0.02	-0.22;0.19
	Injection and pour-on (housing)	14 (1.0)	2 (4.9)	-	-
	Drench and injection (grazing and housing)	38 (2.6)	2 (4.9)	-0.10	-0.27;0.07
	Drench and pour-on (grazing and housing)	12 (0.8)	2 (4.9)	0.16	0.03;0.34
	Injection and pour-on (grazing and housing)	70 (4.8)	6 (14.6)	-0.27*	-0.40;-0.15
Age at first turn-out (m)		1,453 (99.9)			
	<6	280 (19.3)	7 (17.1)	ref	-
	>6	1,173 (80.7)	41 (100)	0.05*	0.01;0.09
Season of turn-out		1,453 (99.9)			
	Spring only	916 (63.0)	37 (90.2)	ref	-
	Summer only	174 (12.0)	15 (36.6)	-0.03	-0.09;0.03
	Spring and summer	349 (24.0)	29 (70.7)	-0.08*	-0.12;-0.04
	Spring and autumn	14 (1.0)	3 (7.3)	-0.14*	-0.28;-3E-3
Total time of co-grazing (cows) (d)		1,454 (99.9)			

0	750 (51.6)	37 (90.2)	ref	-
Milking and dry >14	248 (17.1)	20 (48.8)	0.07*	6E-3;0.14
Dry ≤14	100 (6.9)	6 (14.6)	0.01	-0.06;0.08
Dry >14	104 (7.2)	14 (34.1)	0.12*	0.05;0.19
Milking ≤14	59 (4.1)	12 (29.3)	-2E-3	-0.09;0.09
Milking >14	193 (13.3)	17 (41.5)	0.01	-0.06;0.07
Contamination pasture (sheep)	1,451 (99.8)			
No	746 (51.3)	34 (82.9)	ref	-
Gr_1 only	218 (15.0)	21 (51.2)	0.08*	0.02;0.14
Gr_2 only	28 (1.9)	9 (22.0)	0.18*	0.07;0.28
Gr_1 and Gr_2	405 (27.9)	22 (53.7)	0.10*	0.04;0.16
Gr_3/Gr_1 and Gr_3 /Gr_2 and Gr_3	9 (0.6)	3 (7.3)	-0.09	-0.32;0.14
All	45 (3.1)	9 (22.0)	0.06	-0.06;0.19
Co-grazing (sheep)	1,453 (99.9)			
No	1,350 (92.8)	41 (100)	ref	-
Gr_2 only	51 (3.5)	7 (17.1)	0.04	-0.07;0.14
Gr_1 and Gr_2	38 (2.6)	5 (12.2)	-0.04	-0.16;0.09
Gr_3/Gr_1 and Gr_3 /Gr_2 and Gr_3/ all	14 (1.0)	3 (7.3)	-0.20*	-0.40;-4E-3
Minimal stocking rate in Gr_1[(b)] (an/ha)	1,429 (98.3)	41 (100)	0.05*	0.03;0.06
Number pasture mowed in Gr_2[(b)]	1,441 (99.1)	41 (100)	-1E-3*	-2E-3;-3E-4
Bulk tank milk ODR[(b)] (second sampling)	1,454 (100.0)	41 (100)	0.17*	0.04;0.30
Bulk tank milk PP[(b)] (second sampling)	1,454 (100.0)	41 (100)	3E-4	-1E-5;7E-4
Staff (N)	1,454 (100.0)	41 (100)	-0.01*	-0.02;-2E-3
Age at weaning (w)	1,454 (100.0)	41 (100)	0.02*	0.01;0.03
Quality colostrum tested	1,454 (100.0)			
No	953 (65.5)	30 (73.2)	ref	-
Yes	384 (26.4)	9 (22.0)	-0.10*	-0.18;-0.02
Vary	117 (8.0)	2 (4.9)	-0.11*	-0.20;-0.02
Heifer sent away during the grazing	1,455 (100.0)			
No	1,130 (77.7)	32 (78.0)	ref	-
Yes	324 (22.5)	9 (22.0)	-0.07*	-0.13;-0.01

[(a)]CI=confidence interval; SE=standard error; E*x*= 10^x; *=significant (P-value≤0.05);

[(b)]SCC=somatic cell count; Gr_i=grazing season *i*; ODR=*O. ostertagi*; PP=*F. hepatica*

Table 4-2: Significant correlation coefficients (Pearson, Fisher exact or Spearman) (p-value≤0.05) between the predictors in the final model and other demographic and management variables ($N_{Heifers}$=1,454; N_{Farms}=41)

	Farmer age	Grazing time (d)	Pasture Gr_2 (n)	V_{col}[(a)] 24h (l)	Farmer Education	System prod.[(a)]	Cattle prod.	Cow purchase	System calving	Cow deworming	Cow housing	In-calf DS[(a)]	Age at calving	Dead calf	Risp. IN	BVD	IBR	Clost.	Huskvac
Model Predictors																			
Milk yield		-0.27	-0.23		0.11	-0.21		0.09	-0.11	-0.15	**-0.32**		-0.12	-0.07	-0.07	0.19	0.17	0.13	0.09
Herd size	-0.18	**-0.31**	-0.20	0.22	-0.11	**-0.34**		**0.43**	-0.20	-0.23	**-0.60**	-0.07	**-0.38**		0.24	**0.38**	**0.50**	**0.37**	-0.12
Dairy breed					0.20	0.07		-0.10	0.06	0.15	0.16	0.13		0.08	0.11	**-0.39**	-0.29	-0.14	
Grazing season (N)	0.08	**0.74**	**0.56**		0.11	0.24	0.20	-0.10	0.20	0.21	**0.30**	**0.31**	**0.51**		0.21	0.18	0.24	0.09	0.17
Deworming (N)	**-0.32**	**0.35**	**0.35**	-0.20	0.25	-0.11	0.07	0.05		0.15	**0.34**		0.24	0.08	0.25	-0.12		**-0.52**	-0.11
Deworming protocol		-0.06																	
Turn-out									0.08								0.11		
Contamination (sheep)								**0.36**				0.11							
Co-grazing (sheep)								0.25				0.11							
Min. stocking rate Gr_1		0.12	0.15		0.19	-0.09	-0.09	0.11		-0.15	-0.12	0.06	-0.07		0.13	0.13	0.19	-0.26	0.13
Mowed Gr_2		**0.74**	**0.99**	-0.11		0.13	0.09	0.09	0.22		0.20	-0.10	0.27		0.18		0.05		-0.12
BTM ODR (second sampling)	-0.06	**0.60**	**0.51**	-0.17		**0.41**	-0.06	-0.22	0.14	0.11	**0.73**	-0.07	0.28		-0.19	-0.21	**-0.31**	**-0.30**	
Staff		**-0.42**	**-0.30**	0.21	-0.12	-0.27		0.21		-0.25	**-0.54**	0.11	-0.29		**0.35**	**0.41**	**0.44**	**0.44**	
Weaning age			-0.13		-0.24	0.15	0.16	0.20			0.05		0.24		-0.07			**0.30**	-0.21
Colostrum test	-0.14	-0.25	-0.10		0.25	0.25	0.10	0.19	0.25	**0.42**	0.28	**0.41**	-0.26		**0.45**	0.21	0.29	**0.53**	**0.42**
Heifer sent out	**0.46**	-0.12	-0.08		0.24	0.18	-0.18	-0.11	0.07	0.20	**0.53**	-0.09			-0.24	-0.11	-0.05		-0.11

***Key: bold=moderate to high correlation (≥0.30);**

[(a)]V_{col}=volume colostrum; prod.=production; DS=diet supplementation

After addition of the final model predictors, a significant reduction in the random variation at level 2 (farm) was observed (Table 4-3). After taking into account the model predictors fixed effects, 2% of the unexplained outcome variable was attributed to the farms and 98% to the heifers.

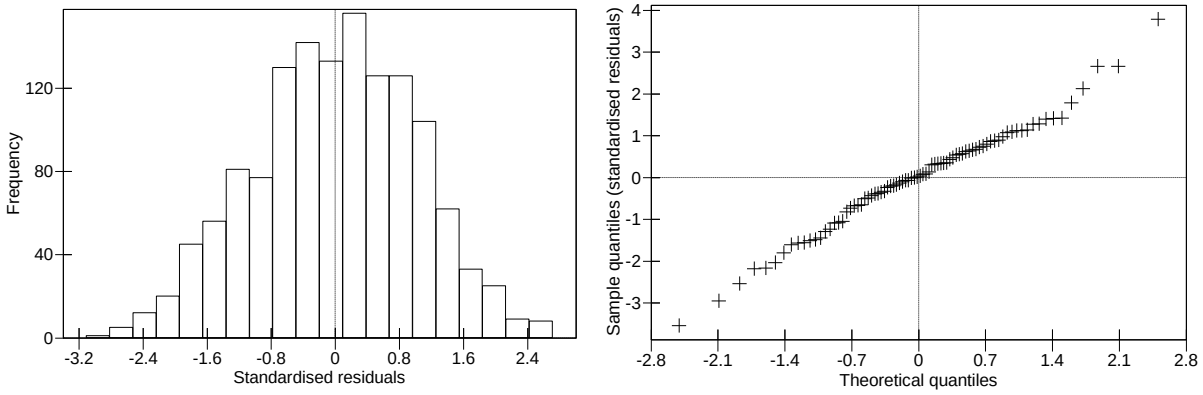
Table 4-3: Distribution of variances at farm level and heifer level in the null model and the final multilevel linear regression model

	Null model		Multilevel final model	
Random effects	**Variance**	**SE[(a)]**	**Variance**	**SE[(a)]**
Farm level	0.032	0.007	0.001	0.001
Heifer level	0.061	0.002	0.057	0.002

[(a)]SE=standard error

Final model residuals indicated a good overall fit at both levels (Figure 4-1). There was only one outlier in the final model, and it did not have any influence on the coefficients. Therefore it was left in the model.

Figure 4-1: Diagnostic plots of standardised residuals at heifer level (left) and farm level (right)



4. Discussion

This is the first longitudinal study that identifies associations between individual milk antibody levels against *O. ostertagi* in heifers and heifer management history from birth to sampling. The discussion of this chapter focuses on the demographics and management factors that were associated with heifer individual milk ODR. The section excludes the discussion related to (1) the study representativeness (previously discussed in Chapter 2., section 8.2.); (2) the association between heifer individual and BTM ODR (previously discussed in Chapter 3., section 4.2.) and (3) the association between heifer individual milk ODR and milk production parameters (to be discussed in Chapter 5.).

4.1. Association between heifer individual milk ODR and heifer characteristics

4.1.1. Stage of lactation

Despite choosing a short period of lactation (i.e. between 30 and 90 DIM), a significant negative association was observed between heifer individual milk ODR and heifer DIM at sampling. This result is in contradiction with previous observations made in adult dairy cows, where individual milk ODR remained constant from 30 to 200 DIM (Sanchez et al., 2004b). It is possible that the different physiology (e.g. IgG synthesis and transport) existing between young and old cattle explain such observation (Sanchez et al., 2004b). This result suggests that in heifers, DIM should always be taken into account when interpreting ODR from individual milk samples.

4.1.2. Breed

In this study, dairy crossbred heifers had significantly lower ODR than dairy purebred heifers. As reported in available literature, such an observation could be related to some genetic differences affecting the ability of heifers to mount an immune response against *O. ostertagi* (Gasbarre et al., 1993; Hayhurst et al., 2010). Moreover, evidence suggests that there is a genetic component in the efficiency of IgG transport into the mammary gland (Kloosterman et al., 1993; Hurley and Theil, 2011). Finally, previous research focused on sheep highlighted that the selection of breed for increased productive traits can have an impact on the host resistance to gastro-intestinal infections (immune response) (Greer, 2008). However, to date, such

evidence has not been reported in cattle and there is a need for further research on this matter (Greer, 2008).

4.1.3. Somatic cell count

The positive association reported between SCC and heifer individual milk ODR is similar to that observed in previous research (Sanchez et al., 2004b; Sanchez et al., 2005; Charlier et al., 2006). In the case of mastitis (as reflected by high SCC in milk), the leakage in tight junctions and the disruption to the integrity of the mammary epithelium can increase the passage of *O. ostertagi* antibodies into the mammary gland (Hurley and Theil, 2011; Lehman et al., 2015). Moreover, evidence suggests that there is some level of antibody competition between *O. ostertagi* antibodies and specific antibodies against mastitic bacteria. As a result, this could, first, prevent the passage of specific antibodies against mastitic bacteria in the milk; second, reduce the capacity of milk neutrophils to phagocytose mastitic bacteria; and, ultimately, promote inflammation and presence of SCC in milk (Burton and Erskine, 2003). It is also worth noting that a potential test cross-reactivity between *O. ostertagi* ELISA antigens and the specific antibodies against mastitic bacteria should not be excluded (Burton and Erskine, 2003; Charlier et al., 2006).

4.2. Association between heifer individual milk ODR and farm contextual variables

4.2.1. Herd size and age at weaning

After controlling for variables related to grazing and anthelmintic treatments, heifers from larger dairy herds had significantly higher ODR. This result is contrary to that reported in previous studies and could be related to the use of different markers of infection to explore associations among variables, i.e. individual *versus* BTM markers (Charlier et al., 2005a; Forbes et al., 2008; Almeria et al., 2009; Bennema et al., 2010; Pablos-Tanarro et al., 2013). Since there was in the current study a significant negative moderate correlation between the size of the herd and the total length of heifer grazing (r=-0.31), heifers coming from larger herds were probably housed for longer. This way, it is possible that heifers coming from larger herds received more silage-based diet while being housed, which can significantly increase their levels of milk antibody and confounds the results (Bloemhoff et al., 2015b). Moreover,

evidence suggests that different levels of animal chronic stress in terms of day-to-day management can stimulate the production of cattle antibodies (Griffin, 1989). However, it was out of the scope of this study to explore association between levels of animal stress and size of the herds.

After controlling for the season and the age of heifer at first turn-out, heifers weaned older had higher ODR. In sheep, evidence suggests that the age at weaning influences the development of immunity against nematodes (Shaw et al., 1995). In fact, milk is a source of active immunological factors that can promote the early development of high immunity in lamb (Shaw et al., 1995). Moreover, the secretion of hormones induced by weaning can stimulate the development of immunity (Shaw et al., 1995). The changes of behaviour and diets that occur at weaning can also potentially distort the metabolic status of the offspring and, ultimately, the development of resistance to helminths in young animals (Shaw et al., 1995; Bloemhoff et al., 2015b). This is the first report of its kind in cattle, for which there is a need to further explore and validate these findings.

Importantly, it is worth noting that size of the herd and the age at weaning could have acted, like other predictors included in the final model (e.g. heifers sent away during the grazing), as surrogate for management variables not captured in the current study.

4.2.2. Farm labour and farmers attitudes

To date, analytic studies on cattle ostertagiasis have mostly focused on variables related to farm production (i.e. type and level), grazing, anthelmintic treatment and climate (Charlier et al., 2005a; Bennema et al., 2010; Pablos-Tanarro et al., 2013). Although these variables are important, they do not allow the anticipation of the influence of other economic and behavioural variables on cattle helminth exposure (e.g. labour, education, and facilities) (Morley and Donald, 1980; Charlier et al., 2015; Wilson et al., 2015). However, it is known that management decisions unrelated to helminth control might modify the extent to which cattle helminth infections are controlled (Morley and Donald, 1980). In the current study, for example, an increasing number of staff was significantly associated with a decrease in heifer ODR. Different approaches can be used in the control of cattle helminth infections, including treatment and cattle movement (i.e. pasture rotation), which can compete for labour with other management decisions (Wilson et al., 2015). With an insufficient number of staff, cattle helminth control can be overlooked due to other farm priorities (Morley and Donald, 1980). In

line with what has been previously highlighted in the literature (O'Kane et al., 2016), the current study also suggests that farmer conscientiousness (i.e. testing the quality of the colostrum, vaccinating and supplementing animal with feed) can have a significant impact on the overall parasite burden of the herd by lowering heifer exposure to helminths on pasture. It is possible that the systematic approach that goes with conscientiousness may foster farmers' practices related to cattle helminth control. Moreover, conscientious farmers are more likely to take the time to search for information and to remain updated on the most efficient practices for cattle helminth control (O'Kane et al., 2016).

4.3. Association between heifer individual milk ODR and grazing variables

4.3.1. Exposure and immunity

Heifer milk ODR was significantly positively associated with heifer total length of grazing, i.e. heifer total number of grazing season(s) and early start of grazing season (spring). This agrees with previous reports (Guitian et al., 1999; Sanchez and Dohoo, 2002; Charlier et al., 2005a; Forbes et al., 2008; Bennema et al., 2010; Pablos-Tanarro et al., 2013; Bloemhoff et al., 2015b) and confirms the importance of both length and repetition of exposure for the development of cattle immunity against *O. ostertagi* (Klesius, 1988; Vercruysse and Claerebout, 1997). The absence of a significant difference in heifer ODR between heifer that had two and more than two grazing seasons also suggests that heifers might have reached a plateau level of antibodies against *O. ostertagi* during their second year of grazing (Mayer, 2016). As highlighted in previous studies (Klesius, 1988; Ploeger et al., 1990b), cattle immunity against *O. ostertagi* develops slowly and takes around two years to become noticeable.

4.3.2. Alternate and mixed grazing

Previous research suggests that the sharing of pastures by cattle belonging to different groups of age can improve cattle helminth control on-farm (Vanderstichel et al., 2012). This is based on the assumption that when older cattle co-graze with young-stock, their presence on pasture decreases the shedding of eggs and, ultimately, the pressure of young-stock exposure to helminths (Waller, 2006). In a recent study, the level of BTM ODR changed when heifers were

co-sharing their pasture with either dry or milking cows (decreasing ODR) or with both dry and milking cows (increasing ODR) (Vanderstichel et al., 2012). It is possible that the lack of precision in the information collected, i.e. the absence of distinction between different types of adult cows (dry and milking), specification of periods (e.g. days, weeks, and months), and sequence in time (e.g. previous and co-grazing), limited the scope and quality of findings in this type of research. By including this complexity and considering different scenarios, the current study shall propose a more robust approach for identifying reliable and valid factors in terms of pasture sharing associated with heifer helminth exposure. There was no significant association between the previous contamination of pasture by cows and heifer milk ODR. In contrast, if heifers had co-grazed at least for 14 days with either (1) dry cows or (2) dry and milking cows, their ODR was significantly higher. Given that most heifers co-grazed with mature cows just prior to calving, it is possible that their higher infection susceptibility at this time of the cycle contributed to such observations (Armour, 1980). It is also possible that different climates, cow deworming practices and stocking densities influenced the ecology of the free-living larvae, the grazing behaviour and the physiological state of the different groups of cattle at the time of the co-grazing (Waller, 2006; Vanderstichel et al., 2012).

Alternate and mixed grazing of cattle and sheep have previously been reported as an effective practice for cattle helminth control (Waller, 2006). Moreover, this represents one of the recommendations included in the guidelines for cattle nematode control sustainability in the UK (COWS, 2010). In the current study, the effects of sheep grazing on heifer milk ODR varied if sheep had (1) previously contaminated heifer pasture (increasing ODR) or (2) co-grazed with heifers (decreasing ODR). With regards to the first observation (pasture contamination), cattle and sheep share different nematode species (e.g. *C. punctata* and *D. viviparus*) (Roberts, 1942). This way, different nematode antigens could have cross-reacted with *O. ostertagi* ELISA (Keus et al., 1981; Klesius, 1988) increasing heifer milk ODR. With regards to the second observation, reports suggest that co-grazing cattle and sheep can reduce the burden of *O. ostertagi* on pasture, since sheep act as a dead-end host for this infection (Waller, 2006). Moreover, different grazing behaviours in sheep and cattle has previously been reported at the time of co-grazing and could influence the level of cattle exposure to helminths (ADAS, 2011).

4.3.3. Stocking rate and mowing of grass

Increasing heifer milk ODR was only significantly associated with increasing heifer stocking rate at first grazing. Increasing stocking rate has commonly been reported as a major driver for livestock exposure to parasites (Waller, 2006). When stocking rate is high, the contamination of the pasture increases the risks of cattle exposure to infective larvae (Armour, 1980). This is particularly true in the case of nematode infections (such as the one due to *O. ostertagi*) where the parasite does not multiply outside the final host (Armour, 1980). In line with the current results, evidence suggests that the effect of stocking rate is particularly important in naive animals, i.e. during their first grazing season (Armour, 1980).

The current study suggests that increasing the frequency of grass mowing in heifer pastures during their second grazing season significantly decreases the risk of heifer exposure to *O ostertagi* (lowering ODR). In fact, mowing of grass can lower the availability of infective larvae on pasture (Waller, 2006; Bennema et al., 2010; Charlier et al., 2010a). This can be due to (1) different characteristics of grass after cutting (e.g. density and height) that determine specific microclimates (i.e. light, moisture, and temperature) and decrease the survival of infective larvae on pasture (Armour, 1980; Waller, 2006); and (2) a mechanical removal of infective larvae from pasture via mowing (Rattray, 2003; Waller, 2006). However, the latter is more questionable given that lowering the sward height can also increase the availability of infective larvae close to the ground (Armour, 1980). Moreover, since there is a variability in forage cutting height, time of mowing and grass regrowth, there is a need to cautiously consider the effect of the approaches to mowing of grass on cattle exposure to helminths (Rattray, 2003).

4.4. Association between heifer individual milk ODR and anthelmintic practices

This is the first study that uses complete records of past anthelmintic treatments in heifers to measure the effects of drug protocols on *O. ostertagi* milk antibody levels. Both long-acting anthelmintic drugs administration at turn-out and pour-on/injection treatments throughout grazing and at housing were significantly associated with lower heifer ODR. This agrees with previous studies and confirms the positive effect of anthelmintic drugs in lowering individual milk ODR (Guitian et al., 1999; Sanchez and Dohoo, 2002; Charlier et al., 2005a; Bennema et al., 2010; Vanderstichel et al., 2012; Bloemhoff et al., 2015b). Differences in anthelmintic drug

compounds (e.g. pharmacokinetic and spectrum of activity), form, time of treatment and quality of application contribute to differences in drug efficacy, especially considering the complexity of the parasite sensitivity to the drug (Ploeger et al., 2000; Sutherland and Leathwick, 2011; Bloemhoff et al., 2014). However, no information on this matter was available to explore any related patterns.

4.5. Limitations of the diagnostic tool used in the study

One of the main challenges in identifying factors associated with cattle helminth infection remains the choice and use of a diagnostic tool to detect the infection in cattle (Charlier et al., 2014). The current study applied the ELISA technique for the detection of milk antibodies against *O. ostertagi*. Although evidence suggests that this technique is reliable, straightforward and safe, there are some limitations to this approach (Roeber et al., 2013). In this regard, the design of the study and the selection of the sample can minimise the bias included in the analysis (Dohoo et al., 2009). Taking previous evidence into account (Charlier et al., 2007a; Blanco-Penedo et al., 2012), the current study based its observations on heifer individual milk antibody levels, including the variability that exists in host response to helminth infections and the confounding effect of the parity of the cow on milk ODR. Moreover, information about other potential confounders (e.g. breed, SCC, milk yield and DIM) were collected at the time of the sampling to minimise bias in the analysis of milk ODR (Roeber et al., 2013). Finally, the analysis included the farm status of *F. hepatica* infection to reduce the effect of *O. ostertagi* ELISA cross-reactivity with this parasite (Bennema et al., 2009). In this regard, the cross-reactivity of the crude antigen used for the ELISA against *O. ostertagi* with other helminths antibodies (e.g. *C. oncophora* and *D. viviparus*) (Klesius, 1988) suggests that the associations identified in the current study may reflect the effect of factors on heifer helminthic exposure in general. It is worth noting that although correlations between *O. ostertagi* milk antibody levels and worm burden in natural infections have been reported in previous studies (Berghen et al., 1993; Dohoo et al., 1997), these correlations are considered weak due to the delay that exist between the current infection and the immune response (Charlier et al., 2014). This way, the detection of milk antibodies does not allow to distinguish between current and past-infection (Roeber et al., 2013). Moreover, it is often not possible to anticipate the severity of the infection

from looking at the level of antibodies only (Roeber et al., 2013). As a result, the observations of the current study would need to be tested in the field to confirm the findings.

5. Conclusions

This is the first longitudinal study that explores the association between several risk factors and heifer individual milk antibody levels against *O. ostertagi*. The research was based on a wide range of variables (e.g. demographics, management, and milk parameters) and sequential information of young-stock grazing management from birth to time of sampling, allowing the minimisation of bias in the analysis and the explanation of a reasonable amount (37.6%) of the variability of antibody levels against *O. ostertagi* in heifer individual milk. The results confirm that it is necessary to take several individual parameters into account when interpreting heifer milk ODR, complementing previous research focused on adult dairy cows. These factors include the breed, DIM, SCC and milk yield of heifers at sampling.

This study suggests that, even after controlling for anthelmintic treatments, several grazing management practices remain significantly associated with heifer individual milk ODR. Therefore, anthelmintic drugs should not be considered as the only option available to prevent or regain production losses due to helminth infections in young-stock. Alternatives to the use of these treatments include the control of heifer length of grazing, the mowing of grass in heifer pasture and avoiding the exposure of heifers to contaminated pasture during periods of higher disease susceptibility (e.g. heifer first grazing season and prior to calving). The use of mixed-grazing with sheep could also be considered by farmers in relation to different risk contexts of helminth infections (i.e. high risk of *O. ostertagi* infections but low risk of *F. hepatica* infections).. Since the diagnostic marker used in the current study (milk antibody levels) did not allow distinguishing between past and current-infections and different levels of infection severity, further field studies are however needed to validate the current findings. Moreover, it is worth noting that some of the predictors included in the final model may have acted as surrogate for other variables not captured in the current study.

Given that cattle exposure to helminths is influenced by the interplay of a wide range of factors of different nature (e.g. climate conditions, management practices, availability of resources, and farmers' attitudes), there is a need to expand the empirical relevance of the current study

by integrating in the analysis other farm dimensions of cattle helminth control (e.g. economic, social). This way, different scenarios could be built and discussed with farmers in order to ensure an efficient and sustainable control of cattle helminth infections in England.

Chapter 5.
Associations between individual milk antibody levels against *Ostertagia ostertagi* and milk production, reproduction and health performances in heifers

1. Introduction

Heifers represent the future of a dairy herd and a capital investment made by farmers to ensure the future of their business. Once born, dairy heifers require the best management for optimised fertility, milk production and disease resilience (AHDB, 2015a). It is suggested that treating heifers against nematodes can increase heifer growth rate, puberty onset and the rate of first-service conception (Purvis and Whittier, 1996; Mejia et al., 1999; Loyacano et al., 2002). Moreover, nematode anthelmintic treatments may also prevent adverse effects on heifer body weight at first calving, which could lead to a decrease in milk production, as suggested in the case of infection by *O. ostertagi* (Ploeger et al., 1996). However, the impact of *O. ostertagi* infection on milk production during first lactation remains to be clarified (Blanco-Penedo et al., 2012; Liedtke et al., 2013).

Most studies investigating the impact of cattle helminth infections on the productivity of milking herd have focused on adult cows. A number of these studies have indicated that increasing exposure to *O. ostertagi* is significantly associated with decreasing milk production in adult cows (Guitian et al., 1999; Sanchez and Dohoo, 2002; Charlier et al., 2005b). Moreover, evidence suggests that the presence of high antibody levels against *O. ostertagi* (Sanchez et al., 2002a) or the absence of treatments against nematodes (Walsh et al., 1995) can negatively impact the reproductive performance of these animals, i.e. lowering the hazard of conception and increasing the number of breeding needed for conception. However, contradictory observations exist in the literature, which makes difficult to draw firm conclusions on these associations (i.e. the negative effect of helminth infections on adult cow milk production and reproduction performance) (Sithole et al., 2006; Derouen et al., 2009;

Charlier et al., 2010b; Ravinet et al., 2016). Many experimental studies have also indicated that *O. ostertagi* causes a non-specific immunosuppression in cattle that can result in an increase in disease susceptibility in cattle (Gasbarre, 1997). In the field, some reports suggest that increasing bulk tank milk (BTM) antibody levels against *O. ostertagi* are significantly associated with increasing offspring mortality (Delafosse, 2013). However, this evidence is still inconclusive and there is a need for research and fieldwork on the topic (Gasbarre, 1997).

In order to better understand the impacts of helminth infections on cow milk production, reproduction performance and health, the choice of the diagnostic marker used to detect infected or exposed animals to helminths is determinant. Previous research has confirmed that the detection of anti- *O. ostertagi* antibodies in cattle milk by ELISA technique is a very convenient and reliable approach for such investigations (Keus et al., 1981). Because cow levels of *O. ostertagi* antibody are highly varied within a farm, the use of individual milk samples can be more informative than that of BTM samples (Charlier et al., 2007a). Moreover, the concentration of individual milk antibodies is influenced by different factors, such as the age of the animal (Sanchez et al., 2004b). This factor can confound the interpretation of ELISA results due to different helminth exposure and antibodies crossing into the mammary gland (Kloosterman et al., 1993; Sanchez et al., 2004b). Because of this, the use of younger animals (i.e. heifers) can be a better approach for exploring associations between levels of antibodies in milk and other collected variables.

The current study explores the associations between the levels of antibodies against *O. ostertagi* in individual heifer milk and several parameters of milk production, reproduction and health.

2. Materials and Methods

2.1. Farm and heifer samples

The detail of the sample size estimation is presented in Chapter 2 (Section 2.) and the final number of heifers included in this study was 1,454 (Chapter 2., section 7.3.2.). The selection of the dairy farms involved both statistical (e.g. optimum heifer sample size and seasonal distribution of sampling) and non-statistical considerations (e.g. time and recording). The dairy

farmers participating in this study were all members of QMMS Ltd. Herds calved all-year-round or at least for two different seasons during the year. The heifers were reared on farms and had access to pasture during the summer months. There were no restrictions on the type of cattle housing (i.e. housed all-year-round, in the winter only, and varied) and the practices of anthelmintic treatments in this study. All herds had computerised records on production, reproduction and health and their records on milk production were at least bi-monthly. Farmers had to provide access to their herd record for a two-year period. To comply with research ethics requirements, farmers were asked to sign an informed consent form. This is detailed in Appendix 4.

The study was conducted on 39 dairy farms from England (Chapter 2., section 7.2.). The size of the herds ranged from 23 to 890 dairy cows with a median size of 150 dairy cows. Dairy cows were predominantly Holstein Friesian. Moreover, in order to (1) account for *O. ostertagi* seasonality (i.e. collection of samples during a year) and (2) collect for each heifer a one-year follow-up of production, reproduction and health, this longitudinal study lasted from March 2014 (i.e. start of the heifer milk sample collection) to April 2016 (i.e. at least one year after the collection of the last heifer sample).

2.2. Sample collection and laboratory methods

From March 2014 to March 2015, all the heifers present on farms between 30 and 90 DIM were routinely sampled by QMMS (monthly). Individual composite milk samples were preserved using bronopol/natamycin and kept at ambient temperature until arrival at the laboratory. In the laboratory, the samples were (1) processed, (2) tested for SCC and milk constituents (e.g. fat and protein), (3) frozen and (4) stored at -20°C (±2°C). Heifer milk samples were frozen within the first 48h after their collection on farms. A stratified random sampling approach was used for the selection of the heifer samples to be analysed for *O. ostertagi* antibodies. Samples were selected in order to be uniformly distributed across farms and seasons (i.e. spring, between April and June; summer, between July and September; autumn, between October and December; and winter, between January and March) (Chapter 2., section 4.). Each heifer was tested once. In the case where multiple samples had been collected from a heifer, only the sample with the lowest DIM was kept. Selected milk samples

were defrosted, defatted by centrifugation (2,000 x g, 2 min) and their supernatant collected for the detection of *O. ostertagi* antibodies.

The ELISA test used the Svanovir® *Ostertagia*-Ab ELISA kit (Svanova Biotech, Uppsala, Sweden), which is based on a crude adult antigen of the GIN *O. ostertagi*. The proceeding of the ELISA tests followed the manufacturers' instructions and the results were expressed as ODR according to the formula (1):

$$ODR = (ODsample - NC)/(PC - NC) \qquad (1)$$

Where: ODsample is the OD reading of a heifer individual milk sample at 405nm and NC and PC are, respectively, the optical densities of the negative (N) and the positive (P) control samples included in each plate of the kit.

Two sets of BTM sample pots were also sent to farmers in June and in October 2014 to estimate the herd antibody levels against *O. ostertagi* and *F. hepatica*. BTM tests against *F. hepatica* were used in the subsequent analysis as a control for potential test cross-reactivity with *O. ostertagi* kit antigens (Klesius, 1988; Bennema et al., 2009). The detection of *F. hepatica* antibodies in BTM used the commercially available POURQUIER® ELISA kit (IDEXX, Institute Pourquier, Montpellier, France) and results were expressed as a PP (Chapter 2., section 5.4.2.). All ELISA tests were conducted by the same laboratory technicians, using the same batch of ELISA kit for each parasite.

2.3. Farm and production data

Farm and heifer production data were extracted from the TotalVet recording programme of QMMS Ltd. at the end of the study, i.e. in April 2016, (Sum-It Computer Systems Ltd., Oxfordshire). The heifer individual records included at the time of sampling were the season, age, breed, milk yield, fat, protein, DIM, SCC, calving date and status of offspring (i.e. alive or dead). The milk, protein and fat yields at day 305 were obtained if heifers had reached this stage at the time of the data extraction. The interval between heifer's first and second calving was computed from the corresponding calving dates, if present. Only accurate health variables with a sufficient number of observations were extracted from TotalVet and considered for the analysis, since the assiduousness of farmers to record varied according to farms and variables

(e.g. lameness, mastitis, and Johne's test results). These included heifer last titre against Johne's disease in the first lactation and heifer farm status at the time of the data extraction (i.e. present, dead and absent (culled or dead)). Finally, farmers were regularly contacted during the study by telephone to provide information on the grazing management and the anthelmintic treatments of their adult cows.

2.4. Statistical analysis

Data were coded, checked and entered into a database (Microsoft Excel 2010). A descriptive analysis was conducted in STATA 12.1 (STATA Inc., Texas, USA) to summarise heifer production, reproduction and health parameters. Three sets of statistical modelling analyses were conducted. Since, for all models, several heifers originated from the same herd, the independence of the observations could not be assumed and the models had heifer individual milk sample ODR nested within herds. Therefore, all statistical models incorporated two hierarchical levels: level 1 (i), a heifer level, level 2 (j) a farm level. The scale of the coefficient of the ELISA predictors (i.e. heifer ODR and BTM ODR and PP) were converted to be interpreted as the effect of a 0.1 unit increase of the ELISA predictor on the outcome (i.e. milk production, reproduction and health parameters)

2.4.1. Association between heifer individual milk antibodies against Ostertagia ostertagi and heifer milk production

The association between heifer individual milk ODR and heifer milk production parameters were estimated using six multilevel linear regression models with the outcomes: (1) milk yield at sampling; (2) protein yield at sampling; (3) fat yield at sampling; (4) milk yield at day 305; (5) protein yield at day 305; and (6) fat yield at day 305.

The predictor variables for the first three models (i.e. milk production parameters at sampling) were: *O. ostertagi* individual milk ODR; heifer breed; season at sampling; age at sampling; DIM at sampling; log (SCC) at sampling; herd size and *F. hepatica* and *O. ostertagi* BTM PP and ODR. The effect of days in milk on sample yield was included using the Wilmink's function (Wilmink, 1987). The predictor variables for the last three models (i.e. milk production parameters at day 305) were: *O. ostertagi* individual milk ODR; heifer breed;

season at sampling; age and, depending on the outcome (i.e. milk /protein and fat yield), yield of milk /protein or fat at sampling; length of first lactation; herd size; cow anthelmintic treatment after sampling; cow grazing after sampling and *F. hepatica* and *O. ostertagi* BTM PP and ODR. Possible dilution effects of milk yield on protein and fat were considered in models 2, 3, 5 and 6.

The models were developed using a Reweighted Iterative Generalised Least Squares (RIGLS) algorithm in MLwiN 2.30 (Rasbash et al., 2012). The models were built using a stepwise approach, combining both forward selection and backward elimination of predictor variables and took the form of equation (2) (Rasbash et al., 2012):

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_{0j} + e_{ij} \quad (2)$$

Where: y_{ij} was the outcome, i.e. milk yield/protein yield/fat yield at sampling or at day 305 of the *i*th heifer from the *j*th herd; β_0 was the intercept; β_1 was the coefficient for the effect of the heifer level predictor x_{ij} on the outcome y_{ij}; β_2 was the coefficient for the effect of the farm level predictor x_j on the outcome y_{ij}; u_{0j} was the level 2 random effect (farm) and e_{ij} was the bottom level residual (heifer), both assumed to be normally distributed. The evaluation of the effects of the factors on the outcome was based on Wald tests. A p-value≤0.05 was considered significant. The confounding variables were retained in the final model and two-ways interactions were tested.

The goodness-of-fit of the six multilevel models was assessed at each hierarchical level by the examination of the normal probability and the leverage plots of residuals (Dohoo et al., 2009; Rasbash et al., 2012).

2.4.2. Association between heifer individual milk antibodies against Ostertagia ostertagi and heifer health

- *Johne's disease*

A multilevel linear regression model was built to investigate the association between heifer individual milk ODR and heifer last titre against Johne's disease in the first lactation (i.e. outcome). If the tests against Johne's disease had been performed prior to the individual test against *O. ostertagi*, the heifer sample was excluded from the analysis. The predictor variables

were: *O. ostertagi* individual milk ODR; heifer breed; season at sampling; age at sampling; DIM at Johne's test; time interval between individual milk ELISA against *O. ostertagi* and Johne's test; yield at day 305; herd size; cow anthelmintic treatment after sampling; cow grazing after sampling and *F. hepatica* and *O. ostertagi* BTM PP and ODR.

The model was developed using the same approach, method and steps described in the above section (see above, section 2.4.1.).

- *Heifer status on farm*

Two multilevel logistic regression models were built to investigate the association between heifer individual milk ODR and heifer status on-farm at the time of the data extraction from TotalVet. These were (1) a multilevel binomial logistic regression model (model 1) and (2) a multilevel multinomial logistic regression model (model 2). The outcome variables were, for model 1, 0- present, 1- absent (i.e. culled, sold or dead); and for model 2, 0- present, 1- absent (i.e. culled or sold), 2- dead. The reference category for the two outcomes was the status 0 (i.e. present). The predictor variables were: *O. ostertagi* individual milk ODR; heifer breed; season at sampling; age at sampling; length of first lactation; yield at day 305; herd size; cow anthelmintic treatment after sampling; cow grazing after sampling and *F. hepatica* and *O. ostertagi* BTM PP and ODR. Two farms stopped their recording in 2015 and were excluded from this analysis.

The model was built using a stepwise approach and the evaluation of the effects of the predictors on the outcome was based on Wald tests. A p-value≤0.05 was considered significant. Confounding variables were retained in the final model. Any interactions between variables was tested. Model 1 (i.e. binomial) used a logit link function to express the probability for a heifer to be absent at the time of the data extraction from TotalVet, as shown in equation (3) (Rasbash et al., 2012):

$$logit\left(\pi_{ij}\right) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + \mathrm{u}_{0j} \quad (3)$$

Where: π_{ij} was the outcome, i.e. the probability of the *i*th heifer of the *j*th herd to be present or not in the farm at the time of the data extraction; β_0 was the intercept of the model; β_1 and β_2 represented the heifer level and farm level vectors of coefficients; $x_{ij}\ and\ x_j$ were the heifer

level and farm level vectors of predictor variables and u_{0j} was the level 2 random effect (farm), assumed to be normally distributed.

Model 2 (i.e. multinomial) also used a logit link function to express the ratio probability of a given status to the probability of the reference score, as shown in equation (4) (Rasbash et al., 2012):

$$log\left(\frac{\pi_{ij}^{(s)}}{\pi_{ij}^{(0)}}\right) = \beta_0^{(s)} + \beta_1^{(s)} x_{ij} + \beta_2^{(s)} x_j + u_{0j}^{(s)} \qquad (4)$$

Where: $\pi_{ij}^{(s)}$ was the outcome, i.e. the probability of the *i*th heifer of the *j*th herd to have a status 's', i.e. s=1 (absent: culled or sold); or s=2 (dead), compared to the score 0 (present); $\beta_0^{(s)}$ was the status-specific intercept of the model; $\beta_1^{(s)}$ and $\beta_2^{(s)}$ represented the heifer level and farm level vectors of coefficients; x_{ij} and x_j were the heifer level and farm level vectors of predictor variables and $u_{0j}^{(s)}$ was the level 2 random effect (herd), assumed to be normally distributed.

Both models statistical analyses were performed using MLwiN v2.30. For each outcome, a model was fitted using a second-order penalised quasi-likelihood methods (RIGLS) to produce starting values for the second model using the method of Markov Chain Monte Carlo (MCMC). The convergence of the models was assessed visually (Hamra et al., 2013; Browne, 2015). MCMC chains were run for 100,000 iterations after a burn-in of 5,000 iterations.

2.4.3. Association between heifer individual milk antibodies against Ostertagia ostertagi and heifer reproduction

Two multilevel models were built to investigate the association between heifer individual milk ODR and heifer reproduction parameters. Firstly, a multilevel binomial logistic regression model (model 1) was built to express the probability of a heifer to have a dead offspring at first calving according to several predictors. These included: *O. ostertagi* individual milk ODR; heifer breed; season at sampling; age at first calving; log (SCC) at sampling; milk yield at sampling; herd size and *O. ostertagi* and *F. hepatica* BTM PP and ODR. Secondly, a multilevel discrete time survival model (model 2) was built to express the hazard of a heifer to calve for the second time in an interval *t*, given that the heifer had not calved before the start of this

interval. The time follow-up of the survival analysis was set at 681 days, i.e. one year plus the time of a subsequent gestation. The heifers that had not conceived a second time by that time were considered as censored. The continuous time interval between first and second calving was divided into four discrete categories of time at 120 days intervals (interval 1, 201-321; interval 2, 322-441; interval 3, 442-561; interval 4, 562-681). The time interval was nested within heifers; therefore a third hierarchical level was incorporated in the model (in addition to the heifer level and the farm level). The predictor variables were: *O. ostertagi* individual milk ODR; heifer breed; season at sampling; age at first calving; offspring mortality at first calving; length of first lactation; herd size; cow anthelmintic treatment after sampling; cow grazing after sampling and *O. ostertagi* and *F. hepatica* BTM PP and ODR.

The two multilevel models (binomial and discrete time survival) were built using a stepwise approach and the evaluation of the effects of the predictors on the outcome was based on Wald tests. A p-value≤0.05 was considered significant. Any confounding variables were retained in the final models. Interactions were tested. Model 1 (binomial) used a logit link function, whereas model 2 (discrete time survival) used a complementary log-log function to express the outcome probability, given this function is based on the assumption of the proportional hazards (Dohoo et al., 2009). Both models equations, i.e. equation (5), for model 1, and equation (6), for model 2, are presented below (Dohoo et al., 2009; Rasbash et al., 2012):

$$logit\left(\pi_{ij}\right) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + \mathrm{u}_{0j} \quad (5)$$

Where: π_{ij} was the outcome, i.e. the probability of the *i*th heifer of the *j*th herd to have a dead calf at first calving; β_0 was the intercept of the model; β_1 and β_2 were the heifer level and farm level vectors of coefficients; x_{ij} and x_j were the heifer level and farm level vectors of predictor variables and u_{0j} was the level 2 random effect (farm), assumed to be normally distributed.

$$cloglog\left(h_{tij}\right) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_{0j} + e_{0ij} \quad (6)$$

Where: h_{tij} was the outcome, i.e. the hazard of the *i*th heifer of the *j*th herd to have her second calving in the interval *t* given that this heifer was present at the start of this interval; β_0 was the logit (hazard) in the baseline time period for a baseline individual (heifer); β_1 and β_2 represented the heifer level and farm level vectors of coefficients; x_{ij} and x_j were the heifer

level and farm level vectors of predictor variables; u_{0j} was the farm random effect and e_{0ij} was the heifer level residual, both assumed to be normally distributed.

All statistical analyses were performed using MLwiN v2.30. For each outcome, a model was fitted using a second-order penalised quasi-likelihood methods (RIGLS) to produce starting values for the second model using the method of Markov Chain Monte Carlo (MCMC). The convergence of the models was assessed visually (Hamra et al., 2013; Browne, 2015). MCMC chains were run for 100,000 (model 1) or 500,000 iterations (model 2) after a burn-in of 5,000 iterations. A term for the interaction between predictors and time was also added in model 2 (discrete time survival) to verify that the model satisfied the assumption of proportionality, i.e. a key assumption of the Cox proportional hazard model (Dohoo et al., 2009).

3. Results

3.1. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer milk production parameters

Out of the 1,454 heifer samples tested against *O. ostertagi*, 1,005 (69%) had records for milk production at day 305. The median (p25-p75) yields of heifer milk, protein and fat at sampling were 29.6(25.4-34.1) kg, 0.93(0.81-1.07) kg, and 1.08(0.83-1.30) kg. The corresponding values at day 305 were 8,093(7,039-9,417) kg, 273(238-314) kg and 320(267-371) kg, respectively.

The crude univariable associations between the six outcomes of the multilevel linear regression models (i.e. milk yield/protein yield/fat yield at sampling and at day 305) and other collected variables are presented in Appendix 9. The final multilevel linear regression models are summarised Tables 5-1 and 5.2. The total of the variance explained by the six final multilevel models was 11%, for model 1 (milk yield at sampling); 85 %, for model 2 (protein yield at sampling); 28%, for model 3 (fat yield at sampling); 55%, for model 4 (milk yield at 305 day); 90%, for model 5 (protein yield at 305 day); and 44%, for model 6 (fat yield at 305 day).

Table 5-1: Final multilevel linear regression models of association between milk/protein/fat yields at sampling and demographic variables as fixed effects (N_{Farms}=41; $N_{Heifers}$=1,454)

Outcome		Milk yield (kg) (model 1)			Protein yield (kg) (model 2)		Fat yield (kg) (model 3)	
Fixed effects								
Variables	**Categories**	**N (%)**	**β**	**95% C.I[a]**	**β**	**95% C.I[a]**	**β**	**95% C.I[a]**
Intercept (SE)[a]			-0.26 (45.83)		-0.01 (0.03)		0.04 (0.17)	
Individual milk ODR[b]		1,454 (100)	-0.26*	-0.40;-0.13	1E-3[a]	-1E-3;3E-3	4E-3	-2E-3;0.01
Dairy breed		1,454 (100)						
	Purebred	1,254 (86.2)	*Baseline*		*Baseline*		*Baseline*	
	Crossbred	200 (13.8)	-1.21	-2.54;0.13	7E-3	-0.01;0.02	0.07	-4E-3;0.14
Season at sampling		1,454 (100)						
	Spring	350 (24.1)	*Baseline*		*Baseline*		*Baseline*	
	Summer	357 (24.6)	-0.89	-1.88;0.10	-8E-3	-0.02;0.01	0.01	-0.04;0.06
	Autumn	373 (25.7)	-0.36	-1.35;0.64	0.02	1E-3;0.03	0.02	-0.03;0.07
	Winter	374 (25.7)	-0.35	-1.33;0.63	3E-3	-0.01;0.02	0.09*	0.04;0.14
Age at sampling (m)		1,454 (100)	0.13*	0.03;0.23	3E-4	-1E-3;2E-3	3E-3	-3E-3;8E-3
DIM at sampling		1,454 (100)	0.03	-0.06;0.11	1E-3*	3E-4;1E-3	-3E-4	-1E-3;1E-3
$DIM^{-0.05}$		1,454 (100)	43.46	-60.66;145.59	-	-	-	-
Milk yield at sampling (kg)		1,454 (100)	-	-	0.03*	2.8E-2;2.8E-2	0.03*	3.0E-2;3.5E-2
Log (SCC) (x1,000c/mL)		1,451 (99.8)	-0.73*	-1.38;-0.08	9E-3*	1E-3;0.02	0.07*	0.04;0.10
Bulk tank milk ODR[b] (second sampling)		1,454 (100)	-0.92*	-1.37; -0.48	1E-3	-4E-3;6E-3	-0.03	-0.06;0.01
Bulk tank milk PP[b] (second sampling)		1,454 (100)	-0.01	-0.02;4E-3	1E-5	-1E-5;3E-5	-1E-4	-1E-4;1E-5
Random effects								
		Level	Variance	SE	Variance	SE	Variance	SE
		Farm	7.272	1.909	8E-4	2E-4	0.040	0.01
		Heifer	40.382	1.531	7E-3	3E-4	0.102	4E-3

[a]CI=confidence interval; SE=standard error; E*x*=10^x; *=significant (P-value≤0.05);

[b]ODR=*O. ostertagi*; PP=*F. hepatica*. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Table 5-2: Final multilevel linear regression models of association between milk/protein/fat yields at day 305 and demographic variables as fixed effects (N_{Farms}=38; $N_{Heifers}$=1,005)

Outcome			**Milk yield (kg) (model 1)**		**Protein yield (kg) (model 2)**		**Fat yield (kg) (model 3)**	
Fixed effects								
Variables	**Categories**	**N (%)**	**β**	**95% C.I[a]**	**β**	**95% C.I[a]**	**β**	**95% C.I[a]**
Intercept (SE)[a]			3.96E4		31.78 (10.68)		75.94 (52.47)	
Individual milk ODR [b]		1,005 (100)	12.10	-12.98;37.18	-0.32	-0.69;0.05	0.47	-0.68;1.61
Dairy breed		1,005 (100)						
	Purebred	884 (88.0)	*Baseline*		*Baseline*		*Baseline*	
	Crossbred	121 (12.0)	-215.72	-488.91;57.47	-0.50	-4.52;3.52	7.51	-5.53;20.55
Season at sampling		1,005 (100)						
	Spring	236 (23.5)	*Baseline*		*Baseline*		*Baseline*	
	Summer	239 (23.8)	429.91*	240.25;619.56	3.42*	0.57;6.27	15.47*	6.63;24.30
	Autumn	263 (26.2)	603.48*	413.81;793.14	1.81	-1.04;4.66	8.32	-0.53;17.17
	Winter	267 (26.6)	556.48*	369.36;743.61	0.43	-2.38;3.23	13.84*	5.13;22.54
Age at sampling (m)		1,005 (100)	35.02*	13.66;56.37	0.33*	0.01;0.65	1.08*	0.05;2.10
Related yield at sampling (kg)		1,005 (100)	121.64*	111.58;131.71	-0.03	-0.21;0.15	-9.03	-36.73;18.67
Length of first lactation (d)		1,005 (100)	1.39*	0.50;2.27	0.01*	3E-4;0.03	0.01	-0.03;0.05
Milk yield at day 305 (kg)		1,005 (100)	-	-	0.03*	2.7E-2;2.9E-2	0.03*	2.5E-2;2.9E-2
Size of the dairy herd		1,005 (100)	0.79	-0.54;2.11	0.01	-0.01;0.03	0.10	-4E-3;0.21
Cow grazing after sampling		1,005 (100)						
	No	230 (22.9)	*Baseline*		*Baseline*		*Baseline*	
	Yes	775 (77.1)	-774.41*	-1,528.17;-20.65	3.76	-7.01;14.52	-5.98	-66.69;54.74
Bulk tank milk ODR[b] (second sampling)		1,005 (100)	-121.09*	-226.74;-15.45	-1.38	-2.89;0.14	-6.98	-14.99;1.03
Bulk tank milk PP[b] (second sampling)		1,005 (100)	-0.13	-0.35;0.09	-2E-3	-0.01;0.01	-0.01	-0.03;0.01
Random effects								
	Level		Variance	SE	Variance	SE	Variance	SE
	Farm		3.07E5	7.72E4	61.80	15.84	2,154.36	444.12
	Heifer		9.98E5	4.59E4	217.52	10.00	2,038.65	94.28

[a]CI=confidence interval; SE=standard error; E*x*=10^x; *=significant (P-value≤0.05);

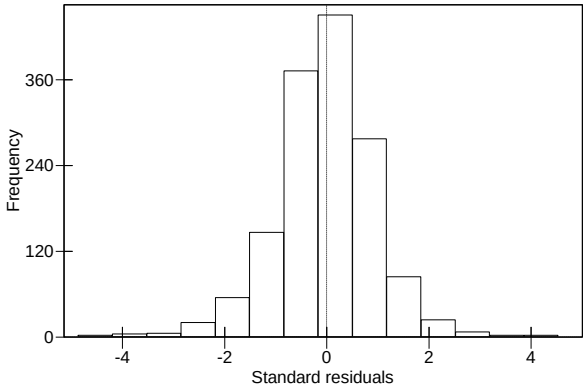
[b]ODR=*O. ostertagi*; PP=*F. hepatica*. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

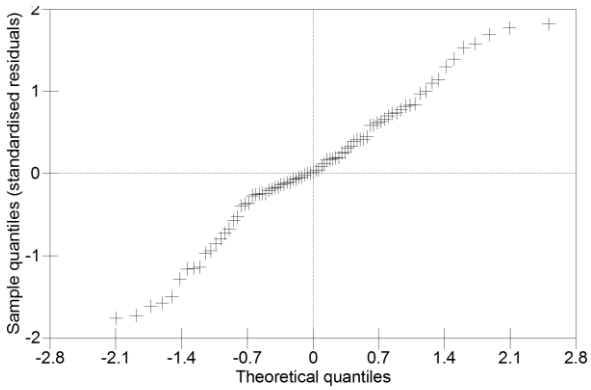
The milk yield of heifer at sampling was significantly associated with both individual and BTM ODR. For each 0.1 unit increase in both individual and BTM ODR (second sampling, i.e. during the period between October and February), heifer milk yield at sampling declined by 0.26kg (95% C.I.: -0.40;-0.13) and 0.92kg (95% C.I.: -1.37;-0.48) from the mean, respectively. Moreover, heifers that originated from highly exposed herds to *O. ostertagi* during the period between October and February 2014 had significantly lower milk yield at day 305. This latter dropped by 121.09kg (95% C.I.: -226.74;-15.45) from the mean for each 0.1 unit increase in BTM ODR. After controlling for the other variables, there was no significant association between heifer yields in protein and fat and the different predictors of helminth exposure, i.e. both individual and BTM ELISA results against *O. ostertagi* and *F. hepatica*.

The visual examinations of the final residuals at each hierarchical level suggested that the six models fitted the data well (Figure 5-1). There was no effect of any outliers and, therefore, they were left in the models.

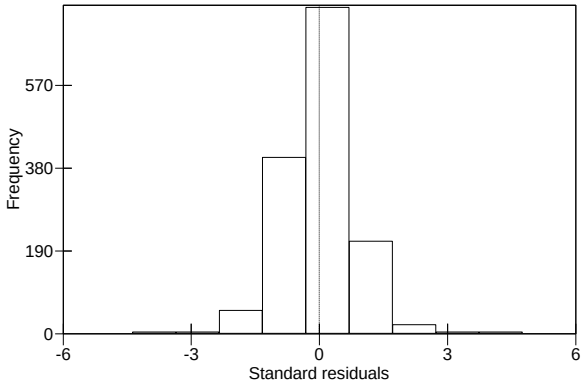
Figure 5-1: Diagnostic plots of final standardised residuals at heifer level (left) and farm level (right) for the six multilevel linear regression models of heifer milk, protein and fat yields at sampling and day 305

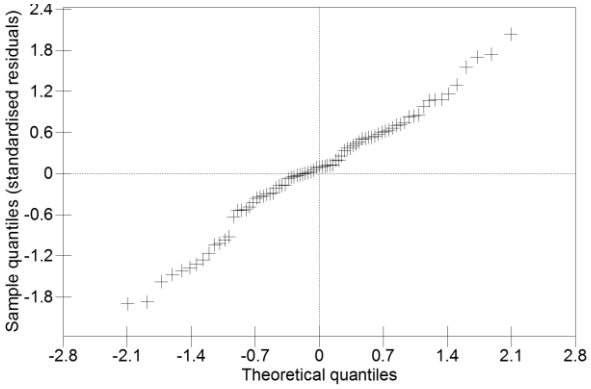
1) Milk yield at sampling (model 1)



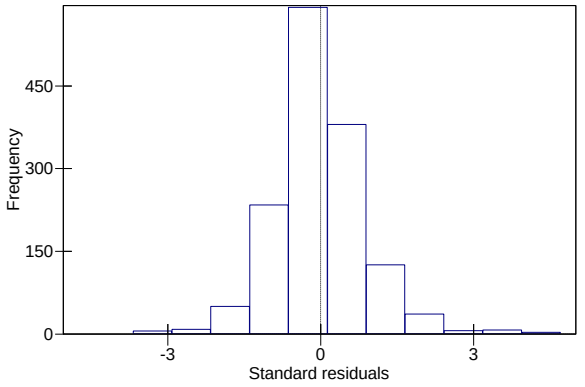


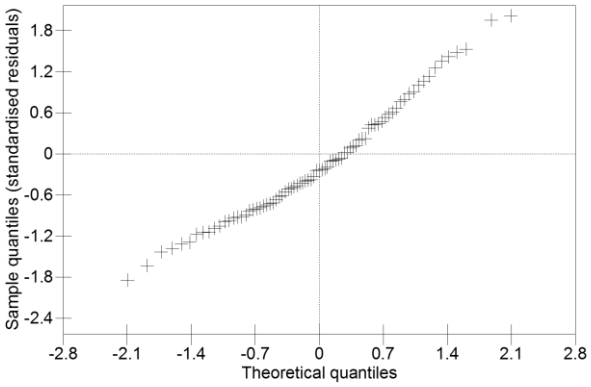
2) Protein yield at sampling (model 2)



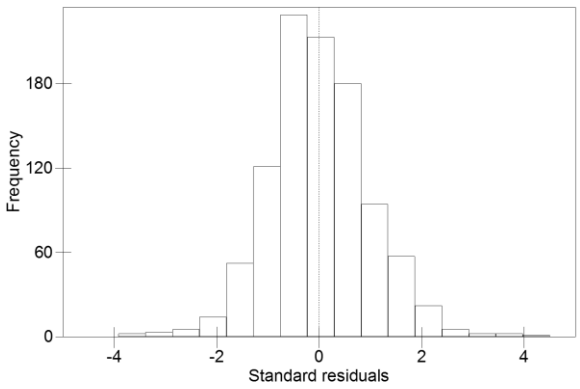


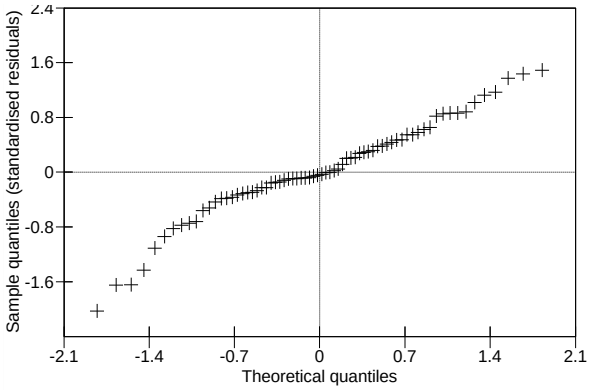
3) Fat yield at sampling (model 3)



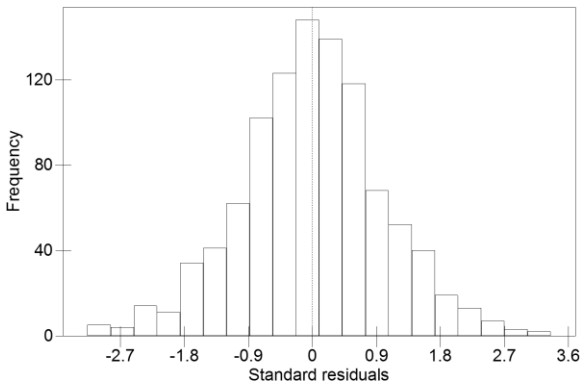


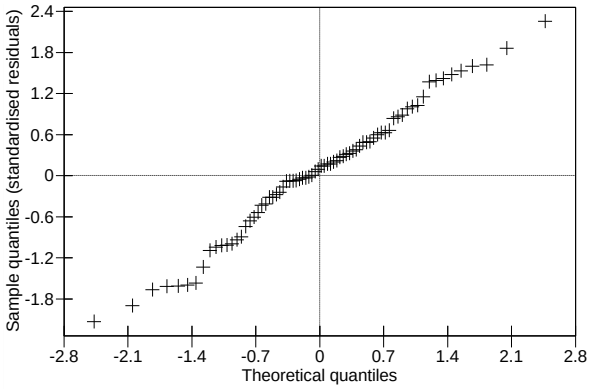
4) Milk yield at 305 day (model 4)



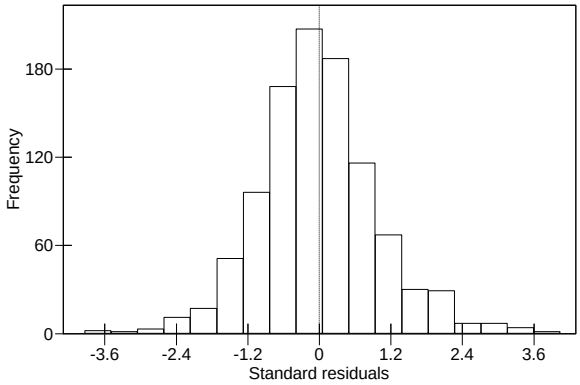


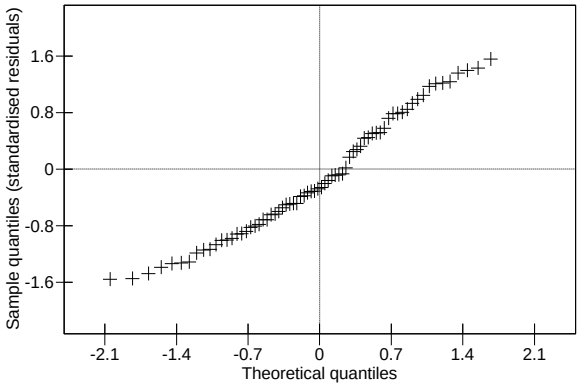
5) Protein yield at 305 day (model 5)





6) Fat yield at 305 day (model 6)





3.2. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer health parameters

3.2.1. Heifer last titre against Johne's disease in the first lactation

The analysis included 78% (N=1,135) of the study samples. The median (p25-p75) value of heifer last titre against Johne's disease in the first lactation was 2.61 (1.73-4.32) unit. A total of 95% (N=1,081), 2% (N=16) and 3% (N=38) of the heifer were diagnosed 'currently negative' (i.e. negative for Johne's disease for all tests conducted during the first lactation), 'currently positive' (i.e. positive for Johne's disease at the last test during the first lactation) and 'provisionally positive' (i.e. positive for Johne's disease at a previous test(s) during the first lactation but negative at the last test), respectively. The median (p25-p75) time interval between heifer individual milk ELISA and Johne's last ELISA was 213 (162- 277) days.

The crude univariable associations between heifer last titre against Johne's disease in the first lactation and other collected variables are presented in Appendix 10. The final multilevel linear regression model is summarised Table 5-3.

Table 5-3: Final multilevel linear regression model of association between heifer last titre against Johne's disease in the first lactation and demographic variables as fixed effects (N_{Farms} =34; $N_{Heifers}$ =1,135)

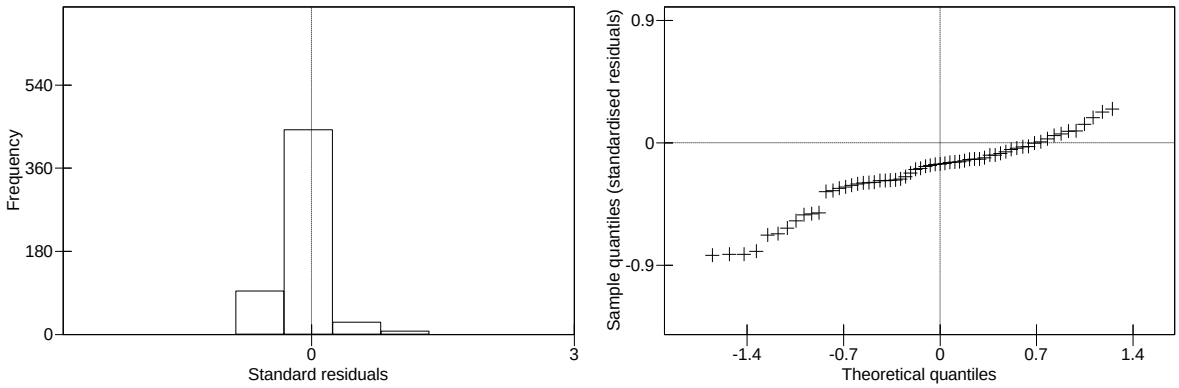
Fixed effects				
Variables	**Categories**	**N (%)**	**β**	**95% C.I[(a)]**
Intercept (SE)[(a)]			0.03 (4.51)	
Individual milk ODR[(b)]		1,135 (100)	0.48*	0.16;0.61
Dairy breed		1,135 (100)		
	Purebred	1,000 (88.1)	*Baseline*	
	Crossbred	135 (11.9)	-0.25	-3.14;2.65
Season at sampling		1,135 (100)		
	Spring	275 (24.2)	*Baseline*	
	Summer	272 (24.0)	1.52	-0.99;4.04
	Autumn	293 (25.8)	0.06	-2.42;3.54
	Winter	295 (26.0)	0.88	-1.56;3.31
Age at sampling (m)		1,135 (100)	-0.06	-0.31;0.18
DIM at the time of Johne's test		1,135 (100)	0.06	-0.01;0.12
Interval between individual ELISA and Johne's test (d)		1,135 (100)	-0.05	-0.11;0.01
Size of the dairy herd		1,135 (100)	4E-3[(a)]	-2E-3;0.01
Bulk tank milk PP[(b)] (second sampling)		1,135 (100)	-1E-3	-3E-3;2E-4
Random effects				
	Level		Variance	SE
	Farm		2.453	2.158
	Heifer		212.876	9.059

[(a)]CI=confidence interval; SE=standard error; E*x*= 10^x; *=significant (P-value≤0.05);

[(b)]ODR=*O. ostertagi*; PP=*F. hepatica.* The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

A 0.1 unit increase in heifer individual milk ODR was significantly associated with a 0.48 unit (95% C.I.: 0.16;0.61) increase in the titre of the last test against Johne's disease in the first lactation. The final model residuals indicated a good overall fit at both levels (Figure 5-2). There was no effect of any outliers in the model results and, therefore, they were left in the model.

Figure 5-2: Diagnostic plots of standardised residuals at heifer level (left) and farm level (right) for the multilevel linear regression model of heifer last titre against Johne's disease in the first lactation



3.2.2. Heifer status on-farm at the time of the data extraction from TotalVet

Out of the 1,454 heifers sampled, 78% (N=1,141) were alive, 18% (N=254) had been sold (culled or sold), and 4% (N=56) were dead at the time of the data extraction (April 2016). If absent, heifer had on average stayed on the farm 392 (280-510) days after sampling.

The crude univariable associations between heifer status on-farm at the time of the data extraction, i.e. for model 1, 'present' *versus* 'absent' (i.e. culled, sold or dead); and, for model 2, 'present' *versus* 'absent'(i.e. culled or sold) or 'dead', and other collected variables are presented in Appendix 11. The final multilevel logistic regression models are summarised Tables 5-4 and 5-5.

Table 5-4: Final multilevel binomial logistic regression model of association between heifer status on-farm at the time of the data extraction from TotalVet (i.e. present/absent) and demographic variables as fixed effects (N_{Farms}=38; $N_{Heifers}$=1,423)

		Present (Reference)	Absent (i.e. culled, sold or dead)		
Variables	**Categories**	**N (%)**	**N (%)**	**O.R[(a)]**	**95 % C.I[(a)]**
Constant (SE)[(a)]				0.37 (0.82)	
Individual milk ODR[(b)]		1,113 (78.2)	310 (21.8)	1.06	0.99;1.12
Dairy breed		1,113 (78.2)	310 (21.8)		
	Purebred	954 (67.0)	282 (19.8)	*Baseline*	
	Crossbred	159 (11.2)	28 (2.0)	0.60	0.32;1.13
Season at sampling		1,113 (78.2)	310 (21.8)		
	Spring	216 (15.2)	118 (8.3)	*Baseline*	
	Summer	263 (18.5)	87 (6.2)	0.57*	0.39;0.83
	Autumn	317 (22.3)	54 (3.8)	0.37*	0.24;0.56
	Winter	317 (22.3)	51 (3.6)	0.29*	0.19;0.45
Age at sampling (m)		1,113 (78.2)	310 (21.8)	1.01	0.96;1.06
Length of first lactation (d)		1,113 (78.2)	310 (21.8)	0.99*	0.992;0.996
Size of the dairy herd		1,113 (78.2)	310 (21.8)	1.00	0.99;1.00
Bulk tank milk PP[(b)] (second sampling)		1,113 (78.2)	310 (21.8)	1.00	0.99;1.00

[(a)]CI=confidence interval; SE=standard error; *=significant (P-value≤0.05);

[(b)]ODR=*O. ostertagi*; PP=*F. hepatica.* The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Table 5-5: Final multilevel binomial logistic regression model of association between heifer status on-farm at the time of the data extraction from TotalVet (i.e. present/absent/dead) and demographic variables as fixed effects (N_{Farms}=38; $N_{Heifers}$=1,423; $N_{Observations}$=2,846)

		Present (Reference)	Absent (i.e. culled or sold)			Dead		
Variables	**Categories**	**N (%)**	**N (%)**	**O.R[(a)]**	**95 % C.I[(a)]**	**N (%)**	**O.R[(a)]**	**95 % C.I[(a)]**
Constant (SE)[(a)]				0.66 (0.95)			-2.25 (1.66)	
Individual milk ODR[(b)]		1,113 (78.2)	254 (17.8)	1.04	0.98;1.11	56 (3.9)	1.12*	1.01;1.25
Dairy breed		1,113 (78.2)	254 (17.8)			56 (3.9)		
	Purebred	954 (67.0)	229 (16.1)	*Baseline*		53 (3.7)	*Baseline*	
	Crossbred	159 (11.2)	25 (1.8)	0.72	0.37;1.41	3 (0.2)	0.31	0.08;1.22
Season at sampling		1,113 (78.2)	254 (17.8)			56 (3.9)		
	Spring	216 (15.2)	103 (7.2)	*Baseline*		15 (1.1)	*Baseline*	
	Summer	263 (18.5)	72 (5.1)	0.54*	0.36;0.80	15 (1.1)	0.78	0.35;1.71
	Autumn	317 (22.3)	43 (3.0)	0.34*	0.22;0.54	11 (0.8)	0.55	0.23;1.28
	Winter	317 (22.3)	36 (2.5)	0.25*	0.16;0.39	15 (1.1)	0.62	0.28;1.35
Age at sampling (m)		1,113 (78.2)	254 (17.8)	1.00	0.95;1.05	56 (3.9)	1.02	0.93;1.11
Length of first lactation (d)		1,113 (78.2)	254 (17.8)	0.99*	0.992;0.996	56 (3.9)	0.99*	0.989;0.997
Size of the dairy herd		1,113 (78.2)	254 (17.8)	1.00	0.99;1.00	56 (3.9)	1.00	1.00;1.01

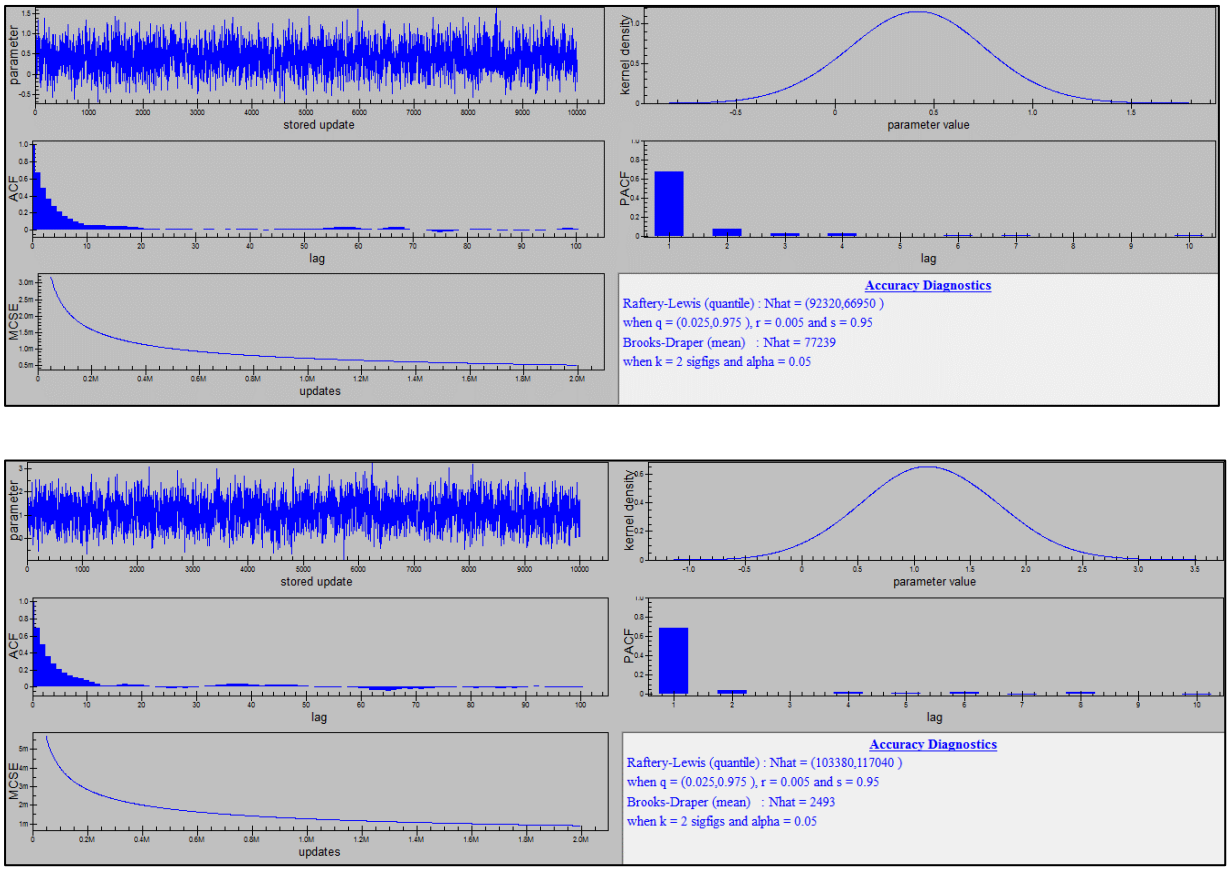
[(a)]CI=confidence interval; SE=standard error; *=significant (P-value≤0.05);

[(b)]ODR=*O. ostertagi.* The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Heifer status on-farm was only significantly associated with heifer individual milk ODR when comparing the groups of heifers ‘present’ and ‘dead’ on-farm at the time of the data extraction (April 2016). After controlling for other variables, a 0.1 unit increase in heifer individual milk ODR increased the odds for a heifer to be dead in April 2016 by 1.12 (95% C.I.: 1.01-1.25). When BTM predictors were included in the model, neither individual nor BTM predictors were significantly associated with the outcome (data not shown).

The visual examination of the MCMC diagnostic plots for each parameter estimated in the models suggested that the two models converged well. Figure 5-3 presents an example of MCMC diagnostic plots obtained for the parameters of ‘heifer individual milk ODR’ in model 2 (heifer ‘present’ *versus* ‘absent’ or ‘dead’).

Figure 5-3: Monte Carlo and Markov Chain diagnostic plots for the multilevel multinomial logistic regression model of heifer status on-farm ('present' *versus* 'absent' or 'dead'). The graphics show the diagnostic plots for the parameters of 'heifer individual milk ODR' for the status 'absent' (top) and 'dead' (bottom)



3.3. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer reproduction parameters

3.3.1. Heifer offspring mortality at first calving

The analysis included 1,385 (95%) of the study samples. Among these, 139 (10%) heifers aborted or had a stillbirth at first calving.

The crude univariable associations between heifer offspring mortality at first calving and other collected variables are presented in Appendix 12. The final multilevel binomial logistic regression model is summarised Tables 5-6.

Table 5-6: Final multilevel binomial logistic regression model of association between heifer offspring mortality at first calving and demographic variables as fixed effects (N_{Farms}=41; $N_{Heifers}$=1,385)

Variables	Categories	Alive (Reference) N (%)	Dead N (%)	O.R[(a)]	95 % C.I[(a)]
Constant (SE)[(a)]				-3.28 (1.08)	
Individual milk ODR[(b)]		1,243 (89.7)	142 (10.3)	1.11*	1.03;1.19
Dairy breed		1,243 (89.7)	142 (10.3)		
	Purebred	1,080 (78.0)	110 (7.9)	*Baseline*	
	Crossbred	163 (11.8)	32 (2.3)	1.40	0.71;2.78
Season at first calving		1,243 (89.7)	142 (10.3)		
	Spring	286 (20.6)	27 (1.9)	*Baseline*	
	Summer	293 (21.2)	38 (2.7)	1.09	0.61;1.96
	Autumn	327 (23.6)	54 (3.9)	1.45	0.83;2.52
	Winter	337 (24.3)	23 (1.7)	0.59	0.32;1.09
Age at first calving (m)		1,243 (89.7)	142 (10.3)	0.99	0.94;1.05
Size of the dairy herd		1,243 (89.7)	142 (10.3)	1.00	0.99;1.00
Bulk tank milk PP[(b)] (second sampling)		1,243 (89.7)	142 (10.3)	1.00	0.99;1.00

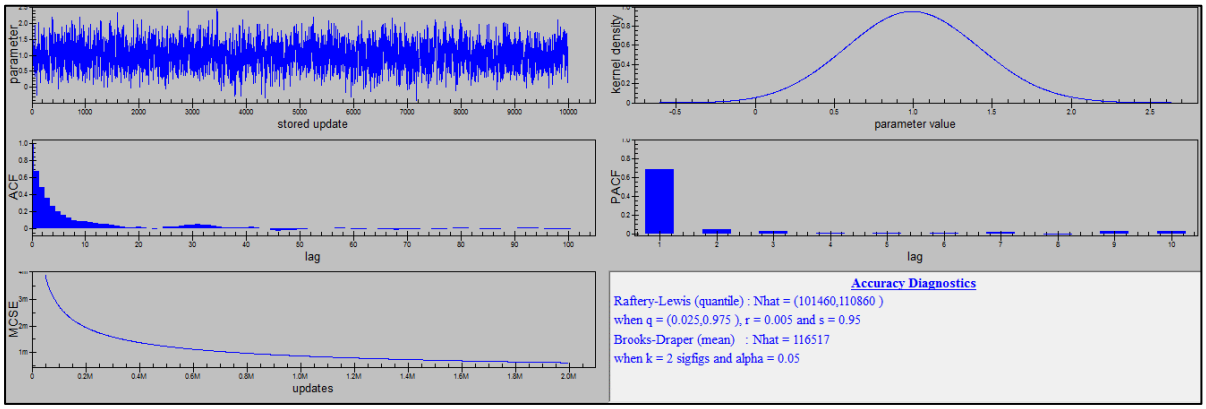
[(a)]CI=confidence interval; SE=standard error; *=significant (P-value≤0.05);

[(b)]ODR=*O. ostertagi.* The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

After controlling for other variables, the odds for a heifer to have a dead calf at first calving significantly increased by 1.11 (95% C.I.: 1.03-1.19) for each 0.1 unit increase in their individual milk ODR.

The visual examination of the MCMC diagnostic plots for each parameter included in the models suggested that the model converged well. Figure 5-4 presents an example of MCMC diagnostic plots obtained for the parameter of 'heifer individual milk ODR'.

Figure 5-4: Monte Carlo and Markov Chain diagnostic plots for the multilevel binomial logistic regression model of heifer offspring mortality. The graphics show the diagnostic plots for the parameter of 'heifer individual milk ODR'



3.3.2. Heifer first-to-second calving interval

A total of 1,423 heifers were included in this analysis, of which 225 (18%) were censored.

The crude univariable associations between the hazard of heifer second calving and the predictor variables are presented in Appendix 13. The final multilevel discrete time survival model is summarised Table 5-7.

Table 5-7: Final multilevel discrete survival time model of association between heifer hazard to calve for a second time in an interval *t* and demographic and time interval variables as fixed effects (N_{Farms}=39; $N_{Heifers}$=1,423; $N_{Observations}$=3,492; $Time_{follow-up}$=681 days)

Variables	Categories	No (Reference) N (%)	Yes N (%)	O.R[(a)]	95 % C.I[(a)]
Constant (SE)[(a)]				-0.17 (0.72)	
Interval (I_i) (d)		2,291 (65.6)	1,201 (34.4)		
	201-321 (I_1)	1,372 (39.3)	57 (1.63)	*Baseline*	
	322-441 (I_2)	443 (12.7)	929 (26.60)	47.70*	33.79;51.65
	442-561 (I_3)	245 (7.02)	201 (5.76)	122.61*	62.11;242.04
	562-681 (I_4)	231 (47.0)	14 (0.40)	16.96*	4.79;60.05
***O. ostertagi* individual milk ODR[(b)]**		2,291 (65.6)	1,201 (34.4)	0.95*	0.90;0.99
Dairy breed		2,291 (65.6)	1,201 (34.4)		
	Purebred	2,033 (58.2)	1,033 (29.6)	*Baseline*	
	Crossbred	258 (7.4)	168 (4.81)	1.26	0.81;1.96
Season at first calving		2,291 (65.6)	1,201 (34.4)		
	Spring	496 (14.2)	282 (8.08)	*Baseline*	
	Summer	531 (84.7)	308 (8.82)	0.43*	0.21;0.89
	Autumn	627 (18.0)	328 (9.39)	0.42*	0.21;0.86
	Winter	637 (18.2)	283 (8.10)	0.24*	0.10;0.56
Interval (I_i) # Season at first calving	**I_1 # Spring**			*Baseline*	
	I_2 # Summer			3.16*	1.43;7.01
	I_3 # Summer			1.06	0.43;2.63
	I_4 # Summer			1.32	0.22;8.12
	I_2 # Autumn			2.47*	1.17;5.25
	I_3 # Autumn			1.89	0.80;4.48
	I_4 # Autumn			0.24	0.01;3.99
	I_2 # Winter			4.42*	1.81;10.81
	I_3 # Winter			1.43	0.53;3.85
	I_4 # Winter			2.42	0.48;12.11
Age at first calving (m)		2,291 (65.6)	1,201 (34.4)	1.00	0.96;1.03
Length of first lactation (d)		2,291 (65.6)	1,201 (34.4)	0.99*	0.993;0.994
Size of the herd		2,291 (65.6)	1,201 (34.4)	1.00	0.99;1.00
Bulk tank milk PP[(b)] (second sampling)		2,291 (65.6)	1,201 (34.4)	1.00	0.99;1.00

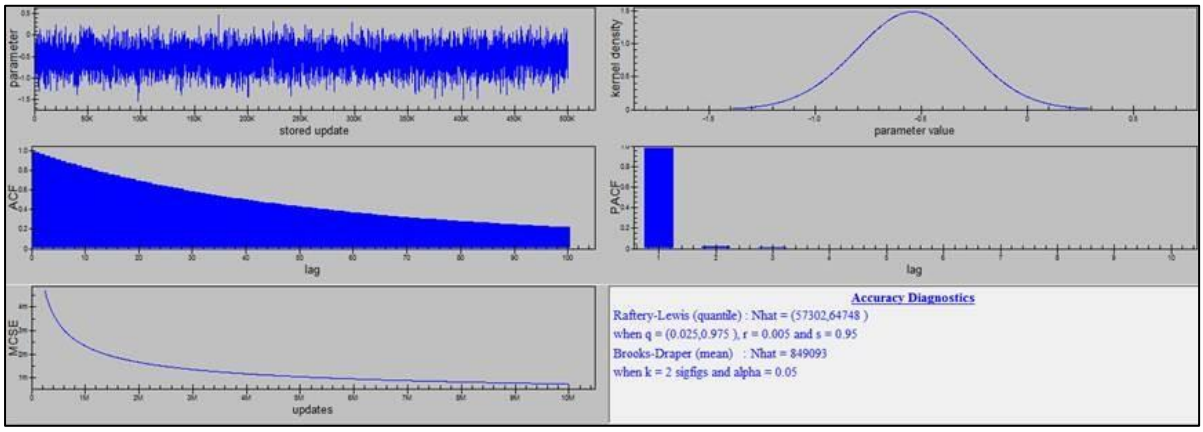
[(a)]CI=confidence interval; SE=standard error; *=significant (P-value≤0.05);

[(b)]ODR=*O. ostertagi.* The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

The hazard for a baseline heifer to calve for a second time between 201 and 321 days after a first calving was 0.84 (0.21- 3.46). Heifer hazard to calve for the second time significantly increased over time. Heifers were significantly more likely to calve a second time during the second (OR (95% C.I.): 47.70 (33.79- 51.65)); third (OR (95% C.I.): 122.61 (62.11- 242.04)); and fourth interval (OR (95% C.I.): 16.96 (4.79- 60.05)), compared to first interval. After controlling for other variables, the hazard for a heifer to calve for a second time at a time *t* decreased by 0.95 (95% C.I.: 0.90- 0.99) unit for a 0.1 unit in heifer individual milk ODR.

Two-way interactions were only significant with the season at first calving. The visual examination of the MCMC diagnostic plots for each parameter included in the model suggested that the model converged well. Figure 5-5 presents an example of MCMC diagnostic plots obtained for the parameter 'heifer individual milk ODR'.

Figure 5-5: Monte Carlo and Markov Chain diagnostic plots for the multilevel discrete survival time model. The graphics show the diagnostic plots for the parameter of 'heifer individual milk ODR'



4. Discussion

4.1. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer milk production parameters

In the current study, heifer individual milk ODR was significantly positively associated with heifer milk yield. However, this association was only present when both parameters were

recorded at the same time. In line with previous reports in adult cows (Kloosterman et al., 1993; Sanchez et al., 2004b), it is possible that an increase in heifer milk yield diluted the concentration of *O. ostertagi* milk antibodies. In a previous study in adult cows (Liua et al., 2009), the correlation between *O. ostertagi* antibodies concentration and stage of lactation was only identified for milk IgG1 and not serum IgG1. Moreover, since the maintenance of an immunity against *O. ostertagi* is highly demanding for the animal in terms of energy (Greer, 2008; Viney and Graham, 2013), it is also possible that this had an adverse effect on heifer milk production. Finally, infection due to *O. ostertagi* decreases the intake of herbage and disturbs the digestion in cattle (Forbes et al., 2000; Charlier et al., 2010b; Viney and Graham, 2013), which, ultimately, can reduce the absorption of nutrients needed for milk production. This is the first study reporting such a negative association between *O. ostertagi* infection and milk production in heifers (Blanco-Penedo et al., 2012; Liedtke et al., 2013). However, this does not necessarily suggest a causal relationship between *O. ostertagi* infection and milk production, for which field intervention studies would be required to validate these findings.

One objective of the current study was to explore if heifer milk ODR at first calving (i.e. between 30 and 90 days in milk) could predict heifer milk production at day 305. The current results suggest that this is not the case. An important factor to consider is that 449 (31%) heifers were excluded from the analysis of milk yield at day 305 given that they had not reached that day at the time of the data extraction. Moreover, considering other results from the current study that suggest that heifers with high milk ODR around first calving are more likely to die during their first lactation, it is possible that the current observation was affected by selection bias. Moreover, the existence of complex relationships between factors often asks from scientists to reduce the sources of uncertainty in their research (Dohoo et al., 2009). In the current study, it is worth noting that a considerable amount of variables might not have been captured during the 305-day period (e.g. nutrition and protocol of anthelmintic treatment). It is therefore possible that a lack of information might have prevented the identification of any significant association between heifer individual milk ODR and milk yield at day 305. This complexity and the need to collect a large amount of data can also explain why research generally relies on coeval parameters to establish an association between *O. ostertagi* infection and cattle milk production (Sanchez et al., 2005; Charlier et al., 2010b; Vanderstichel et al., 2013). In this regard, heifer milk yield at day 305 was significantly associated with BTM ODR. In fact, considering that BTM is a pool of milk samples from all lactating animals including

heifers at day 305, it is possible that BTM ODR acted as a coeval marker of the milk yield at day 305.

The results from the current study did not report any significant association between heifer individual milk ODR and the contents of protein and fat in heifer milk. However, in line with previous research (Charlier et al., 2005b; Sekiya et al., 2013), this association became significant when the effect of milk yield was not accounted for in the model (data not shown). This suggests that associations between milk ODR and milk solid contents were likely to be confounded by the effect of milk yield in heifers (Kloosterman et al., 1993).

4.2. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer health

This is the first longitudinal study that establishes a significant association between heifer individual milk ODR and heifer health. In this study, a significant association was identified between heifer individual milk ODR and heifer probability to die before the end of the study. Given that there is a lack of similar research on this topic, comparison and interpretation of results are limited. The only similar report currently available in the literature suggests that herds with high levels of BTM antibodies against *O. ostertagi* are likely to have higher levels of cattle mortality (Delafosse, 2013). However, this is based on a bivariate analysis of the association between variables that do not account for the confounding effects of other factors. Previous research suggests that *O. ostertagi* induces an important immunosuppression in cattle, which can impact on cattle susceptibility to other diseases and, ultimately, on cattle health (Gasbarre, 1997). In fact, *O. ostertagi* produces excretory-secretory substances that can modulate and suppress cattle immune responses (Klesius, 1993; Vercruysse and Claerebout, 1997). Evidence suggests that *O. ostertagi* increases the establishment of other helminths, such as *Dictyocaulus viviparus*, in calves (Kloosterman et al., 1989). Moreover, some studies reported that other cattle helminths, especially *F. hepatica*, are responsible for increasing cattle susceptibility to intracellular pathogens (e.g. *M. bovis, S. dublin,* and *B. pertussis*) (Aitken et al., 1978; Claridge et al., 2012). As a consequence, it is possible that increasing levels of *O. ostertagi* exposure resulted in an increase in heifer mortality during the current study. In line with other results of this study, it is also possible that the infection due to *O. ostertagi* increased heifer susceptibility to *Mycobacterium avium* subspecies paratuberculosis (MAP) (i.e. Johne's

pathogen). If confirmed, the latter would be another reason to take rapid action against *O. ostertagi* in England, taking into account the increasing number of Johne's cases reported in the region (SAC, 2003).

It is also possible that the significant association reported in the current study between heifer milk ODR and heifer test results against Johne's disease reflected different competencies of heifers to mount an immune response against pathogens. Moreover, differences in terms of farm pressure of infection and management can hinder or facilitate the exposure and/or immune response of heifers to several pathogens. Finally, it is worth noting that the ELISA used for the detection of *O. ostertagi* antibodies could also have cross-reacted with antibodies produced against MAP or other pathogens related to MAP infection.

4.3. Association between heifer individual milk antibodies against *Ostertagia ostertagi* and heifer reproduction

In the current study, there was a positive association between heifer individual milk ODR and heifer offspring mortality at first calving. This result sheds light on previous research and suggests that the impact of *O. ostertagi* on calf mortality is not necessarily due to colostrum quality (Delafosse, 2013). There is a lack of studies on the mechanisms by which *O. ostertagi* could interfere with heifer gestation. Some clinical trials conducted in beef heifers suggested that *O. ostertagi* negatively affects the body condition score and the weight of pregnant heifers during gestation (Loyacano et al., 2002). Moreover, treatments against *O. ostertagi* were reported to significantly increase the pelvic area of infected heifers suggesting that *O. ostertagi* infection may increase the risk of heifer dystocia at first calving (Mejia et al., 1999). Finally, it is possible that the adverse effects of *O. ostertagi* infection on host nutrients absorption (Greer, 2008) deteriorate and prevent good development of the foetus during the gestation.

In the current study, heifers with higher individual milk ODR around first calving were more likely to have their second calving delayed. Such a persistence over time of the negative effects of *O. ostertagi* infection in cattle has been previously reported in the literature, where effects of the parasite could be noticeable for at least a year after the animals' first exposure to helminths on pasture (Ploeger et al., 1990b). This is the first time that an adverse effect of *O. ostertagi* infection on cattle reproduction performance is reported in heifers adding to similar

observations reported in adult cows (Walsh et al., 1995; Stromberg et al., 1997; Sanchez et al., 2002a). This finding suggests that higher exposure to *O. ostertagi* on pasture can impair the reproductive performance of first lactation heifers and, ultimately, reduce the productive lifetime of a milking herd.

4.4. Limitations of this study

Farmers included in the current study were all members of QMMS Ltd laboratory, which granted access to a wide range of high-quality parameters on heifer production, reproduction and health performance. Although the availability of the data was variable according to the assiduousness of the farmers in recording events, most of the variables collected were either directly monitored (e.g. milk yield and calving date) or made available by the laboratory itself (e.g. fat yield, protein yield, Johne's ELISA results), which guarantees their quality. Moreover, since the data were also regularly checked and maintained by highly qualified technicians from the Sum-it computer systems Ltd. (Oxfordshire), potential record errors could be identified and corrected.

Different variables related to heifer management, especially in terms of health and reproduction, could not be captured in this study and might have influenced the current observations. Factors such as delay in the care of heifers parturition (i.e. causing calf anoxia) and the quality of heat observations are known to be important causes of calf mortality (Greene, 1978) and reproduction impairment. Moreover, the study did not include information on other aetiology related to calves mortality and reduced fertility (e.g. *E. coli*, BVD, IBR, and nutrition).

Finally, although the use of individual milk antibodies as a marker of *O. ostertagi* infection offers better possibilities to explore associations between parasite exposure and production performance (Sekiya et al., 2013), such an approach present some limitations. First, evidence suggests that the correlation between *O. ostertagi* antibody levels and cattle worm burden is weak and that *O. ostertagi* antibody levels do not reflect active infections in cattle (Charlier et al., 2014). As a result, identification of associations and the interpretation of results can prove difficult. Moreover, *O. ostertagi* ELISA test can cross-react with many other helminths (Klesius, 1988). This cross-reactivity was to some extent controlled in the current study while

accounting for *F. hepatica* BTM ELISA results in the models. However, in the case of the model related to heifer health status, the effect of *O. ostertagi* exposure on heifer mortality disappeared while accounting for *F. hepatica* BTM PP. This suggests that this association (i.e. probability of death and level of antibodies against *O. ostertagi* around first calving) may have also accounted for infection by *F. hepatica*. Moreover, since many other nematodes can cross-react with *O. ostertagi* antigens, all associations reported in the current study may have included the effect of other helminthic diseases. Since specific ELISA for *O. ostertagi* is not yet available (Charlier et al., 2009), it is difficult to suggest any improvement related to this issue while conducting longitudinal studies of *O. ostertagi* infections in cattle with this diagnostic tool.

5. Conclusions

This is the first study reporting a significant association between *O. ostertagi* antibody levels and impairments in heifer milk production (i.e. lower milk yield at first calving), reproduction (i.e. higher mortality in offspring at first calving and delay in heifer second calving) and health (i.e. higher antibody levels against Johne's disease in heifer first lactation and higher probability of death after first calving).

These observations do not infer a causal relationship between individual milk ODR and heifer productivity parameters but raise questions about the observed associations. Moreover, considering the existence of multiple cross-reactions which were not accounted for in the different models, the reported associations should be interpreted as possible effects of nematode infections on production, reproduction and health performances in heifers.

Although the current study was limited and did not include several management factors that could also have influenced the results (e.g. biosecurity, reproduction management, and other disease control), if confirmed causal, the observed associations would justify an urgent need for farmers to implement effective and strategic control against helminth infections in heifers. For a confirmation of this causality, further field intervention studies are required.

Chapter 6.
Opening-up cattle helminth infections in England: Exploring farmers' knowledges, practices and values

1. Introduction

Raising concerns over anthelmintic resistance in the UK have contributed to the development of several guidelines for helminth control sustainability in livestock by the industry (SCOPS, 2003; COWS, 2010). Recent reports, however, suggest that the adoption and application of these guidelines by farmers is still poor and that the levels of farmers' engagement with helminth control is highly varied among farms (Heasman et al., 2012; Morgan et al., 2012).

A considerable number of questionnaire surveys have been conducted to shed light on the state of play of helminth control practices in cattle and sheep farms (Borgsteede et al., 1998; Stafford and Coles, 1999; Barton et al., 2006; Bloemhoff et al., 2014; Easton et al., 2016; Moore et al., 2016; Ploeger et al., 2016). Most of these studies suggest that farmers overuse anthelmintic treatments and their decisions on helminth control vary according to farm locations (i.e. geography), climate, herd and flock sizes, farmers' training, animal physical condition and infection diagnosis (e.g. faecal egg counts) (Gasbarre et al., 2001b; Moore et al., 2016). Some studies have also looked at the association between farmers' awareness of anthelmintic resistance and their use of anthelmintic treatments (Moore et al., 2016).

Despite the importance of considering the aspects listed above, these are not the only factors that should be taken into account when investigating farmers' decision-making on helminth control. Other factors include, for example, farmers' attitudes, perceptions, and financial aspects, which could also influence decisions on helminth control (Charlier et al., 2015). As it will be discussed, helminth control practices cannot be considered separately from the rest of the farm-system management, given they might compete with other resources such as labour, finance and skills (Morley and Donald, 1980). For example, the effectiveness of a measure

(e.g. duration of treatment benefits) and its cost are key factors influencing farmers' helminth control practices (Moore et al., 2016).

Although informative, the surveys mentioned above fail to capture in a more comprehensive way the different factors related to farmers' concerns and values that might affect helminth control practices. Even those studies which have argued that more holistic approaches are needed, suffer from limitations in terms of recommendations that are feasible and adequate for farmers. However, if guidelines on helminth control are expected to be accepted and adopted on-farm, researchers focused on the topic need to fully understand farmers' behaviour and their contextual challenges (Charlier et al., 2015).

Over eighty-three theoretical socio-cognitive frameworks have been developed in human psychology to explain behaviour and behavioural processes (Michie et al., 2011). The interest in using these theories in veterinary sciences is recent and aims to support research on farmers' attitudes towards animal health management (Ellis-Iversen et al., 2010; Garforth et al., 2013). Theoretically, this body of research has been informed by frameworks such as the Health Belief Model (HBM), the Theory of Reasoned Action (TRA) and the Theory of Planned Behaviour (TPB); methodologically, it has mostly relied on the use of quantitative surveys based on application of close-ended questionnaires (Carr and Tait, 1991; Beedell and Rehman, 2000; Gunn et al., 2008; Delgado et al., 2012; Sok et al., 2016). The outcomes of these studies widely suggest that farmers' poor uptake of 'best practices' against infectious diseases is primarily due to a lack of awareness of the issue and insufficient knowledge-transfer from their veterinarians (Jansen et al., 2010b; Scrase et al., 2015; Easton et al., 2016). In this study, it is argued that, although these two factors may play a role in farmers' adoption of guidelines, they cannot alone explain the processes through which farmers make decisions in terms of helminth control in their farms.

Doubts have emerged regarding the capacity of theoretical socio-cognitive frameworks to understand human behaviour. This is because approaches often rely on instrumental frameworks and methods that do not leave room for reassessment of research variables and assumptions, despite engagement with research subjects (Ogden, 2003a; Sniehotta et al., 2014). Moreover, without fully understanding the most relevant and common underlying factors influencing the behaviour of a particular population, the use of a single theory to explain the latter can be challenging (Michie et al., 2011).

The current study therefore aims to broaden the scope of analysis of farmers' helminth control practices. For that, through in-depth, qualitative interviews with dairy cattle farmers in England, it explores key aspects that have been overlooked in the available literature. These include how farmers build their knowledges (i.e. the diversity and the changing nature of their knowledge) and define their practices in relation to helminth infections in cattle, and how these intersect with farmers' values and concerns. The following sections first explain the design of the study and its methodology (section 2.), before presenting the results of the analysis in two parts (section 3. and 4.). It then discusses the implications of the findings for helminth control in dairy farms (section 5.). The chapter closes with suggestions for changes in the way helminth control guidelines are developed (section 6.).

2. Research design and methodology

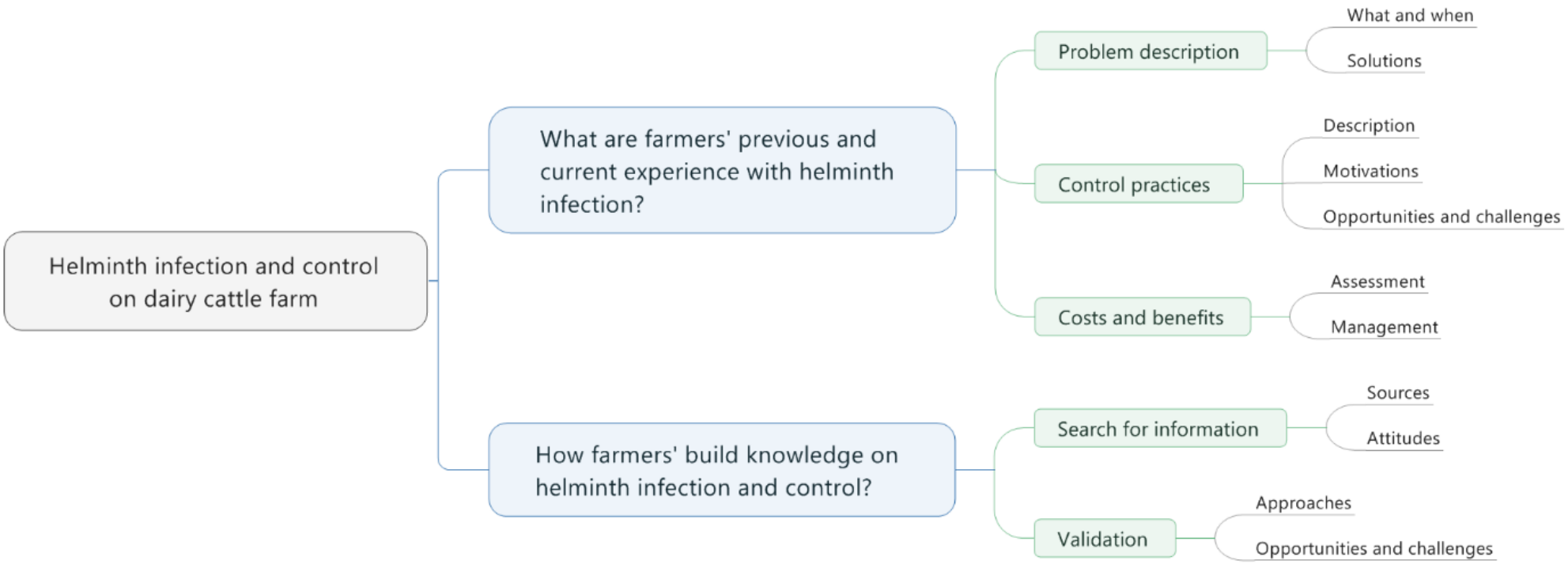
The current study was funded by AHDB dairy (UK) and received an approval from the ethics committee of the School of Veterinary Medicine and Science at the University of Nottingham. A convenience sample of 42 dairy cattle farmers was used, who participated in a quantitative longitudinal study on *O. ostertagi* infection (Chapter 2.). Farms were all members of Quality Milk Management Services (QMMS) Ltd. (Somerset, England). Interviews were conducted by the PhD student during a farm visit from April 2014 to May 2014. All farmers signed an informed consent form beforehand, agreeing with the terms and conditions of the study (Appendix 4.). Semi-structured face-to-face interviews were conducted with the main manager of the herd in the farm. Of the 42 respondents, 36 were male (86%) and 6 were female (14%); 7 (17%) also had sheep on-farm.

The deductive part of the research (Bryman, 2012) builds on the available literature on the epidemiology, prevention, control and impact of cattle helminth infections and what are considered as the drivers behind farmers' decision making in cattle helminth control (Chapter 1.). This body of theory was used to design the qualitative study by informing data collection, i.e. the design of in-depth, semi-structured interviews (Appendix 14) and the initial framework used to analyse the data, which is discussed below. Interview schedules were pilot-tested to identify questions that could be confusing, any missing topics, and to rephrase any sensitive questions. Interviews lasted on average 35 minutes (from 15 to 90 minutes) and were audio-

recorded. The interviews were transcribed by a third party, checked and imported into the software NVivo 11 (QSR, International) for qualitative analysis. The description of the different steps adopted for the qualitative analysis are presented below.

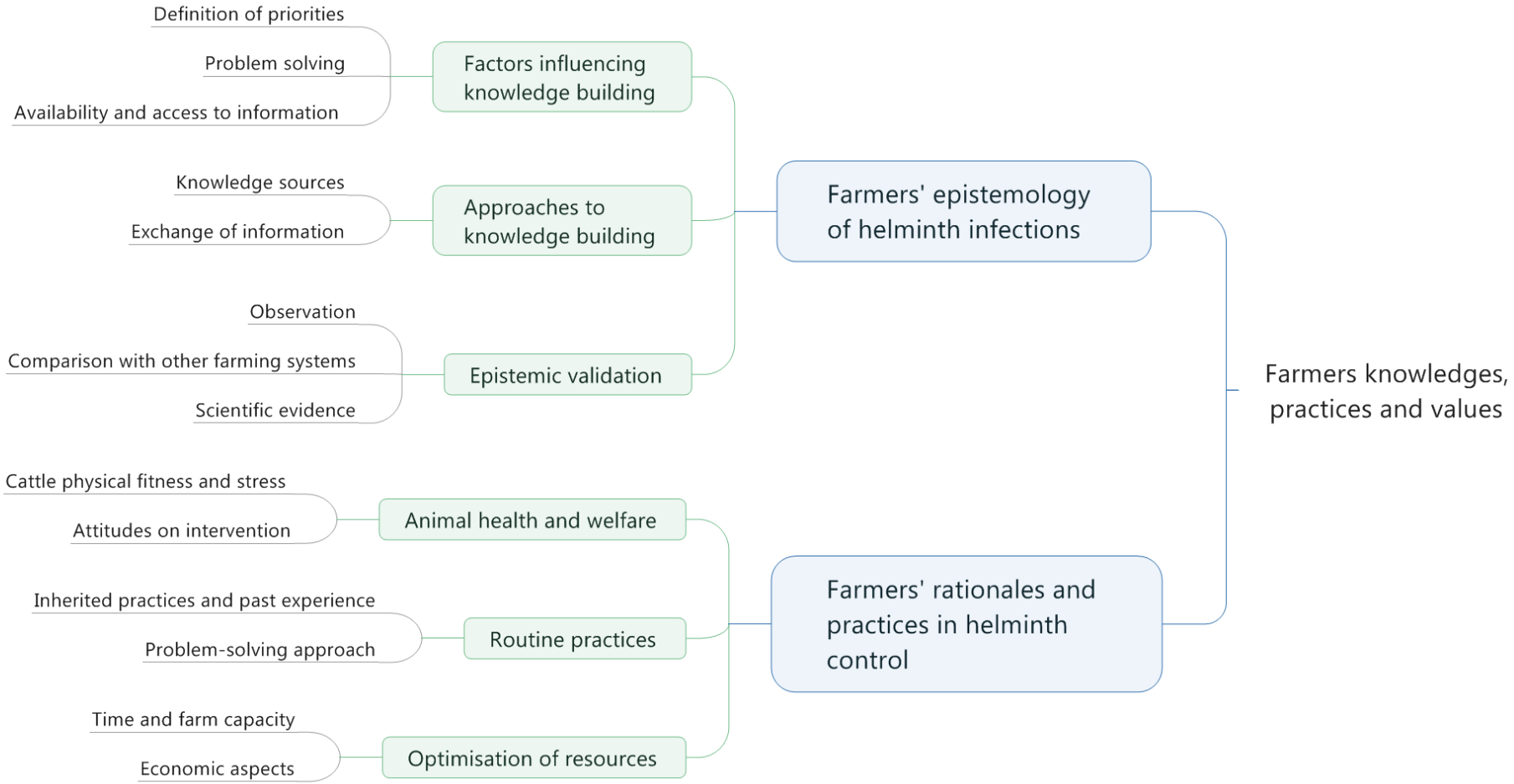
In NVivo, the initial categories that informed the coding scheme for the analysis of interviews' transcripts are shown in Figure 6-1 (please note that these are aligned with the interview questions since they draw on the same literature review). As shown in Figure 6-1**,** categories included how farmers experienced helminth infections in their farm, how they understand the problem and how they would address it; their perceptions on the challenges and opportunities of helminth control and their evaluation of the costs and benefits of control practices; and how farmers build knowledge in order to address helminth infection, both in terms of search and validation of information.

Figure 6-1: Analytical framework of the qualitative analysis (deductive part)



The inductive part of the qualitative analysis, i.e. the process through which, from the data, a model or a theory about farmers' decision making on cattle helminth control is developed (Bryman, 2012), used a systematic coding process to identify emerging themes in the interview transcripts (i.e. thematic analysis) (Coffey and Atkinson, 1996). Supported by the initial framework (i.e. parental nodes in NVivo), but not limited to it, emerging themes (i.e. child nodes in NVivo) added detail and content to the initial categories that had been drawn from the theory. Through an iterative process, emerging themes and sub-themes were identified and refined (i.e. excerpts of the interview transcripts) until data saturation was achieved (Silverman, 2014). A sole coder developed the thematic analysis (PhD student, CB), although the reliability of the coding scheme was discussed with the research team before and during the coding process. Figure 6-2 presents the emerging themes and sub-themes generated from the inductive part of the research. These represent not only *what* farmers' know and do about cattle helminth infections but also *how* farmers assess situations, engage with others and make decisions on helminth control (Johnston, 1995). The following sections of this chapter (sections 3. and 4.) detail and discuss these emerging themes.

Figure 6-2: Emerging themes of the qualitative analysis (inductive part)



3. Dairy farmers' epistemology of helminth infections

Individuals' understandings of reality are based on the particular values and beliefs they have about the world, which shape and are shaped by experience and knowledge. Epistemology aims at understanding the nature of this knowledge and focuses on what is considered as valid knowledge by particular groups of individuals (Bryman, 2016).

In this work, farmers' epistemology refers to *what* farmers know about cattle helminth infections and *how* they build this knowledge. The following sections report on the qualitative analysis of the interviews conducted with farmers. These interviews explored different aspects of farmers' epistemology, including the motivations that guide knowledge building; the sources they use to look for information; and, finally, the way they validate (i.e. include and/or exclude) the information deemed relevant or useful in relation to their concerns and interests.

3.1. Factors influencing farmers' knowledge building

At least three factors can be identified as key drivers motivating farmers' to gather information on helminth infections. These are the consideration of helminth infections against other farming priorities; the need to solve problems that emerge in routine farming activities; and, third, the availability and access to relevant information for addressing problems related to helminth infections on the farm.

3.1.1. Defining priorities

Most farmers expressed a low concern - hence interest - in cattle helminth infections. They acknowledged being mostly passive in gathering information on the topic, given the presence of other more important issues on-farm. Overall perception was that cattle helminth infections were neither a worry nor a priority compared to other topics, such as fertility, lameness, mastitis or Johne's disease. The main reason for farmers to regularly look for information on helminths was related to their concerns about costs. In fact, some farmers explained that they met with other fellows in order to do market studies of available products, as mentioned below by Farmer 37:

> "The way our group works, is each person, in that group, has a job; **he has something he has to buy**… and it's their job, responsibility, **to find the best price** they can and when you go to the meeting, they present their case for using that supplier."

Helminth infections were often normalised by farmers, who considered them as natural and inevitable processes that were part of the "puzzle" of the dairy cows (Farmer 32). The fact that animals would be infected by helminths was even seen as beneficial for some farmers, who considered that the animal could hence build a natural immunity and become more resistant to subsequent infections:

> "I accept the worms create less production, but if you want the animal to create its own immunity, you may have to take a hit on production on some animals… not on all, but on some of them, just so some others can get the immunity. If you tried to clear them of all worms, you would have great production, but then, if worms came in from somewhere and hit them, it would be on your doorstep… so **keeping the tolerance** and **allowing a small drop in production** and **creating immunity, everyone is happy**." (Farmer 34)

As a consequence, farmers showed a certain level of acceptance, tolerance and/or resignation concerning the presence of a minimum level of helminth infections on-farm:

> "Well, because I think all cattle have a certain amount of gut worms and **it's natural for them to do so**. Unless the animal gets something else wrong with it, **I don't think they are a particular issue**." (Farmer 18)

Farmers remained however curious on the topic. Most of them explained that the reason they participated in this study was because they wanted to understand and know a bit more about the current situation of helminth infections in their farm. However, they admitted that the difficulty of grasping the importance of something that is mainly invisible (i.e. subclinical) affected the way they would engage with the topic. Interestingly, here farmers demonstrated to be reflective and self-critical by indicating that they should rethink the way they define priorities in the farm (i.e. paying more attention to the issue of helminth infections).

3.1.2. Solving problems

When farmers were asked why they would seek for information on cattle helminths, the majority of them (N=32; 78%) would explain that this was because they had been concerned and had noticed a health problem with their animals. 'Visual' signs (i.e. clinical) would indicate a deterioration in animals' physical condition and/or loss of weight, a decrease of calving index or even a "shinny coat, as if the coat of the animal had gone a bit straggly" (Farmer 26). These observations would trigger farmers to look for information in the available literature or immediately seek for external advice (e.g. through consultation with veterinarians), as pointed out by Farmers 32, 3 and 34, respectively:

> "Literature… off the internet… **it depends if I've got a problem**. If I've got a problem, I go and find the information."

> "(…) when we had that roundworm, **I telephoned her up (the veterinarian) straightaway and let her know we had a problem**, she came out, she thought it would be roundworm before she tested them anyway but then that test proved that it was."

> "**When I have a concern**; last year, he was called out (the veterinarian) to these calves that were a month old; and he comes out and **we have a conversation and he gave me possibilities to discuss the treatment, discuss the prevention**."

Therefore, farmers suggest that the search for information or advice from externals is almost exclusive to those cases where problems have been identified and need to be solved. It is worth noting that some farmers preferred not to actively look for problems possibly related to helminths (e.g. subtle loss of animal physical condition), which could, consequently, prevent them of looking for information and building their knowledge on helminth infections. For example, an absence of obvious clinical signs would convince farmers that animals "are in too good a condition to have a problem" (Farmer 31) and prevent them from looking for information on the topic. Likewise, as stressed by farmer 29 and 32:

> "Well, hopefully, I mean the cattle are generally healthy**, if you start looking for problems sometimes you find some** but, no, I'm sort of happy with what's going on, **so I don't look for any problems**."

> "If I felt I was getting (worm) resistance and I just needed to find another way around it, then I'd probably have a different attitude **but if it's not broken, don't fix it**."

Problem-solving motivations can therefore influence knowledge building in two ways. Firstly, by prompting farmers' interest in learning about the issues with the objective of solving a problem that is typically immediate and that could be disruptive of farms' proper functioning. Secondly, by preventing them from looking for information either because farmers would adopt a problem-averse approach or because there would not be a justification for looking for information or consulting with others.

3.1.3. Availability and access to information

Farmers can face challenges when it comes to availability and access to information that could help addressing helminth infection. Such information can be obtained from external sources or be generated on-farm through farmers' self-recording of events. As for the former, the availability of information was a reason indicated by farmers that could be connected to a lower level of interest in cattle helminth infections. The majority of the farmers (N=35; 83%) indeed considered that current literature on cattle helminth infections was either too scarce or out-of-date, especially when compared to sheep and other diseases. Both Farmers 41 and 29 highlighted this fact, suggesting a need for improvement on this matter:

> "I think that's probably the issue, there's more sheep than cattle and it's highlighted in sheep, in the industry generally, that there are worm resistance; whereas **it's not been highlighted so much in the cattle**… so perhaps, you know, perhaps, **it's something that may be highlighted,** I don't know." (Farmer 41)

> "Well, there's always bits and pieces floating about in the farm press on worms, **but there's never really anything new**, you know… worms are there and you deal with them." (Farmer 29)

Some of the farmers interviewed explained that they could not afford losing time and energy in trying to access or decipher information. Some of them argued that even when they were motivated and wished to be more informed, they faced obstacles. One farmer mentioned complaining and constantly ringing veterinarians to get a copy of worm results without success (Farmer 39). Another concern voiced by farmers was that leaflet containing information on helminth infections are way too long. Given time limitations, this could lead farmers to "miss the important points" (Farmer 11). Interviewees also suggested that low quality of knowledge-transfer, in particular with veterinarians, resulted in unintelligible and contestable information. For instance, milk antibody results can be seen as a 'black box' for farmers (Farmer 19).

In terms of information generated on-farm that could be useful for helminth control, although many farmers recognised the value of recording on-farm events (e.g. treatments and disease outbreak), they admitted not applying this thoroughly for helminths. When done, recording usually related to mandatory aspects such as anthelmintic treatments (e.g. compound and time of administration), which are relevant to insurance policies (e.g. organic inspection and milk insurance). Most farmers justified themselves by saying that recording activities were time-demanding, "probably tripling the amount of their time" (Farmer 26); and something they could not afford (Farmer 1). This explains why "the deworming (recording) tend to slip through the net" (Farmer 29). Moreover, other farmers said to feel discouraged to enter data in the computer. This seemed like a very complex task for some of them (Farmers 10, 13, and 32), who preferred to keep paper notes; however, they pointed out that such notes could be easily lost or were unintelligible. In this regard, the few farmers who used computer recording emphasised how essential this was to avoid being "completely lost" (Farmer 4). Here, for example, they praised the role of software technicians in helping them to keep the records up to date, therefore facilitating availability and access to information:

> "I find it is (computer recording) easy to use and **mainly because of the helpline**… I just think they're really good, at the end, of the Summit Helpline and, **if you're not really technologically minded, you can telephone them** and, you know, there's a small team." (Farmer 19)

3.2. Approaches to build knowledge

Farmers use a range of sources when looking for information on cattle helminths. The most popular sources indicated by those interviewed were veterinarians (N=28; 67%), the media (i.e. internet and farming press) (N=21; 50%) and farmer fellows (N=20; 48%); other less popular sources were pharmaceutical supplies (N=7; 17%) and past education in agro-farming (N=5; 12%).

It was possible to identify three different approaches to information exchange when farmers build knowledge through engagement with veterinarians. These can be characterised in terms of the information flow (i.e. one-way or two-way communication) and its direction (i.e. top-down, bottom-up or a mix of both, regarding those responsible for making the decision). The first of them is represented by a top-down (i.e. from expert to farmer), one-way flow of information, where farmers expect advice to be provided by veterinarians, as illustrated in the passages below from interviews with Farmer 29 and 2, respectively:

> "Well, yes, basically **they (veterinarians) need to go out there and keep their knowledge up** … they (veterinarians) have got to be constantly researching and seeing what's going on **and, then, passing on that information (to us)**."

> **"**CB: So in terms of other information you get regarding worm control?
>
> Farmer: **We don't**.
>
> CB: You don't?
>
> Farmer: **Down to the vet.**"

A second approach, which is characterised by a two-way flow of information (i.e. dialogue), is based on a more horizontal relationship between farmers and veterinarians. These dialogues can be constructive, with both actors welcoming an exchange of perspectives and points of view when discussing helminth control in the farm. For example, as Farmer 17 and 12 would put it:

> "I'm quite happy with how we are with our vets at the moment. They're proactive enough, **they understand me, I understand them,** you know.

> They understand, sometimes **they'll suggest I do something and sometimes, we won't do it, because we can't, you know, but also they understand** (…)".

> "(…) he (the veterinarian) is following our milk samples, he went to meetings with X (farmer) and you know, **(they) come up with a plan together**".

Finally, a more bottom-up approach would consist of farmers' engaging with veterinarians (either one-way or two-way flow of information), but making the decision independently after consulting with experts, as mentioned by farmers 34 and 1:

> "Yes, we have the discussions, yes, **and I decide that we are alright as we are** (…) so your vet is there to be spoken to and discuss things with and **if you think he is right** then you go down that route **and if you don't you look for somebody else** to get advice from."

> "**I would listen to it and put my own views on it**, I will listen to anything and then I will think about it. I will look at the pros and the cons **and come to my own conclusion**."

3.3. Farmers' epistemic validation

Farmers' showed different strategies to validate information so as to make decisions in helminth control. These include visual 'evidence' as 'proof' to confirm hypotheses; comparison with other cases and farmers' experiences; and the use of scientific evidence (including expert advice).

3.3.1. Observation

Without exception, all farmers stressed the importance of experience and learning-by-doing in farming, especially in the case of helminth control. In the case of epistemic validation, farmers would stress the importance of visual evidence of helminth infection. This means they would favour confirmation of hypotheses through visual scrutiny of clinical signs:

> "I think **it's because you can't see them (worms)**… if you've got mastitis, you see it and think, why is this, and **when you don't see something**, you are just doing this to prevent it; as **I don't see worms coming out of the back of cows**… But if you say, you need to change that because it's not working well, the only evidence I have got is that you are telling me that you tested them and looked at the milk… **but I haven't seen it myself**… (...)." (Farmer 26)

Such observations can be direct, with the presence of sick animals on farm as suggested by the quote above. Indeed, farmers indicated that a fundamental factor guiding management practice is evidence of animal's health condition, as visually confirmed by them (e.g. Farmer 5, 8, 21, and 26). In this sense, where helminth infections were mostly silent (i.e. absence of clinical signs), farmers would believe it would be unlikely that they would harm their animals and have a negative impact on production:

> "**If I start seeing problems, I'll worry about it**… but if my cattle are well and they are milking well and **they are looking well and they are not dying, then I don't worry about it**." (Farmer 42)

3.3.2. Comparison with other farming systems and the influence of the media

Livestock industry's figures and concerns in the UK influenced the way farmers considered helminth infections. Many farmers mentioned the considerable economic impact that helminths already had in the sheep sector, explaining this made them more concerned about the topic in the case of cattle. Farmers believed it was crucial for their business to avoid such situation and said that they, as cattle farmers, were highly responsible for this not to happen:

> "I mean… you know… **where the sheep industry's got itself, we don't want it happening the same**. Going down the same route as the sheep industry if we can help it." (Farmer 8)

Such a sensitivity to the issue was mainly seen among farmers who either had reared/were rearing sheep (44%) or had a level of farm production higher than the average (300 to 890 *versus* 150).

Trends in helminth infection in other contexts than their own farms (e.g. impacts on other livestock systems and experiences with other types of worms) would contribute to farmers' awareness and interest on the topic. Those farmers who could not relate their experience with those being discussed elsewhere (e.g. in the media, specialised literature, and public debates) were doubtful of whether this was really something worth looking at. As an example, farmer 11 believed that "lungworm would be a greater problem than the Fluke" because he could "hear a lot more about the stomach worms and lungworms than you do Fluke" in the "trade magazines".

Frequently, farmers would look for similar or comparable experiences of cattle helminth infection from other farms around their area, as explained by farmers 9 and 5:

> "I see my vet… I would be asking him things like, **has there been a problem in their locality**? For example, like this year… he (veterinarian) said yes, there's been a lot more problems in this area… so yes… (…)"

> "(…) we kind of listen to our vet and listen to other people in the area as to, you know, **whether there's issues that are starting to take hold and then we might change what we do** (…)"

3.3.3. Scientific evidence

Farmers were frequently doubtful of the impartiality of the information provided by externals (i.e. industry representatives and veterinarians). They often considered that their advisers were, like them, individuals who were trying to maintain their business alive, hence following their own interest in selling more services or drugs:

> "**I'm very sceptical about big business creating things so that they can make money**… especially in terms of health… human health especially, but also animal health. So they'll, and because they've got huge amounts of money behind them… **they're very, very clever and they've got very good people working for them and they'll make a story and they'll convince you**. You'll see an article in the press saying, if you wormed all your dairy animals you'd get ten percent milk, which personally I don't believe. I'm sure in some cases

> they can prove it, but I'm sure, because **they're so clever at manipulating the data**…" (Farmer 25)

> "But it's very hard to get the vet on board cos' he thinks… he thinks the answer is always the drugs (laughs) … you know… vets sell drugs… **It's quite hard sometimes to get the vet on the preventative, rather than the … sell medicines**." (Farmer 35)

Nevertheless, in general, veterinarians' advice played an important role in farmers' decision-making on cattle helminths. Even in the case of very sceptical farmers, veterinarians were well-regarded since they had a "scientific side" (Farmer 2) and a duty to "be more independent" (Farmer 32), which would make farmers be more inclined to take their advice on board. Farmers would generally approach their veterinarians after learning any concerns about worms being raised, for example, in the media, through drug companies' communications, and seminars. Additionally, participating in scientific studies often helped farmers to take the time to focus on helminths and validate their knowledge, avoiding the topic to be the 'fifth wheel' of their farming, as mentioned by Farmer 19:

> "So I suppose that was one, but I needed encouragement to do it**… it was my job to do the worm egg counting**, I should have been doing it… it was just something that always got forgotten and… so part... you know… **participating in the study made us have to do it each week**."

Some farmers said they directly applied veterinarian's advice on worms, given their low interest in the subject and lack of time (Farmer 26). Others, more obstinate and independent, would challenge veterinarian's information prior to any validation. Farmer 8 explained that he started his own clinical trial because he wanted to prove whether the deworming policy recommended to him was right or wrong. Many farmers emphasized the need for veterinarians to provide evidence for their advice, rather than using a "belt and braces approach based on gold standard" (Farmer 35). In fact, most farmers believed in their veterinarians and were receptive to their challenges, when they could feel goodwill and most importantly that they felt understood in terms of their specific ways of farming, as highlighted by Farmer 25:

> "He (veterinarian) is much better, **because he's a friend**… much better… that's why I stopped the other vet… they were too… basically, they had a business model and everything was to make the last bit of money."

4. Farmers' rationales and practices in cattle helminth control

Historically, experts have provided farmers with recommendations for improving the functioning of their farming systems and obtaining desirable outcomes (McCown, 2001). However, despite continued efforts to facilitate the adoption of guidelines, evidence suggests that there is a gap between theory and practice in agriculture, and especially in the case of cattle helminth control (McCown, 2001; Van de Ven and Johnson, 2006; Heasman et al., 2012). Such a gap has been traditionally framed as a knowledge-transfer problem in which, through guidelines, experts (e.g. policy makers and veterinarians) would fail to deliver scientific knowledge about management to farmers (Van de Ven and Johnson, 2006; Jansen et al., 2010b; Easton et al., 2016). However, the issue could be far more complex since, as discussed in this study, farmers seem to mobilise different sets of knowledge, values and concerns when making decisions about cattle helminth control. Therefore, besides understanding farmers' epistemology, investigating other factors that shape the practices of helminth control is also much needed (Van de Ven and Johnson, 2006). Following the previous section, which focused on how farmers build their knowledge on cattle helminths, this section explores farmers' rationales behind helminth control. Three key rationales were identified: animal health and welfare; routine practices and optimisation of resources. The different practices embedded in these rationales are also outlined in the following sections.

4.1. Animal health and welfare

When explaining their reasons behind cattle helminth control, most farmers (N=36; 86%) indicated that "this was part of the job to do" (Farmer 1), since farmers felt responsible for maintaining their animals "healthy" and "happy" (e.g. Farmers 5, 34, and 15). Many of them

explained that they did not like to "highly stress" their cattle (Farmer 41). On the contrary, as put by Farmer 23:

> "**I like to see healthy animals**; I don't like to see poor animals, **I like to see my animals looking well**."

In this regard, deworming practices reflected an overall farmers' view of "always putting the cattle first" (Farmer 1). Some farmers even argued that preventing helminth infections was an ethical aspect of farming (Farmer 4) and that maintaining animal welfare was mandatory for them (Farmers 12 and 29). Helminth control was often seen as an evidence for farmers to prove that they had the capacity to look properly after their cattle (Farmers 29 and 5). Here, farmers believed they understood their animals better and cared more about their well-being than anyone else:

> "We have to have a diet plan now, by nutritionists, and I said that won't last 2 minutes… the cow will be useless (…) so I said, **we will have to do without the milk yield then, because it won't be healthy for the cow and they don't understand it**." (Farmer 34)

Farmers' choice of different anthelmintic treatments was highly influenced by how they perceived the safety of the treatment and by how much stress they considered it would put on the cattle. To illustrate this point, Farmer 17 explained that even though pour-on methods were more expensive, he would prefer them in order to prevent "stress for the animals". Interestingly, this same farmer also established a connection between animal stress and that of farm staff responsible for handling the animal: "(…) Lots of people getting bumped and bruised and, for an easy life, pour on seemed to be the way to go." Other farmers would also agree that what would be less risky and stressful for the animal would be "less dangerous" (Farmer 25) and "easier" for farmers (Farmers 17 and 29), highlighting that animal stress and farm proper-functioning seem go hand in hand.

Farmers showed different attitudes in relation to interventions (i.e. for helminth control) in the context of animal health and welfare. These can range from practices that would favour the use of anthelmintic drugs only in the case that is absolutely necessary (i.e. where animal health is deteriorating), to the systematic use of anthelmintic drugs or constant control to prevent cattle exposure to helminths on pasture, independently of the identification of a need to do so.

For example, Farmer 25 believed cattle well-being and health was dependent on natural processes and therefore required as less intervention as possible on the part of the farmer:

> "I'm more inclined to think that the animal, if you leave it to their own devices and don't interfere with it, most of the time that **they'll do reasonably well**… and **I try to interfere as little as I can with them**. **That doesn't mean that I won't treat them** (…)."

Conversely, Farmer 32 and 14 considered intervention as a constant need (in both healthy and unhealthy animals) in order to prevent helminth infections and ensure animal health and welfare:

> "**My cows don't go out at all. I'm in control of them and that works very well**. (…) as soon as I let anything out to graze, I've got a higher risk of interaction with wildlife… and I don't see that, that's a good thing…" (Farmer 32)

> "(…) well the cattle after their first grazing season **are wormed regardless**, whereas years ago it used to be, oh well, yes, they don't look very good **it was a hit and miss, and now they are wormed**." (Farmer 14)

4.2. Routine practices

Farmers' practices in relation to helminth control are many times routine-based. Indeed, inherited practices and past experience were fundamental aspects of the farming systems studied. Although some aspects of helminth control had slightly changed over the years (e.g. drug compound or form), farmers assured that their practices remained overall the same (e.g. Farmers 1 and 7); or that this was at least "as far as they could remember" (Farmers 17 and 2). In this regard, farmers always referred to past experience, i.e. the time they were "young" (Farmer 28) or a "little boy" (Farmer 29) and started participating in the activities of the farm. They explained they would follow their father's practices back in those days (e.g. Farmers 4 and 5) and since then, as a "tradition" (Farmer 15) that was "deep routed in them" (Farmer 39), they have continued to apply the same practices:

> "I mean, **when I was a little boy**, sort of, well, as long as I can remember, we used to keep beef cattle and always, all summer, the practising of deworming cattle at grass **is something that, you know, (has) always been done**" (Farmer 29)

> "**Since father's been farming**, he's always taken that sort of side of things (deworming) very seriously and I suppose **we've just sort of carried it on and carry on with that same protocol**" (Farmer 5)

Farmers mentioned that part of a farmer's mentality and "old mind set" is to do what they always have done and that they are very good at it (Farmer 4). Farmers tended to translate helminth control into routine practice, which could simplify it. They would suggest that, because they could be a "bit lazy" (Farmer 37), helminth control needed to be as simple as possible in order to be implemented on the farm:

> "I guess, because **it's straightforward and it's easy to follow** and not to miss doses, because you do the groups at the certain times of year, and you don't miss treatment. **It's a lot easier to keep track** of because there are a lot of things, getting a lot of vaccinations and it's tricky to keep track of it all." (Farmer 38)

The nature of the infection (i.e. subclinical) would further support these routine-based processes. This way, the culture of farming and the nature of the disease seem to reinforce themselves in reproducing routine-based practices. For example, farmers' indicated that their practices regarding helminth control rarely relied on any "proof" (Farmer 26); although a problem would not be "pinned down" to worms, farmers would often treat animals against worms (Farmer 27). In fact, farmers would keep on applying what "seemed to work" (e.g. Farmers 26, 27, and 42), as illustrated in the quote below:

> "There was maybe one or two with breathing problems, which the vet put down to lungworm, but it was never, it was never actually clarified, that that was the problem… so I mean, like I said **I'm happy with Panacur bolus, it seems to, it seems to work, if it didn't work I'd be worried**." (Farmer 42)

In the context of the application of inherited practices, it is worth noting the importance of problem-solving as the driver for reassessing routine-based approaches. Farmers considered their helminth control practices as part of their farm routine that they ruled on their own terms and rarely challenged, unless they would identify any visible problems with their animals. Farmers would 'call for help' (i.e. advice from a veterinarian) only if they "had wormed animals and failed (to control helminths)" (Farmer 21). In the case farmers would experience a worm problem, the memory of it and its management would be then set in stone for years. Farmer 31 explained having had, twenty-five years ago, an important mortality rate in cows that had drunk the water from ditches. Since then, he systematically fenced all the ditches present on farm. Likewise, another farmer expressed how an experience made him change his earlier practices, adopting a new routine of systematic helminth control:

> "Well, I know what happens if you don't do it (deworming)… you get dead animals… **we've been there**, and yes, **I am quite religious about it now**… so everything gets done (treated)… We don't take any chances." (Farmer 27)

4.3. Optimisation of resources

Another key aspect of farmers' rationales behind helminth control is the overarching goal of optimising resources within the farming system. These include considerations on time, financial resources and farm capacity (i.e. staff, area, and infrastructure).

4.3.1. Time and farm capacity

Time is an important factor determining farmers' decision-making on cattle helminth control, both in relation to the application of the practice itself (i.e. implementing it or not) and its 'type' (e.g. frequency, form of treatment, and recording). The majority of farmers decided to implement a given helminth control practice on-farm because they considered as being "convenient and easier" (e.g. Farmers 9, 28, and 31) and therefore "quicker" (e.g. Farmers 3 and 31) than other alternatives. As explained by Farmer 4:

> "(…) a lot of people use the auto-dewormer (i.e. bolus) **because it is very convenient** and you can put it inside the heifer in the grazing season and turn

> them on to the same ground and forget it. (Even though) there is an expense to that…, **it is also easier**."

Another farmer indicated his preference for using anthelmintic drugs while avoiding what seemed for him to be more complicated alternatives for helminth control (in this case, rotational grazing): "you could keep moving them (cattle) to a fresh field, but it would be a lot more complicated and a lot more work, quite honestly" (Farmer 30). Relatively simple practices and some degree of automatism; for example, farmers often used markers, especially seasons and time of harvest/reseed, to plan and remind about helminth control. This would help farmers saving time while ensuring they complete their tasks, without having to think too much about them, as illustrated below:

> "I think once you've got it into a routine, and **you kind of stick to that routine**, **it just becomes part of the course really**. You don't really look at it as a sort of ... it's kind of another job but it's a part of the ... part of the whole package, I suppose." (Farmer 5)

In terms of optimising farm activities, they explained "always looking to incorporate" (Farmer 9), and "coincide" (Farmer 14) multiple farming management practices together, in order to save time and make processes easier. Here, many farmers (N=33; 79%) commented on the fact that their animals were treated against helminths at the same time as "TB (i.e. tuberculosis) testing" (Farmer 6), "service" (i.e. breeding) (Farmer 1) or other vaccination practices, as illustrated by the quote below:

> "An Arm and Hammer system… give them a dewormer and very often, at the same time, we might be doing a vaccine, as well for BVD, Lepto or IBR. They have one of those every three months so, **normally, you would do more than just one thing anyway**..." (Farmer 8)

This was predominantly motivated by a lack of staff (Farmers 11, 18, and 26) and the poor facilities that would make worm control difficult and time-consuming (Farmers 15 and 40):

> "The problem is with livestock farms these days is that they're so heavily stocked and lowly, **low numbers of staff**, **that you all run around trying to**

> **get everything done** and reminders of things that are gonna need doing" (Farmer 18)

4.3.2. Economic aspects

The majority of farmers (N=36; 86%) explained that the utmost reason for treating cattle against helminths was the fear they had of facing "bigger costs" if they were not controlling them (e.g. Farmer 15, 21, and 34). They mostly controlled the infection in heifers, given it would enable them to "produce more milk over their lifetime" (Farmer 3). Farmers generally supported the idea that controlling worm was "a cheap insurance" (Farmer 21) or an "investment" (Farmer 10) that guaranteed economic benefits and financial security. Some stressed the fact that although "they wished" to stop worming, "it was impossible not to treat" (Farmer 34). As illustrated by the quote below:

> "The only way we could lower it (drug use), is by taking risks… wouldn't we… because we would have to stop using one of the medicines that we use at the present time and we might regret that… mightn't we… so **it's a bit of an insurance policy** almost, isn't it?" (Farmer 13)

> "It seemed the easiest thing to do, again, **it is cost-benefit**… by the time you've seen two or three (sick animals), it is the tip of an iceberg… so **it's more cost effective for me to be preventative**. It might not be the best way of using drugs, but it is the best way of doing it, because once you start to get clinical signs, you know you've got a much bigger problem underneath." (Farmer 32)

Worm control was considered as a duty, a practice farmers "should be doing" (Farmer 26) to maintain their business. Overall, farmers admitted they did "not have any method to prove it", but liked to think their practices benefitted their business by preventing a loss in productivity (Farmer 15). Drugs ensured them that their animals were "kept healthy", which was "their (economic) interest" (Farmer 24). Although some farmers would indicate they noticed production improvements after treatment (e.g. milk yield and heifers calving at the right age), most of them admit there was no concrete evidence of such improvements (Farmer 38).

However, they would simply assume that this would help them being more economically efficient.

5. Discussion

5.1. Improving helminth control in cattle farming by bridging the gaps between experts and farmers knowledges

Over time, scientists have predominantly advocated the need for better communication of science to farmers in order to facilitate the uptake and implementation of expert guidelines in the farm and ensure disease prevention and control (Jansen et al., 2010b; Garforth et al., 2013; Toma et al., 2013; Ploeger et al., 2016). This assumption is based on the notion that farmers' practices should be exclusively determined by expert knowledge (as included in the guidelines) and that a reason to do so is because farmers' knowledge is either inadequate or lacking. Experts often see farmers as *technicians* and the farming community as a *ground* for applying what is considered as being 'best-practice' to improve farmers' businesses and the sustainability of livestock industry (McCown, 2001). This way, experts believe that providing farmers with more information will overcome their knowledge 'deficit', while promoting acceptance and adoption of their recommendations (Sturgis, 2004). Despite years of experts' attempts (considering that 2003 was the first year of publication of a guideline on livestock helminth control in the UK), such a strategy has not yet proved to produce any significant changes and/or improve farmers' helminth control practices in the UK (Heasman et al., 2012; Morgan et al., 2012). Drawing on the results of the current study, this section aim to understand the reasons behind this gap between theory and practice in order to advance the comprehension of what might be preventing the advancement of cattle helminth control in the UK.

5.1.1. Farmer tacit knowledge

As previously reported in the literature (Ohlmer et al., 1998; Sligo and Massey, 2007; Heffernan et al., 2008; Jansen et al., 2010b; Kaler and Green, 2013), the current study confirms that farmers actively seek for information on animal disease prevention and control. Farmers have access to a substantive amount of information (Sligo and Massey, 2007; Jansen et al.,

2010b). When searching for information on particular topics, including helminth infections, farmers use different types of public sources of information, such as the farming press and the internet, and highly rely on a network of informal knowledge gathering (i.e. speaking to veterinarians, as well as consulting with other fellow farmers and theirs peers). As suggested by the current study, farmers build their knowledge interacting with others, sharing both their experience and understanding, to revisit and produce new knowledge (Oreszczyn et al., 2010). They critically assess information in terms of its relevance to farm management and business sustainability. They look for specific scientific evidence and prioritise 'reliable data' to inform their practice. Through this active search for information and engagement with their social community, farmers shape their identities and local practices, and build their tacit and robust knowledge on cattle helminth infections (Oreszczyn et al., 2010). In the particular case of helminth infections, the literature confirms that farmers know how to recognize and differentiate different types of helminth infections (Ploeger et al., 2016). As observed in the current study, farmers' learning is enhanced by *mutual engagement* (i.e. constructive dialogues); *joint enterprise* where farmers and their community would work together to achieve a negotiated common goal (e.g. farmers discussion groups and farm staff) and a *shared repertoire* where actors are sharing a common history and culture built over time through interactions (e.g. members of the same family, organic farmers, and cattle farmers) (Oreszczyn et al., 2010). Farmers demonstrate to highly value their own expertise, which was often built through years of practice and tradition. Given that farmers are embedded in a relational network, it is worth noting that their adoption of experts' recommendations on cattle helminth control is unlikely to happen if most of the interaction between expert and farmer is based on a top-down, one-way flow of information (i.e. with a lack of consideration of farmers' views).

As mentioned above, the sense of identity gained from belonging to a community is determined by farmers' *mutual engagement*, *joint enterprise* and *shared repertoire* (Oreszczyn et al., 2010). In this study, the relational networks created by farmers to build knowledge with externals and make decisions on cattle helminth control, included or excluded people according to their technical competence and the impartiality of their advice. Such network of shared practice creates, over the years, informal boundaries between those belonging to the community and those who are not part of it (Oreszczyn et al., 2010). Although it is possible for different networks of practice to co-exist, their specific characteristics can make the flow of knowledge from one network to another rather challenging. This is especially the case when

considering different community professions with different technical languages and objectives. As shown in the current study, farmers could be sceptical of expert advice and question the underlying interests of authorities responsible for designing and implementing cattle helminth control 'best practices' (e.g. believing that they would be solely pursuing their interests in selling more drugs). Although farmers highly relied on their own judgment and that of their peers, they also often gathered information on cattle helminth control from veterinarians. Implementation of the advice provided by veterinarians could be: (1) imperative (i.e. farmers would always apply what veterinarians recommend); (2) advisory (i.e. farmers would make their own final decision without necessarily attaining to expert recommendations) and (3) participatory (i.e. farmers and veterinarians engage with each other and are co-responsible for the final decision). Whatever the case, farmers would always consider receiving veterinarians' advice, as previously reported in the literature (Gasbarre et al., 2001b; Garforth et al., 2013; Kaler and Green, 2013). In fact, farmers seem to establish a special relationship in terms of trust with veterinarians based on proximity and the status of the profession, which legitimates their advice. Farmers believed in their veterinarians and were receptive to their critique when they felt understood in the context of their farming.

In sum, farmers would distinguish two types of information while building their knowledge: (1) an 'internal' information coming from their own community (e.g. on farm and surrounding farms) that would be directly available, accessible and judged qualitatively through their own observation; and (2) an 'external' information coming from outside the boundaries of farmers' community (e.g. veterinarians, the media, and guidelines) that would require evidence and reliable data to be validated, given farmers' higher level of exigency in this case. To ensure the flow of knowledge between scientists and farmers, both communities need to engage with one another and understand their mutual differences. The tradition by which experts favour expert over lay knowledge (i.e. farmers' knowledge), taking the former as the legitimate and, ultimately, best form of knowledge to inform on-farm practice seems therefore misleading.

5.1.2. Farmer rationales

Farming practices are driven and influenced by contextual trends, which include particularities of the farm system, business and family dynamics (Garforth, 2015; Wilson et al., 2015). Time and financial resources are key determinants of farmers' attitudes towards cattle helminth control, as it is for other farm activities (Ohlmer et al., 1998; Kaler and Green, 2013; Visschers

et al., 2015). In this study, farmers generally grouped and coincided several farming activities in order to be more efficient (e.g. deworming at the time of TB testing and/or vaccination). Their decision making processes were reflexive and based on prioritisation (e.g. animal clinical signs and objectives of production), as well as management of often competing challenges on farm (e.g. effective use of time and money and compliance with the legislation). As frequently suggested in the literature (Vercruysse and Claerebout, 2001), cattle helminth infections were rarely part of farmers' priorities. Aligned with the findings of this study, although farmers reflect critically on their cattle helminth control practices (e.g. problem perception, ideas of options, plans and expectations) and adapt them against contextual factors, such as the weather, animals' clinical signs and reports of worm cases (e.g. Faecal egg counts and condemned carcases), they do not see worms as a problem when confronted with more concerning issues on farm, such as TB and mastitis. Moreover, farmers often believe that the recommendations included in the guidelines are mainly obsolete and not adapted to their farming. As reported in other sheep and cattle producer surveys (Gasbarre et al., 2001b; Ploeger et al., 2016), farmers are faced with challenges when managing sub-clinical parasite disease and trying to implement guidelines that are often inadequate to their farming system. Despite their intentions to do so, the lack of grazing surface (for pasture rotation), facilities (for treatment or for laboratory testing), time and financial resources work as a barriers to implement recommended 'best practices'. As a result, already embedded and easier 'solutions', such as routine anthelmintic treatments, tend to be the preferred choice for helminth control. Previous studies suggest that authorities (e.g. government, the industry, and veterinarians) fail to understand farmers' context and priorities, while requiring adoption of disease policies and interventions (Edwards-Jones, 2006; Charlier et al., 2015; Garforth, 2015). This tendency to overlook and even neglect farmers' epistemology and the contextual difficulties they face, undermines their trust on expert opinion and might, ultimately, compromise the credibility of guidelines.

5.2. Evolving paradigms in research and animal disease prevention and control: from guidelines to dialogues

The understanding of farmers' knowledges, attitudes and beliefs has been the objective of much research, particularly in the field of agriculture and the development and implementation of

technology (Tironi et al., 2013; Mudege et al., 2015). This has been mostly driven by stakeholders' interest (e.g. government, agencies, and the industry) in assessing farmers' practices and contextual factors that shape farming in order to guarantee their involvement and subsequent adoption of new technologies (Stirling, 2008).

In veterinary sciences, such participatory approach in the social appraisal of farming is relatively new and has mainly focused on animal disease prevention and control; more specifically, on farmers' acceptance of new animal health legislations and guidelines (e.g. vaccination, welfare, and biosecurity). Most of these studies have relied on quantitative surveys (i.e. close-ended questionnaires) and were based on socio-cognitive frameworks, of which the most prominent ones are the Health Belief Model (HBM), the Theory of Reasoned Action (TRA) and the Theory of Planned Behaviour (TPB) (Carr and Tait, 1991; Beedell and Rehman, 2000; Heffernan et al., 2008; Delgado et al., 2012; Sok et al., 2016). This literature suggests that one of the main reasons behind farmers' poor uptake of animal health and welfare recommendations is a lack of knowledge-transfer and poor communication (Heffernan et al., 2008; Delgado et al., 2012; Garforth et al., 2013; Toma et al., 2013). The assumption on which this argument builds is that stakeholders' knowledge, in particular scientist or expert knowledge, is necessarily 'good' and 'adequate', and that farmers' practices should be informed by it (Van de Ven and Johnson, 2006; Stirling, 2008). The extensive amount of research that has been conducted to explore farmers' knowledge, levels of trust in expert knowledge and the quality of communication with their veterinarians agree with such standpoint (Sligo and Massey, 2007; Jansen et al., 2010a; Jansen et al., 2010b; Easton et al., 2016).

Scientists' rationales and values shape the design of studies, which includes decisions on sampling and the methods used in social appraisal processes. Indeed, researchers' motivations and concerns can frame social appraisals in instrumental ways (Stirling, 2008). Here, approaches such as close-ended questionnaires and socio-cognitive frameworks as the ones indicated above, can limit the scope of appraisals by pre-defining the questions and variables to be assessed, while not letting room for these to be reframed (Ogden, 2003b; Sniehotta et al., 2014). In this sense, it has been suggested that socio-cognitive frameworks create and shape cognitions rather than allow *access* to cognitions (Ogden, 2003b).

This is the first qualitative study on cattle helminth infections that attempts at "opening up" social appraisal (Stirling, 2008) by providing an in-depth account of dairy farmers' views. Engagement with farmers was carried out by open qualitative interviews where farmers were given the opportunity to actively participate and shape the discussion. In contrast to past research on the subject, this study has therefore sought to prioritise farmers' voices and perspectives as much as possible. In any case, some of the limitations of the research include the use of a convenience sample in which farmers were clients of a laboratory, which could have a positive effect on their knowledge levels. However, it is important to note here that the size of the sample was considered as adequate for the qualitative study since it included a diversity of farmers' profiles (e.g. farm's size, type and system of production, and types of helminth control practices). In regards to assessing the quality of the data, it is worth noting that the criteria used for quantitative research such as reliability and validity, work differently in the case of qualitative research. Following the framework proposed by Mays and Pope (2000), it is believed that the current study attains to the relevant criteria used to assess quality in this kind of research, i.e. fair dealing by including the perspectives of a range of participants without any preferences, clear exposition of methods for data collection and analysis, attention to negative cases (i.e. identifying and giving consideration to outliers).

6. Conclusions

To date, this is the first qualitative study on cattle helminth infections that provides an in-depth account of dairy farmers' views on cattle helminth infections. The results suggest that farmers actively search and access a substantive amount of information on cattle helminth control. This is done through interaction with others (e.g. veterinarians and fellow farmers) and sharing both their experience and knowledge. Farmers therefore constantly revisit and produce new knowledges. Such a process of knowledge building allow farmers to shape their identities, local practices and build their tacit and comprehensive knowledge on cattle helminth infections. Moreover, farmers critically assess information in terms of its relevance to farm management and business sustainability. Despite their intentions to better control helminths in the farm, they face challenges when managing sub-clinical parasite disease and have difficulties in adopting guidelines that usually prove to be inadequate to their farming system.

To ensure and foster understanding between experts and farmers, both communities need to engage with one another in more constructive ways. The tendency of experts to overlook and even neglect farmers' epistemology and the contextual difficulties they face, undermines their trust on expert opinion and, ultimately, compromises the credibility of guidelines on cattle helminth infections. It is only through constructive dialogues between experts, as those responsible for developing guidelines, and farmers, that opportunities will be created for these actors to move forward on the governance of cattle helminth control. Guidelines for best practice must be informed by farmers' perspectives and take into account their contextual challenges if they are expected to be adopted.

Part II. Beef study

Chapter 7.

Ostertagia spp., rumen fluke and liver fluke single and poly-infections in cattle: An abattoir study of prevalence and production impacts in England and Wales[1]

1. Introduction

Recent projections of the world population growth have emphasised the urgent need to increase worldwide food production, in particular, annual meat production (FAO, 2009) while reducing environmental impacts and maintaining high levels of animal health and welfare. In the UK, parameters such as increased growth rate, higher carcase weight and low-cost grazing systems are key determinants of increased production since animal numbers are expected to decline (Thornton, 2010). In this context, production-limiting diseases such as helminth infections are of major concern. In temperate areas, helminth infections in grazing livestock are not only an important cause of reduced productivity but also a driver of poor welfare and greenhouse gas' emissions (Sargison, 2014). Evidence of increase in prevalence and spread of endemic helminths have already been reported in the UK (Skuce and Zadoks, 2013; Sargison, 2014).

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Part II. Chapter 7.

Helminth infections are seasonal, ubiquitous on livestock farms and responsible for major impacts on both animal production and reproduction performances (Charlier et al., 2014). In the UK, beef cattle are particularly susceptible to helminths since their rearing is predominantly pasture-based (AHDB, 2009; Sargison, 2014). To date, however, research is scant on the epidemiology and the impact of helminth infections in beef cattle (Charlier et al., 2009). In fact, in England and Wales especially, there has been no published abattoir survey on cattle helminth infections since the eighties (Froyd, 1975; Bairden and Armour, 1981).

In temperate areas such as the UK, two of the most economically important helminth parasites affecting cattle are the nematode, *O. ostertagi*, and the trematode , *F. hepatica* (Charlier et al., 2014). The recent increasing number of rumen fluke cases that have been reported in cattle in Western Europe also raised concerns about the potential production impact this parasite could have. However, data on rumen fluke remain scarce, the reports on its impacts on cattle production are mainly inconclusive (Sargison et al., 2016) and information in terms of prevalence in England and Wales is absent (Gordon et al., 2013).

Although several diagnostic tools have been developed to detect host exposure to helminths, current methods often have poor specificity (e.g. antibody ELISA and FEC) and a lack of correlation over time with the actual parasite burden in the host (Charlier et al., 2014). Moreover, current methods often cannot discriminate between different levels of infections' severity and profiles (Chapter 1., section 3.). To date, very few studies have been published on cattle helminth poly-infections and none have investigated the impact of such poly-parasitism on cattle production, especially for infections due to *Ostertagia spp.*, *F. hepatica* and rumen fluke (Murphy et al., 2006). In this context, since specific post-mortem examinations is considered as the 'gold standard' for assessing prevalence and pathology in infected animals (Rapsch et al., 2006; Larraillet et al., 2012; Sanchez-Vazquez and Lewis, 2013; Toolan et al., 2015), such an approach could aid in widening and refining our current knowledge on cattle helminth infections.

Therefore the current chapter aims to (1) estimate the prevalence and severity of *Ostertagia spp.*, *F. hepatica* and rumen fluke single and poly-infections in cattle at slaughter in England and Wales; (2) identify demographics and environmental factors associated with different profiles and severities of infections due to these three helminths infections; and (3) explore the associations between these infections and carcase performance in prime beef cattle.

2. Materials and Methods

None of the scoring data described below were collected by the PhD candidate. The candidate's role was mostly limited to the building of the different databases, the data analysis and the results' interpretation.

2.1. Sample collection and viscera scoring

Abomasa, reticulorumens and livers from commercial cattle were collected and examined post-mortem quarterly over a twelve-month period between March 2014 and January 2015 in an abattoir slaughtering up to 1,500 cattle per week in the South-West of England. On each four visits at slaughter (i.e. March 2014, June 2014, October 2014 and January 2015), specific viscera from all cattle were inspected on the slaughter line. Livers were examined on-line with the meat inspectors at the abattoir. The liver was examined and scored for the presence of typical cholangiohepatitis lesions ('pipe stem' appearance) (chapter 1. section 2.2.2.) and its surface incised as deemed appropriate to detect the presence of liver fluke. Reticulorumens and abomasa were examined in the 'gut room', where they were excised and the contents expelled. The internal surfaces of the reticulorumen were visually assessed for the presence of adult rumen fluke and, if present, for their numbers. The abomasum was dissected from the omasum, everted and rinsed to expose the mucosal surface and estimate the number of typical lesions due to *Ostertagia spp.* on the fundus and pylorus of each abomasum.

Abomasum gross lesions were classified into four categories (scores 0-3) based on the number of gastric gland lesions characteristic of *Ostertagia spp.* (chapter 1. section 2.1.2.) (Larraillet et al., 2012): 0- no lesions; 1- less than 100 lesions; 2- between 100 and 1,000 lesions; and 3- more than 1,000 lesions. Each reticulum and rumen were thoroughly examined and classified on a numerical scale according to the number of adult rumen fluke (scores 0-3): 0- no fluke; 1- between 1 and 10; 2- between 11 and 100; 3- between 101 and 200; and 4- more than 200 fluke. The presence of liver fluke, i.e. 0- no fluke (neither fluke nor liver fluke lesions); 1- actual presence (presence of fluke and liver fluke lesions); and 3- historical presence (no fluke but presence of liver fluke lesions), as well as the lesions due to liver fluke, i.e. 0- no lesions; 1- moderate lesions; and 2- severe lesions, were scored based on gross-pathological scales previously used in other studies (Sanchez-Vazquez and Lewis, 2013). The scoring of gross

lesions was conducted by the same group of operators at each visit, who were blinded to the identity of the animal or farm.

Before the commencement of the study, the scoring system was pilot-tested in the same abattoir as a feasibility check. At the same time, a sample of adult rumen fluke specimens was collected from two animals and preserved in 70% methanol and was sent for speciation (Moredun Research Institute, UK), applying PCR amplification and DNA sequencing of the ITS-2 region using generic primers (Rinaldi et al., 2005) with subsequent sequencing of purified PCR amplicons (Gordon et al., 2013).

2.2. Animal demographics and carcase parameters

Data from the abattoir information management system were used to provide additional information on each animal, using the kill number as the unique identifier. The following demographics information was extracted: date of birth; date of slaughter; farm; breed; sex (i.e. male and female); category (i.e. mature bull, cow, heifer, steer, and young bull); cold carcase weight (CCW) (kg); carcase conformation; fat classification; and liver condemnations (i.e. yes and no). No additional information on the history of the animals in relation to previous grazing, housing and anthelmintic treatments was available. To determine the geographic origin of the farm the animals were submitted from, the postcodes of each farm were used and related latitude, longitude and altitude extracted from 'Google Maps' (Map data ©2016 Google). The breed information was classified in four categories: dairy purebred, dairy crossbred, beef purebred and beef crossbred, using the information provided on the passport and DEFRA (Department for Environment, Food and Rural Affairs) breed classification list (DEFRA, 2014). The age of the animal at slaughter was calculated in months and defined as the time between the date of birth and the kill date. Carcase conformation and fat classifications were evaluated referring to the EUROP scale (Pritchard et al., 2013), where conformation classes range from E=Excellent to P=Poor with P, O, and U further sub-divided into – and +; and fat, from 1 (very lean) to 5 (very fat) with grades 4 and 5 sub-divided into L (leaner) and H (fatter).

2.3. Statistical analysis

Data were coded, checked and entered into a database (Microsoft Excel 2010). A preliminary descriptive analysis was conducted using STATA 12.1 (STATA Inc., Texas, USA) to summarise the data. Sample carcase traits were compared to the ones reported by Pritchard et al. (2013) for the British beef and dairy cattle. Then, three sets of analysis were conducted, as described below:

2.3.1. Prevalence and severity of helminth infections

Descriptive statistics were conducted to summarise the prevalence of *Ostertagia spp.*, adult rumen fluke and liver fluke infections at farm and cattle levels. This was based, respectively, on the presence in the carcases of abomasal lesions, adult rumen fluke and both lesions and parasite for liver fluke. For each helminth, the carcases were summarised based on the severity scores of the helminths, the season and the category of animal. Where scores were available for all the three helminths, the percentage of co-infected animals was calculated.

2.3.2. Factors associated with the presence and severity of helminth infections

Three multinomial logistic regression models were built (i.e. one for each helminth) to investigate the relationship between the carcase severity scores for helminths and other collected variables (Dohoo et al., 2009). Since several carcases originated from the same herd, observations could not be considered independent. Therefore, the three models incorporated two hierarchical levels: level 1 (i), the cattle level, level 2 (j) the farm level. The outcome variables were (1) for model 1, the scores of *Ostertagia spp.* lesions, i.e. 0- no lesions; 1- less than 100 lesions; 2- between 100 and 1,000 lesions; and 3- more than 1,000 lesions; (2) for model 2, the scores of adult rumen fluke, i.e. 0- no fluke; 1- between 1 and 100 fluke; and 2- more than 100 fluke; and (3) for model 3, the scores of liver fluke lesions, i.e. 0- no lesions; 1- moderate lesions; and 2- severe lesions. For all three models, the reference category for the outcome was score 0 and the predictor variables were: the breed; category; age; month of sampling; altitude and presence of co-infections.

The models were built using a stepwise approach, combining both forward selection and backward elimination of demographic and scoring variables. The evaluation of the effects of significant factors on the three outcomes was based on Wald tests. A P-value$\leq$0.05 was

considered significant. Confounding variables were retained in the final model. Any interactions between variables were tested. The multilevel multinomial models 1, 2 and 3 used a logit link function to express the ratio probability of a given helminth score to the probability of the reference score, as shown in equation (1) (Rasbash et al., 2012):

$$log\left(\frac{\pi_{ij}^{(s)}}{\pi_{ij}^{(0)}}\right) = \beta_0^{(s)} + \beta_1^{(s)} x_{ij} + \beta_2^{(s)} x_j + u_{0j}^{(s)} \qquad (1)$$

Where: $\pi_{ij}^{(s)}$ was the probability of the *i*th carcase of the *j*th herd to have a score 's' (for model 1, 's' =1, 2, 3; for model 2 and 3, 's' =1, 2) compared to the score 0; $\beta_0^{(s)}$ was the score-specific intercept of the model; $\beta_1^{(s)}$ and $\beta_2^{(s)}$ represented the cattle level and farm level vectors of coefficients; x_{ij} and x_j were the cattle level and farm level vectors of predictor variables and $u_{0j}^{(s)}$ was the farm level random effect, assumed to be normally distributed. All statistical analyses were performed using MLwiN v2.30. All the calculations were based on a Reweighted Iterative Generalized Least Squares (RIGLS) procedure and a second-order approximation by penalized quasi-likelihood (Rasbash et al., 2012).

2.3.3. Prime beef carcase traits associated with the presence and severity of helminth infections

The impact of helminth past/current infections on prime beef carcase performance was estimated using three multilevel linear regression models with the following outcomes: (1) the Cold Carcase Weight (CCW); (2) the carcase conformation; and (3) the carcase fat classification. Since several carcases originated from the same herd, the models had carcases nested within herds. Only steers, heifers and young bulls from 12 to less than 36 months were included in this analysis, as these represented the population of cattle reared for prime beef in the UK (AHDB, 2009). Both conformation and fat classifications were converted into a 15-numerical scale with (1) the conformation numerical scale ranging from 42 (i.e. E=Excellent) to 6 (i.e. P=Poor); and (2) the fat numerical scale ranging from 6 (i.e. 1=very lean) to 42 (i.e. 5=very fat) (Pritchard et al., 2013). The predictor variables for the three multilevel models were: the breed; category; age; carcase parameters; month; altitude and an eight-level categorical variable for presence of co-infections (i.e. no helminths; lesions due to *Ostertagia spp.* only; presence of adult rumen fluke only; lesions due to liver fluke only; lesions due to *Ostertagia spp.* and presence of adult rumen fluke; lesions due to *Ostertagia spp.* and liver

fluke; presence of adult rumen fluke and lesions due to liver fluke; lesions of *Ostertagia spp.*, presence of adult rumen fluke and lesions due to liver fluke).

The models were built using a stepwise approach. The evaluation of the effects of significant factors on the three outcomes was based on Wald tests. A P-value≤0.05 was considered significant. Confounding variables were retained in the final model and interactions between variables were tested. Models were developed using a Reweighted Generalised Iterative Least Squares (RIGLS) algorithm in MLwiN 2.30 (Rasbash et al., 2012). The models took the form of equation (2) (Rasbash et al., 2012):

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j \, u_{0j} + e_{ij} \qquad (2)$$

Where: y_{ij} was the outcome (i.e. CCW, carcase conformation, or carcase fat classification) of the *i*th carcase from the *j*th herd; β_0 was the intercept; β_1 and β_2 represented the cattle level and farm level vectors of coefficients; x_{ij} and x_j were the cattle level and farm level vectors of predictor variables; u_{0j} was the farm level random effect and e_{ij} was the bottom level residual (cattle), both assumed to be normally distributed.

The model goodness-of-fit was assessed at each hierarchical level by the examination of the normal probability and the leverage plots of residuals (Dohoo et al., 2009; Rasbash et al., 2012).

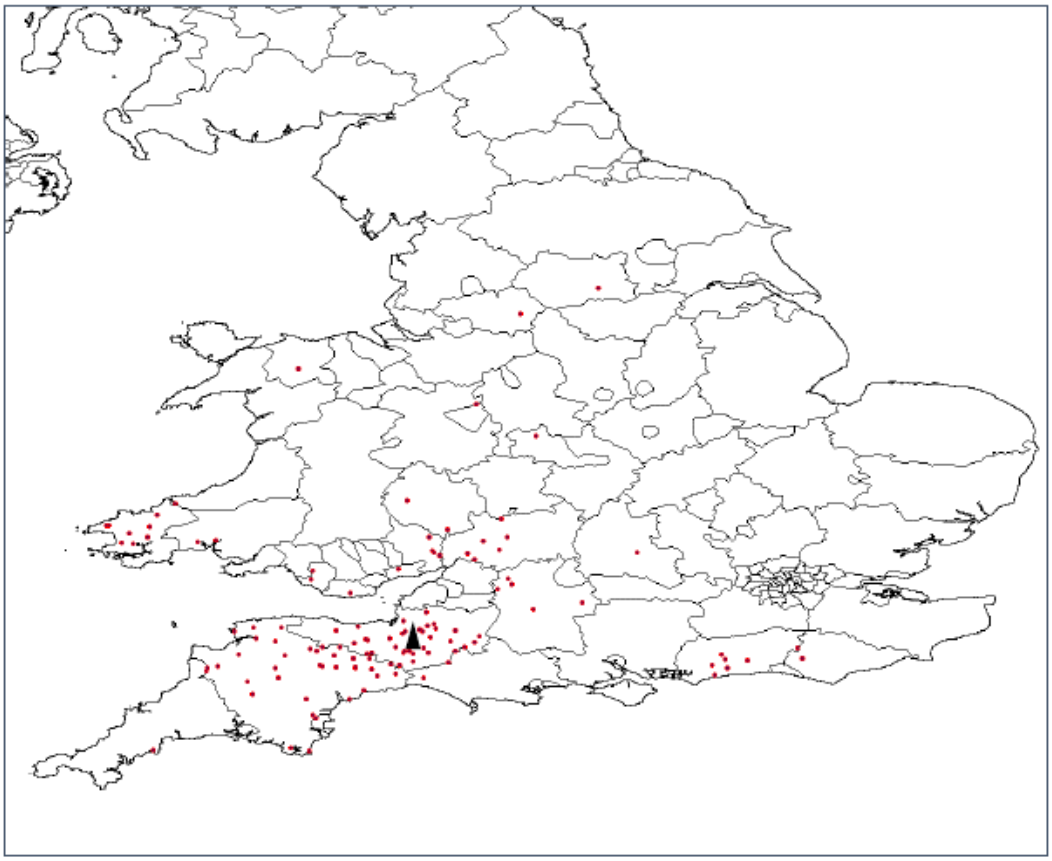
3. Results

3.1. Description of animal and carcase parameters

3.1.1. Carcase traits

A total of 974 carcases were sampled from March 2014 to January 2015: 298 (31%) in March, 233 (24%) in June, 230 (24%) in October and 213 (22%) in January. The carcases originated from 156 UK farms, localised in 23 counties. A total of 134 (86%) farms could be geo-localised, of which 82% (N=110) were from England and 18% (N=24) from Wales (Figure 7-1).

Figure 7-1: Locations of 86% of the beef producers enrolled in the abattoir cross-sectional study (N=134)



***Key: Dot, beef producers in the sample; Triangle, abattoir**

The median (p25-p75) number of carcases per farm was 4 (2-8). The four predominant cattle breeds were Holstein Friesian (28% (N=272)), Limousin cross (12% (N=118)), British Blue cross (11% (N=108)) and Charolais cross (9% (N=87)). Fifty percent (N=484) of the carcases were from beef crossbreds, 36% (N=353) from dairy purebreds, 9% (N=83) from beef purebreds and 4% (N=42) from dairy crossbreds; the rest (N=12) belonging to either dual-purpose or other breeds. The sample (N=974) included 64% males and 36% females, of which 53% (N=518) were steers, 20% (N=193) cows, 16% (N=155) heifers, 11% (N=106) young bulls and less than 1% (N=2) mature bulls. Considering the number of mature bull included in the sample (<1%), these were excluded from the subsequent analyses. Table 7-1 presents, by cattle category, the median (p25-p75) age, CCW, conformation, fat classification, and percentage of liver condemnations of the carcases included in the study.

Table 7-1: Median (p25-p75) age, cold carcase weight (CCW), conformation and fat classifications, and percentage of liver condemnations by category of cattle included in the abattoir cross-sectional study (N=972; mature bull (N=2) excluded)

Variables (N)	Cows (193)	Heifers (155)	Steers (518)	Young Bulls (106)
Age (m)	79 (56-113)	29 (26-31)	29 (26-31)	14 (14-15)
CCW (kg)	323 (283-346)	314 (290-334)	344 (307-384)	294 (267-334)
Conformation	P^+ (P^+-O^+)	R (O^+-R)	O^+ (O^+-R)	O^+ (O^+-R)
Fat classification	3 (2-4L)	4L (3-4L)	3 (3-4L)	2 (2-3)
Liver condemnation (%)	31.6	12.9	14.1	9.4

3.1.2. Comparison of the sample with available information on the characteristics of beef and dairy cattle carcase traits in England and Wales

This section aimed to see whether the sample population included in this cross-sectional study were representative of British carcases, taking the work of Pritchard et al., 2013 as a reference. A total of 52 breeds were identified in the study. The top-five breed codes in the current study were Holstein Friesian (HF), Limousin cross (LIMX), British Blue cross (BBX), Charolais cross (CHX) and British Friesian (BF), of which three were similar to that reported by Pritchard et al. (2013) (i.e. LIMX, CHX and HF). The two other codes identified in Pritchard et al. (2013) study were Aberdeen Angus cross (AAX) and Limousin (LIM), which were both within the top-ten breed codes of the current study. Like for Pritchard et al. (2013), the study data: (1) were obtained from younger (i.e. animals mostly reared for beef production) and mature animals (i.e. cows and mature bulls); (2) had a larger proportion of males than females under 48 months of age (as more females are kept for reproduction on-farm); and (3) the average weight of male carcases was higher than female carcases slaughtered at the same period of the year (in the current study, a significant difference was observed, i.e. with p-value<0.001). Taking only into account animals from 12 to 36 months (i.e. prime beef), the mean (SE) net carcase weight and classifications of conformation and fat (conversion into numerical scale) for both sexes were: (1) for the current study: 334 (55) kg, 19 (6) and 26 (6); and (2) for Pritchard et al. (2013): 324 (51), 21 (6) and 28 (6).

3.2. Description of carcase presence and/or lesions of helminths

3.2.1. Prevalence and severity of helminth infections as defined by scores

Adult rumen fluke specimens isolated from the two carcases sampled in the pilot study were identified as *Calicophoron daubneyi.*

Out of 972 carcases (i.e. mature bulls excluded), a total of 933 abomasa, 936 reticulorumens and 951 livers were scored for lesions due to *Ostertagia spp.*, presence of adult rumen fluke and lesions due to liver fluke, respectively; the others being either condemned or lost. There was a large variation in the prevalence of helminth infections with: (1) at cattle-level, 89% (828/933), 25% (231/936) and 29% (272/951) of the carcases; and (2) at farm level, 97% (149/154), 48% (73/153) and 64% (98/152) of the producers with at least one carcase with signs of ostertagiasis, adult rumen fluke and lesions due to liver fluke, respectively. The distribution by cattle category of the severity scores for the three helminth infections is presented in Table 7-2.

Table 7-2: Distribution of the three helminth severity scores by category of cattle included in the abattoir cross-sectional study (N=972; mature bull (N=2) excluded)

	Cows (%)	Heifers (%)	Steers (%)	Young Bulls (%)	TOTAL (%)
Lesions due to *Ostertagia spp.* (N=933)					
0- No lesion	16 (9)	12 (8)	65 (13)	12 (12)	105 (11)
1- ≤100	48 (28)	36 (25)	136 (26)	33 (32)	253 (27)
2- 101-1,000	43 (25)	34 (23)	126 (25)	37 (36)	240 (26)
3- >1,000	65 (38)	64 (44)	186 (36)	20 (20)	335 (36)
Presence of adult rumen fluke (N=936)					
0- No fluke	135 (77)	112 (76)	361 (70)	97 (95)	705 (75)
1- ≤100	17 (10)	23 (16)	75 (15)	4 (4)	119 (13)
2- >100	23 (13)	12 (8)	76 (15)	1 (1)	112 (12)
Lesions due to liver fluke (N=951)					
0- No lesion	94 (51)	116 (75)	367 (72)	102 (98)	679 (72)
1- Moderate	62 (34)	32 (21)	128 (25)	1 (1)	223 (23)
2- Severe	28 (15)	6 (4)	14 (3)	1 (1)	49 (5)
Presence of liver fluke (N=950)					
0- No fluke	115 (63)	119 (77)	380 (75)	103 (99)	717 (76)
1- Actual presence	22 (12)	13 (9)	82 (16)	1 (1)	118 (12)
2- Historical presence	47 (25)	22 (14)	46 (9)	0 (0)	115 (12)

Forty per cent (40%) of the abomasa with lesions of ostertagiasis had more than 1,000 lesions (score 3). There was a similar percentage of carcases with less (score 1; 51%) and more (score 2; 49%) than 100 adult rumen fluke. Liver fluke was identified in approximately 86% of the livers with lesions due to liver fluke.

A seasonal variation was present for the prevalence of helminths in carcases, with the highest prevalence of lesions due to *Ostertagia spp.* observed in January (98%), compared with 84% in March, 85% in June and 89% in October. A similar pattern was observed for the lesions due to liver fluke and adult rumen fluke with the lowest relative prevalence in March (22% and 17% respectively) and highest prevalence in January (34% and 28% respectively) and October (33% and 31% respectively). The prevalence of liver fluke and adult rumen fluke infections in June was 28% and 25% respectively.

3.2.2. Presence of co-infections

Out of the 972 carcases, 909 (94%) had a score available for all three helminths. Of these (N=909), 92% (N=837) had at least the signs of one helminth infection. A total of 39% (N=351) of the animals had co-infection, of which 15% (N=138) with lesions due to *Ostertagia spp.* and adult rumen fluke, 12% (N=111) with lesions due to *Ostertagia spp.* and liver fluke, 11% (N=97) with signs of all three helminths and 1% (N=5) with only adult rumen fluke and lesions due to liver fluke. The presence of adult rumen fluke and lesions due to liver fluke were mainly concurrent with other infections, with only 3% (6/219) and 6 % (15/255) of infected animals having single-infection with adult rumen fluke and liver fluke respectively, compared to 57% (465/811) with only *Ostertagia spp.* Out of 219 animals (24%) infected with adult rumen fluke, 47% (N=102) also had signs of liver fluke infection. The prevalence of co-infected animals was highest in October with 50% (104/206) of the carcases presenting signs of at least two parasites, compared with 44% (83/189) in January, 35% (81/229) in June and 29% (83/285) in March. The highest prevalence of co-infections was observed in cows with 51% (83/162) of the carcases infected with at least two helminths, compared with 42% (210/502) for steers, 35% (51/145) for heifers and 7% (7/100) for young bulls.

3.3. Demographics and environmental factors associated with the severity of cattle helminth infections

The number of observations for predictor variables per model is presented in Table 7-3. The three final multilevel multinomial models are presented in Table 7-4.

Table 7-3: Cattle and farm level variables included in the final multilevel multinomial models with the severity scores of *Ostertagia spp.*, rumen fluke and liver fluke infections as the outcomes

		Model 1: lesions due to *Ostertagia spp.* (N=933)				Model 2 : presence of adult rumen fluke (N=936)			Model 3 : lesions due to liver fluke (N=951)		
		None	<100	100-1,000	>1,000	None	≤ 100	>100	None	Moderate	Severe
Variables	**Categories**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Breed	**Pure dairy**	30 (29)	76 (30)	97 (41)	135 (40)	263 (37)	38 (32)	41 (37)	255 (38)	64 (29)	20 (41)
	Pure beef	9 (9)	22 (9)	21 (9)	26 (8)	54 (8)	14 (12)	10 (9)	55 (8)	25 (11)	3 (6)
	Cross dairy	65 (62)	145 (58)	106 (44)	152 (46)	354 (51)	59 (50)	54 (49)	331 (49)	123 (56)	24 (49)
	Cross beef	0 (0)	8 (3)	14 (6)	19 (6)	29 (4)	7 (6)	5 (5)	30 (5)	9 (4)	2 (4)
Category[(a)]	**Cow**	16 (15)	48 (19)	43 (18)	65 (19)	135 (19)	17 (14)	23 (20)	94 (14)	62 (28)	28 (57)
	Heifer	12 (11)	36 (14)	34 (14)	64 (19)	112 (16)	23 (19)	12 (11)	116 (17)	32 (14)	6 (12)
	Steer	65 (62)	136 (54)	126 (52)	186 (55)	361 (51)	75 (63)	76 (68)	367 (54)	128 (57)	14 (29)
	Young Bull	12 (11)	33 (13)	37 (15)	20 (6)	97 (14)	4 (3)	1 (1)	102 (15)	1 (1)	1 (2)
Age (Month)	**<24**	29 (28)	53(21)	59 (25)	41 (12)	165 (23)	13 (11)	4 (1)	166 (25)	17 (8)	2 (4)
	24-30	46 (44)	91 (36)	82 (34)	137 (41)	264 (38)	58 (48)	34 (11)	273 (40)	79 (35)	7 (14)
	>30	30 (28)	110 (43)	99 (41)	158 (47)	277 (39)	49 (41)	274 (88)	240 (35)	129 (57)	40 (82)
Month	**March**	46 (44)	62 (25)	72 (30)	111 (33)	238 (34)	35 (29)	16 (14)	232 (34)	57 (25)	8 (16)
	June	33 (31)	74 (29)	49 (20)	74 (22)	173 (24)	31 (26)	27 (24)	166 (24)	58 (26)	8 (16)
	January	23 (22)	72 (28)	60 (25)	59 (18)	148 (21)	26 (22)	40 (36)	148 (22)	53 (24)	21 (43)
	October	3 (3)	46 (18)	59 (25)	92 (27)	147 (21)	28 (23)	29 (26)	133 (20)	57 (25)	12 (25)
Altitude (m)	**≤60**	-	-	-	-	194 (31)	49 (45)	43 (48)	187 (30)	79 (43)	18 (46)
	>60	-	-	-	-	438 (69)	60 (55)	46 (52)	432 (70)	104 (57)	21 (54)
O[(a)]	**None**	-	-	-	-	89 (13)	5 (4)	6 (5)	79 (12)	23 (11)	1 (2)
	Present	-	-	-	-	613 (87)	111 (96)	106 (95)	576 (88)	193 (89)	44 (98)
RF[(a)]	**None**	89 (89)	180 (71)	185 (77)	248 (74)	-	-	-	540 (82)	128(60)	27 (59)
	Present	11 (11)	74 (39)	55 (33)	88 (26)	-	-	-	120 (18)	85 (40)	19 (41)
LF[(a)]	**None**	79 (77)	170 (69)	158 (68)	248 (75)	540(78)	73 (62)	47 (44)	-	-	-
	Present	24 (23)	77 (31)	76 (32)	84 (25)	155 (22)	44 (38)	60 (56)	-	-	-

[(a)]Mature bull excluded; O=lesions due to *Ostertagia spp.*; RF=presence of adult rumen fluke; LF=presence of liver fluke

Table 7-4: Final multilevel multinomial models of association between the severity scores of *Ostertagia spp.*, rumen fluke and liver fluke infections and demographic and concurrent helminth infection variables as fixed effect

Variables	Categories	Model 1: lesions due to *Ostertagia spp.* (154 Herds, 933 cattle, 2,697 Obs.)						Model 2: presence of adult rumen fluke (153 Herds, 936 cattle, 1,584 Obs.)				Model 3: lesions due to liver fluke (153 Herds, 951 cows, 1,584 Obs.)			
		<100		100-1,000		>1,000		≤ 100		>100		Moderate		Severe	
		O.R[a]	95% C.I[a]	O.R	95% C.I	O.R	95% C.I	O.R	95% C.I	O.R	95% C.I	O.R	95% C.I	O.R	95% C.I
Breed	**Pure dairy**	*Baseline*						*Baseline*				*Baseline*			
	Pure beef	1.40	0.86;2.27	0.79	0.46;1.34	0.69	0.44;1.09	1.87	0.87;4.00	1.73	0.69;4.35	1.99	1.00;3.96	0.92	0.20;4.32
	Cross dairy	1.14	0.83;1.55	0.49*	0.35;0.69	0.45*	0.34;0.61	0.91	0.53;1.56	1.13	0.64;2.02	2.30*	1.46;3.64	3.18*	1.42;7.11
	Cross beef	7.29*	4.48;11.88	8.63*	5.16;14.42	6.20*	3.92;9.78	2.03	0.80;5.11	1.01	0.29;3.51	1.03	0.36;2.96	0.79	0.09;7.33
Category[b]	**Cow**	*Baseline*						*Baseline*				*Baseline*			
	Heifer	2.16*	1.35;3.45	4.34*	2.52;7.46	7.11*	4.38;11.53	2.55*	1.07;6.12	2.15	0.81;5.70	0.43*	0.21;0.86	0.08*	0.01;0.41
	Steer	1.24	0.84;1.85	2.06*	1.32;3.20	2.54*	1.72;3.75	2.51*	1.20;5.28	3.95*	1.91;8.18	0.64	0.38;1.10	0.13*	0.05;0.33
	Young Bull	2.08	0.99- 4.37	3.15*	1.48;6.66	2.01	0.95;4.22	0.92	0.21;3.93	1.21	0.11;13.94	0.04*	0.01;0.38	0.14	0.01;2.80
Age (m)	**<24**	*Baseline*						*Baseline*				*Baseline*			
	24-30	1.59	0.93;2.71	1.60	0.95;2.70	2.82*	1.70;4.67	1.50	0.68;3.31	3.08	0.88;10.72	1.07	0.54;2.10	0.66	0.06- 6.78
	>30	2.72*	1.56;4.75	2.27*	1.31;3.94	4.40*	2.59;7.46	1.35	0.57;3.16	5.48*	1.56;19.21	1.87	0.92;3.80	3.75	0.43- 32.94
Month	**March**	0.08*	0.05;0.12	0.07*	0.04;0.10	0.06*	0.04;0.08	*Baseline*				*Baseline*			
	June	0.11*	0.07;0.16	0.05*	0.03;0.08	0.04*	0.03;0.05	1.24	0.69;2.23	2.32*	1.13;4.75	1.09	0.66;1.82	1.25	0.36;4.34
	January	*Baseline*						1.11	0.58;2.12	2.82*	1.34;5.92	1.75*	1.04;2.94	3.20*	1.16;8.86
	October	0.20*	0.13;0.29	0.09*	0.06;0.14	0.06*	0.04;0.09	2.01*	1.08;3.72	4.45*	2.12;9.38	2.06*	1.21;3.50	1.81	0.63;5.17
Altitude (m)	**≤60**	-	-	-	-	-	-	*Baseline*				*Baseline*			
	>60	-	-	-	-	-	-	0.58*	0.36;0.92	0.44*	0.26;0.72	0.56*	0.38;0.82	0.63	0.29;1.33
O[b]	**None**	-	-	-	-	-	-	*Baseline*				*Baseline*			
	Present	-	-	-	-	-	-	2.40	0.93;6.18	1.51	0.58;43.94	0.90	0.49;1.65	3.42	0.41;28.22
RF[b]	**None**	*Baseline*						-	-	-	-	*Baseline*			

	Present	3.01*	2.27;4.00	1.92*	1.38;2.67	2.27*	1.70;3.03	-	-	-	-	2.71*	1.83;4.02	4.08*	1.95;8.50
LF[(b)]	**None**	*Baseline*						*Baseline*				-	-	-	-
	Present	1.06	0.80;1.41	1.57*	1.13;2.19	0.92	0.68;1.25	1.79*	1.08;2.96	3.21*	1.93;5.34	-	-	-	-

[(a)]CI=confidence interval; SE=standard error; O.R=odds ratio; *=significant (P-value≤0.05)

[(b)]Mature bull excluded; O=lesions due to *Ostertagia spp.*; RF=presence of adult rumen fluke; LF=presence of liver fluke;

3.3.1. Model 1 (abomasal lesions due to Ostertagia spp.)

Compared to dairy purebreds, dairy crossbreds were significantly more likely to have *Ostertagia spp.* lesions of all severities [OR (95% C.I.): 7.29 (4.48-11.88); 8.63 (5.16-14.42); 6.20 (3.92-9.78)]. Whereas beef crossbreds were significantly less likely to have *Ostertagia spp.* lesions of higher severity (≥100 lesions) [OR (95% C.I.): 0.49 (0.35-0.69); 0.45 (0.34-0.61)].

Compared to cows, heifers were significantly more likely to have lesions due to *Ostertagia spp.* of all severities [OR (95% C.I.): 2.16 (1.35-3.45); 4.34 (2.52-7.46); 7.11 (4.38-11.53)], steers were more likely to have more than 100 lesions [OR (95% C.I.): 2.06 (1.32-3.20); 2.54 (1.72-3.75)] and young bull between 100-1,000 lesions [OR (95% C.I.): 3.15 (1.48-6.66)].

There was a significant effect of age; compared to animals slaughtered younger than 24 months of age, animals slaughtered older than 30 months were at significantly higher risk of having lesions due to *Ostertagia spp.* of all severities [OR (95% C.I.): 2.72 (1.56-4.75); 2.27 (1.31-3.94); 4.40 (2.59-7.46)] and animals slaughtered between 24-30 months more likely to have more than 1,000 lesions [OR (95% C.I.): 2.82 (1.70-4.67)].

Compared to January, there were significantly reduced numbers of lesions due to *Ostertagia spp.* of all severities in March (OR: 0.06-0.08), June (OR: 0.04-0.11) and October (OR: 0.06-0.20).

The presence of adult rumen fluke was significantly associated with all severities (OR: 1.92-3.01) of lesions due to *Ostertagia spp.* There was no significant association between the presence of lesions due to *Ostertagia spp.* and the presence of liver fluke.

3.3.2. Model 2 (presence of adult rumen fluke)

There was no significant association between the presence of adult rumen fluke and the type of breeds.

Compared to cows, steers were significantly more likely to have adult rumen fluke infestation of all severities (OR: 2.51-3.95) and heifers more likely to have 1 to 100 adult rumen fluke [OR (95% C.I.): 2.55 (1.07-6.12)].

Animals slaughtered older than 30 months were significantly more likely to be heavily infected with adult rumen fluke (>100) than animals slaughtered younger than 24 months [OR (95% C.I.): 5.48 (1.56-19.21)].

Compared to March, there were increased numbers of animals infested with more than 100 adult rumen fluke in June [OR (95% C.I.): 2.32 (1.13-4.75)], October [OR (95% C.I.): 2.82 (1.34-5.92)] and January [OR (95% C.I.): 4.45 (2.12-9.38)]. Carcases originating from higher altitude farms (>60m) were significantly less likely to have adult rumen fluke compared to carcases originating from lower altitude farms (≤60m) (OR: 0.44- 0.58).

The presence of lesions due to liver fluke was significantly associated with the presence of adult rumen fluke (OR: 1.79-5.34). There was no significant association between the presence of lesions due to *Ostertagia spp.* and the likelihood/severity of adult rumen fluke infection.

3.3.3. Model 3 (liver lesions due to liver fluke)

Compared to dairy purebreds, beef crossbreds were significantly more likely to have both moderate and severe lesions due to liver fluke (OR: 2.30-3.18).

Compared to cows, heifers were significantly less likely to have lesions due to liver fluke (moderate and severe) (OR: 0.08-0.43), steers less likely to have severe lesions due to liver fluke [OR (95% C.I.): 0.13 (0.05-0.33)] and young bulls less likely to have moderate lesions due to liver fluke [OR (95% C.I.): 0.04 (0.01-0.38)].

After controlling for the other variables, there was no significant association between the age of the animal at slaughter and the presence of lesions due to liver fluke.

Compared to March, there were significantly higher numbers of carcases with lesions due to liver fluke of all severities in January (OR: 1.75-3.20) and of moderate severity in October [OR (95% C.I.): 2.06 (1.21-3.50)]. Carcases originating from higher altitude farms (>60m) were significantly less likely to have moderate lesions due to liver fluke compared to carcases originating from lower altitude farms (≤ 60m) [OR (95% C.I.): 0.56 (0.38-0.82)].

The presence of adult rumen fluke was significantly associated with the presence of lesions due to liver fluke of all severities (OR: 2.71-4.08). There was no significant association between the lesions due to both liver fluke and *Ostertagia spp.*

3.4. Association between helminth infection severity and prime beef carcase performance

The final multilevel linear regression models are summarised Table 7-5. The total variance explained by the different final models was: 50%, for model 1 (cold carcase weight); 33%, for model 2 (conformation); and 64%, for model 3 (fat classification).

After controlling for the effects of breed, category, age and season, animals with single-infection of either ostertagiasis or adult rumen fluke had, on average, significantly lower CCW [Coef. (95% CI): -30.58 (-50.92;-10.24); and -50.34 (-88.50;-12.18)] and lower fat class [Coef. (95% CI): -3.28 (-5.56;-1.00) and -5.49 (-10.28;-0.69)], respectively, than carcases from helminth-free animals.

The presence of lesions due to liver fluke had no significant impact on CCW except when present along with both lesions due to *Ostertagia spp.* and adult rumen fluke, leading to significantly lower CCW [Coef. (95% CI): -48.28 (-88.35;-8.21)], compared to carcases free of the three helminths. Carcases with both lesions due to *Ostertagia spp.* and adult rumen fluke had significantly lower CCW [Coef. (95% CI): -39.99 (-73.09;-6.88)] compared to carcases free of the three helminths.

The presence of lesions due to liver fluke on its own had a significant negative impact on carcase conformation by a 3.65 (-6.98;-0.32) point decrease in the class numerical scale, compared to carcases free of the 3 helminths.

Table 7-5: Final multilevel linear regression models of association between prime beef carcase parameters, i.e. cold carcase weight (model 1), conformation (model 2) and fat classification (model 3) and demographic and concurrent helminth variables as fixed effect

		Model 1: cold carcase weight (115 Herds, 756 cattle, 618 Obs.)			Model 2: conformation (115 Herds, 756 cattle, 709 Obs.)			Model 3: fat classification (115 Herds, 756 cattle, 630 Obs.)		
Fixed effects										
Variables	**Categories**	**N**	**β**	**95% C.I[a]**	**N**	**β**	**95% C.I[a]**	**N**	**β**	**95% C.I[a]**
Intercept (SE)[a]			295.35 (12.49)			14.15 (2.25)			28.30 (1.63)	
Helminth Inf.	**None**	64	*Baseline*		64	*Baseline*		64	*Baseline*	
	O[b] only	401	-30.58*	-50.9;-10.24	401	1.13	-0.53;2.78	401	-3.28*	-5.56;-1.00
	RF[b] only	6	-50.34*	-88.50; -12.18	6	2.41	-1.27;6.09	6	-5.49*	-10.28;-0.69
	LF[b] only	11	-20.39	-50.76;9.98	11	-3.65*	-6.98;-0.32	11	-1.41	-5.71;2.89
	O- RF	102	-39.99*	-73.09;-6.88	102	-1.69	-4.36;0.98	102	-1.72	-5.57;2.14
	O- LF	80	-22.94	-52.89; 7.01	80	-1.26	-3.65;1.12	80	-0.35	-3.91;3.21
	RF- LF	4	-32.41	-73.06;8.24	4	3.48	-0.66;7.64	4	-4.85	-10.19;0.49
	O- RF- LF	57	-48.28*	-88.35;-8.21	57	-1.27	-4.68;2.14	57	-3.81	-8.61;0.99
Random effects										
	Level		Variance	SE		Variance	SE		Variance	SE
	Herd		561.42	101.81		2.31	0.68		4.45	1.26
	Cattle		844.80	56.10		13.34	0.803		20.98	1.34

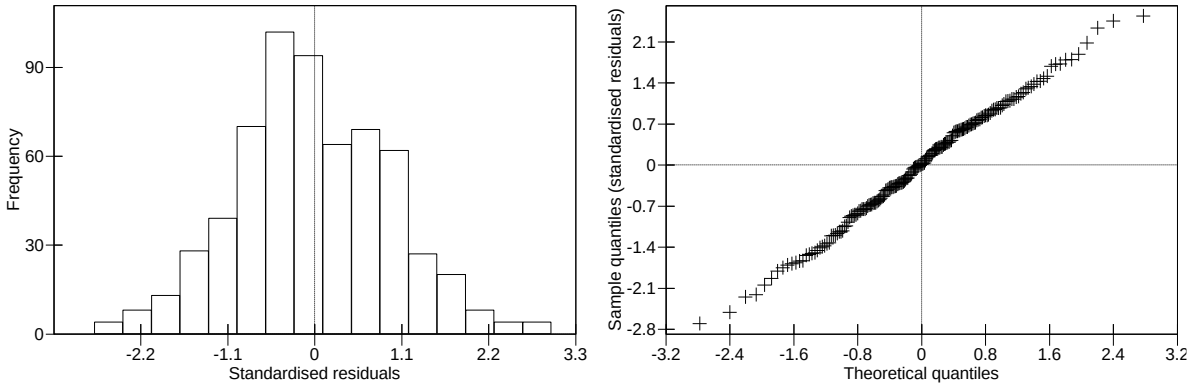
[a]**Breed, category (mature bull excluded), age, cold carcase weight, conformation, fat, month and altitude were included in model as confounders, and results presented adjusted for these variables; CI=confidence interval; SE=standard error; *=significant (P-value≤0.05);**

[b]**O=lesions due to *Ostertagia spp.*; RF=presence of adult rumen fluke; LF=lesions due to liver fluke**

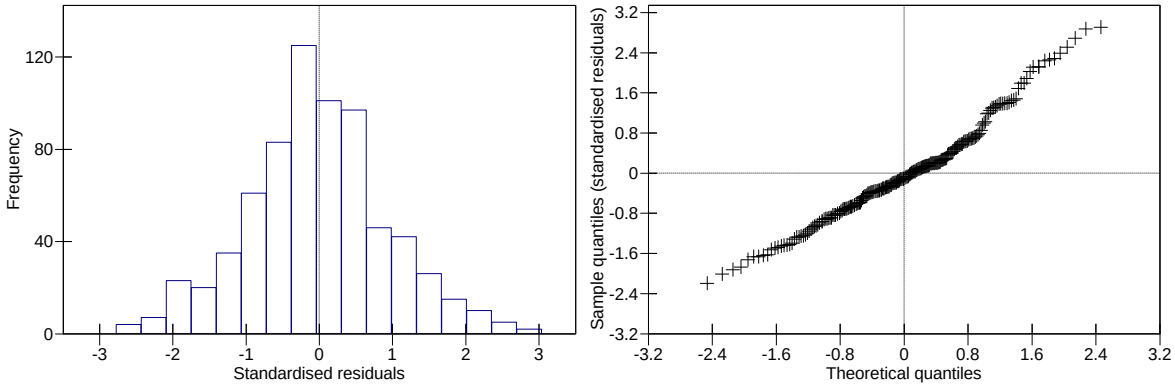
Visual examinations of the three models final residuals at each hierarchical level suggested that the model fits were good (Figure 7-1). Outliers did not have any influence on the coefficients. Therefore these was left in the models.

Figure 7-1: Diagnostic plots of final standardised residuals at cattle level (left) and farm level (right) for the three multilevel linear regression models of prime beef cold carcase weight, carcase conformation and carcase fat classification

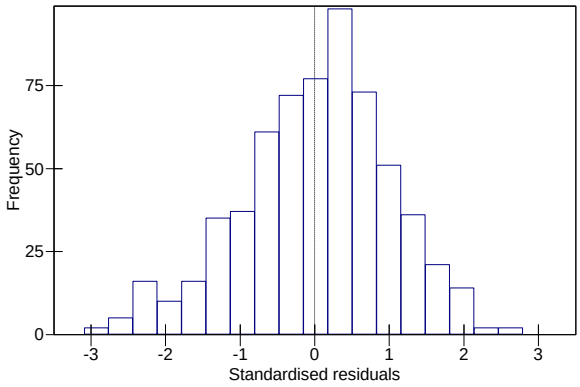
1) Cold carcase weight (model 1)

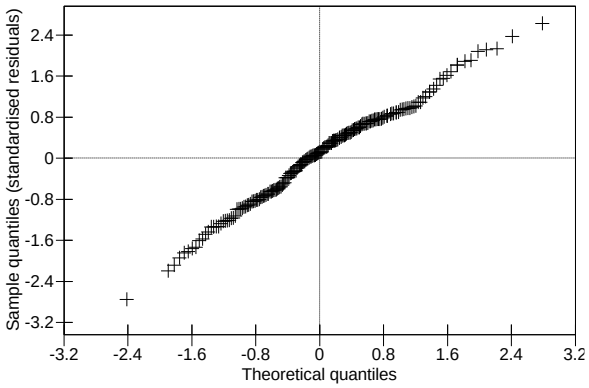


2) Carcase conformation (model 2)



3) Carcase fat classification (model 3)





4. Discussion

This is not only the first abattoir study since the eighties on the prevalence of *Ostertagia spp.* and liver fluke cattle infections in England and Wales (Froyd, 1975; Burrows et al., 1980; Bairden and Armour, 1981; Hong et al., 1981), but also the first abattoir survey on cattle helminths to include rumen fluke and co-infections in this region.

4.1. Prevalence of infections and co-infections

Although interpretation of these data should be cautious given the absence of information on previous anthelmintic treatments and past grazing history, the prevalence of cattle ostertagiasis reported in the current study was 89%, which is similar to that recorded in previous European abattoir surveys (86% to 97%) (Agneessens et al., 2000; Borgsteede et al., 2000) and much higher than that observed in the current study for liver fluke and adult rumen fluke (29% and 25% respectively). Very few farms (3%) in the current study had cattle with no evidence of lesions due to *Ostertagia spp.* compared with 52% and 36% of farms without any presence of adult rumen fluke and lesions due to liver fluke, respectively. These results confirm the predominance and ubiquity of *Ostertagia spp.* infection among cattle farms in England and Wales (Hong et al., 1981), mainly related to the relatively simple direct lifecycle of this parasite compared with the indirect lifecycles of the two trematodes (McCann et al., 2010b; Gordon et al., 2013).

The estimate of prevalence of adult rumen fluke infection in the current study (25%) is quite similar to that previously recorded in cattle at slaughter in mainland Europe (Szmidt-Adjide et al., 2000; Gonzalez-Warleta et al., 2013; Malrait et al., 2005) and confirms the establishment of this trematode in the UK (Gordon et al., 2013; Sargison et al., 2016). Higher prevalence of adult rumen fluke were recently recorded in similar studies in Ireland (52%) (Toolan et al., 2015) and in Scotland (29%) (Sargison et al., 2016) and could be attributed to differences in environment and cattle production systems, especially in the case of Ireland (Murphy et al., 2006; Toolan et al., 2015).

Overall, 29% of the cattle were infected with liver fluke, which is similar to recent prevalence data in Northern Ireland (Byrne et al., 2016). The only similar abattoir survey conducted in England and Wales was more than forty years ago (Froyd, 1975). Given the expected huge variability in climate conditions and the important changes that occurred in UK livestock farming since the eighties (Chapter 1., section 1.1.2.), comparison of both studies is difficult. However, there has been evidence of a recent spread of liver fluke infection in cattle in the region (Pritchard et al., 2005a).

4.2. Specimens of adult rumen fluke

All the specimens of adult rumen fluke isolated in this study were identified as *C. daubneyi* and not *P. cervi,* which was previously assumed to be the predominant rumen fluke species in the British Isles (Gordon et al., 2013). Despite this, the possibility of other species being present in England and Wales cannot be excluded, given that only two carcases were sampled for adult rumen fluke speciation. However, this result complements previous work conducted in Scotland and Ireland (Gordon et al., 2013; Zintl et al., 2014) and emphasises the importance, if not predominance, of *C. daubneyi* in the UK, as it is in mainland Europe (Szmidt-Adjide et al., 2000; Gonzalez-Warleta et al., 2013; Gordon et al., 2013).

4.3. Cattle helminth co-infections

In the current study, 39% of the carcases had signs of co-infections. The similar environmental requirements and common microclimate and microhabitat shared by the three helminths and

their intermediate hosts may explain some of the animals' co-infections, but not entirely (Viney and Graham, 2013). As for instance, cattle anthelmintic treatments or management practices on farms may generate different patterns of co-infections (Gordon et al., 2013). However, this information was not currently available to explore any patterns.

The presence of adult rumen fluke was significantly associated with the presence of lesions due to liver fluke. Because both helminths have very similar lifecycles and both *F. hepatica* and *C. daubneyi* can share the same intermediary host *Galba truncatula* (Zintl et al., 2014), it has been suggested that cattle infected with one fluke would simultaneously be infected with the other (Gordon et al., 2013). Although the presence of both fluke species was associated, only half of the animals (102/219) infected in the current study with adult rumen fluke had signs of lesions due to liver fluke. As reported previously, different lymnaeid communities can act as intermediate hosts for the two helminths and, in the UK, snails other than *Galba truncatula* may play an important role as intermediate host (Dreyfuss et al., 2014). Under these circumstances, competition between either the parasites or the intermediate hosts, especially for food in colonised habitats, could explain the important number of adult rumen fluke single-infections identified in the current study (Dreyfuss et al., 2014). These results raise questions on the current dynamic of helminth infections in cattle in the UK and the need to fully understand host-helminths interactions and co-evolution, especially in the context of specific helminth poly-infections (Gasbarre, 1997; Viney and Graham, 2013).

4.4. Factors associated with the presence and severity of cattle helminth infections

As previously reported in the literature (Myers and Taylor, 1989; McCann et al., 2010a), there was a significantly higher risk of carcase helminth infections in October-January, compared to March-June, which could be related to the specific lifecycles of the three helminths. It is also possible that exposure of animals slaughtered in March-June was reduced, given, in the UK, animals are often housed in the winter and beef cattle often undergo a two-month fattening period while housed before slaughter (AHDB, 2009). Unlike this study, the seasonality of *Ostertagia spp.* was not reported in a similar beef study (Charlier et al., 2009), which could be attributable to its study design and the lack of test specificity of the ELISA technique used.

After controlling for the breed, cows were less likely to present lesions due to *Ostertagia spp.* and adult rumen fluke, but more likely to present lesions due to liver fluke, compared to heifers and steers. In both cases, this is likely to be related to the development of some host immunity that, for both *Ostertagia spp.* (Gasbarre, 1997) and rumen fluke (Diaz et al., 2006), would reduce the worm burden and for liver fluke would cause liver fibrosis (i.e. in order to ensure the survival of the liver fluke in the host) (Mendes et al., 2013).

4.5. Associations between different levels of cattle helminth infections' severity and prime beef carcase performance

The presence of lesions due to liver fluke only was significantly associated with lower conformation, but neither CCW nor fat classification as reported in a previous similar study (Sanchez-Vazquez and Lewis, 2013). There are several studies that have failed to demonstrate the effect of liver fluke infection on cattle growth rate and there is a possibility that *F. hepatica* may alter host performance through mechanisms other than body weight (Loyacano et al., 2002; Charlier et al., 2009). The study by Sanchez-Vazquez and Lewis (2013) reported small significant negative effects of liver fluke on CCW and fat classification. There is a possibility that this effect observed in their study was due to to the impact of the presence of other co-infections that were not investigated, especially, given that, in the current study, liver fluke in combination with *Ostertagia spp.* and rumen fluke did have an impact on CCW.

The current results on *Ostertagia spp.* single effect on CCW and fat classification agree with previous intervention studies on beef cattle (Suarez et al., 1991; Loyacano et al., 2002) but contradict a recent abattoir survey in which no similar association was reported, though there was an effect on conformation (Charlier et al., 2009). It is likely, in this case, that the lower specificity of *O. ostertagi* ELISA used in the latter study, combined with the inclusion of only adult cows and the non-control of other helminth infections in the model, explained such differences. Our results suggest that, compared to no lesion, the adverse effect of *Ostertagia spp.* on CCW was higher on average (coefficient values) when present along with the two other helminths. It is possible, as reported in a previous study (Loyacano et al., 2002), that both gastro-intestinal nematodes and liver fluke negatively affect host performance through different mechanisms and that, if present simultaneously, the resulting effect on CCW might be additive. Further research would need, however, to be conducted to confirm this hypothesis.

There have not been any studies that established a significant effect of adult rumen fluke on carcase weight and classification. Only one attempt has been made to identify a significant association between the presence of adult rumen fluke and cattle carcase performances, but results were inconclusive (Sargison et al., 2016). In the current study, there were significant negative associations between adult rumen fluke and CCW and fat classification. Compared to carcases with no lesion, this effect was seen when rumen fluke was present on its own or along with both *Ostertagia spp.* and liver fluke. These results bring into question the widely held view in Europe that adult rumen fluke are relatively benign and well tolerated by their host, contrary to tropical regions where its high pathogenicity was confirmed (Fuertes et al., 2015). Given in the current study there were only a few animals solely infected by rumen fluke, there is a need for further investigations into the pathogenicity of adult rumen fluke in cattle. In addition, what cannot be ascertained in the current study is whether any of the animals that were positive for adult rumen fluke may also have been infected with juvenile fluke in the duodenum; these stages are known to be highly pathogenic when present in large numbers (Millar et al., 2012; Zintl et al., 2014).

4.6. The limitations of the study

Although highly specific, meat inspection is considered as a poorly sensitive diagnostic tool (Rapsch et al., 2006; Sanchez-Vazquez and Lewis, 2013), which is likely to underestimate the prevalence estimates. Moreover, only the presence/lesions of adult parasites but not juveniles were screened in the current study, which also may have led to underestimation of prevalence. However, this underestimation is less likely to effect the observed associations and co-infection patterns. This cross-sectional study provided us with associations between various factors and presence of helminths but did not infer causality. During this study, steps were taken to minimise bias by validating the feasibility and reliability of the scoring system (pilot study) and by maintaining throughout the study the same group of operators for scoring.

Though the study was only conducted in one abattoir in England limiting its generalisability, this abattoir was one of the largest abattoirs in the region with a relatively high throughput. The farms were localised in 23 counties and given the study sampling occurred throughout the year, it was possible to include different types of cattle production systems. Moreover, the characteristics of the carcases included in the current study presented the same characteristics

as the ones reported by Pritchard et al. (2013) while characterising beef and dairy cattle production profiles in Britain.

5. Conclusions

The observations made in the current study confirm the ubiquity of *O. ostertagi* infections and the significant presence of *F. hepatica* infections in England and Wales. These also suggest that rumen fluke infections are well established and that poly-infections to *O. ostertagi, F. hepatica* and adult rumen fluke are very common in cattle in the region.

The results also highlight the importance, if not predominance, of *C. daubneyi* in the UK, as opposed to *P. cervi*, and emphasise the importance of this specimen in the region, as it is in mainland Europe.

More importantly, the study identified for the first time significant associations between different profiles and severities of helminth infections in cattle and prime beef carcase performance. Results suggest that *O. ostertagi* and adult rumen fluke single-infection can impair cattle cold carcase weight and fat, whereas *F. hepatica* single-infection can impair carcase conformation. Moreover, there is a significant adverse effect of simultaneous infections due to these three helminths that might differ from the effects observed in the case of single-infections. Finally, the current findings also take another look at the presumed benign nature of adult rumen fluke in cattle.

The patterns of infection severity reported in this study in terms of cattle category, age group and season, agree with previous research but also raise the question with regards to the patterns of co-infections existing in England and Wales. However, the exploration of these patterns was limited since no information on cattle anthelmintic or management practices on farms were available. Therefore further investigations are required to better understand the dynamic of *O. ostertagi*, *F. hepatica* and rumen fluke poly-infections in cattle in the UK, considering cattle management, cattle and helminths interactions and species co-evolutions.

Chapter 8.

Concluding remarks: Reflections on cattle helminth control and implications for policy and practice

In order to address the complexity of cattle helminth infections and control in England and Wales (Chapter 1.), this thesis explored the prevalence (Chapters 3. and 7.), risk factors (Chapters 4. and 7.) and impacts (Chapters 5. and 7.) associated with three helminth parasites of economic importance in dairy and beef cattle (i.e. *O. ostertagi*, *F. hepatica* and rumen fluke), and engaged with farmers to explore their knowledges, practices and values around prevention and control of cattle helminth infections in this region (Chapter 6.). The research project draws on a mixed methods approach that included both quantitative and qualitative research studies. The design and the sampling strategies chosen for the quantitative studies aimed to minimise bias in data collection and analysis of associations between predictors and outcomes of interest related to cattle helminth infections (Chapters 2., 4., 5., and 7.). This included the use of reliable markers (i.e. post-mortem examination and individual milk antibody levels) and multiple sources of data collection (i.e. questionnaire, interviews, and recording program). For the qualitative study, data collection followed a framework informed by an extensive literature review and the analytical procedure was based on a systematic and transparent coding scheme that ensured the consistency of the process (Chapter 6.). Overall, the research has shed light on important epidemiological patterns and key factors that influence the control of cattle helminth infections in England and Wales (Vercruysse and Claerebout, 2001). Together, these are fundamental aspects that need to be considered for the development of improved strategies on cattle helminth control.

The potential limitations of the different studies comprised in this project are discussed in each of their related chapters and are not the focus of this chapter. The objective here is to step back and critically reflect on the overall outputs of the project and their implications for the improvement of cattle helminth control in England and Wales, as well as in the UK more broadly. In doing so, this chapter aims to expand the empirical relevance of this thesis by

exploring other complementary levels and scales (i.e. actors and domains) that also need to be taken into account so cattle helminth control can be improved.

1. Main results of the thesis

This project confirms the ubiquity of *O. ostertagi* infections and the significant presence of *F. hepatica* infections, in beef and dairy cattle, in England and Wales. It also suggests that rumen fluke infection is well established and that poly-infections to *O. ostertagi, F. hepatica* and rumen fluke, possibly *C. daubneyi*, are very common in cattle in England and Wales. Importantly, this research is the first of its kind investigating the negative effects (1) of several helminth infra-communities on carcase performance in prime-beef cattle and (2) of *O. ostertagi* on milk production, reproduction and health performances in dairy heifers. The research design and methods proposed here offered a reliable and valid approach to collect data from a wide range of sources, which increased the strength of the measured associations. As a result the underlying biological associations of factors reported in this thesis are likely to be valid and generalizable to the population of heifers and prime-beef cattle in England and Wales. Considering that young-stock is the future of beef and dairy herds, the observed associations, if confirmed causal, would justify on cost-effectiveness ground an urgent need for farmers to implement more effective and strategic control against *O. ostertagi, F. hepatica* and rumen fluke in England and Wales, as well as in the UK more broadly. In this regard, the research suggests that there are alternative strategies, potentially more desirable, for helminth control in dairy young-stock that could replace the use of anthelmintic drugs. For example, different types of grazing management practices can help with reducing dairy heifer exposure to *O. ostertagi* on pasture, in particular when heifers are the most susceptible to diseases. These include for instance to avoid high stock density during a heifer's first year of grazing, to avoid mixing heifers with mature cows for more than a couple of weeks prior to calving and to frequently mow the grass of heifer pasture. However, if we are to guarantee the transition in management practices and improvements on cattle helminth control in England and Wales, as well as in the UK, there is a need to ensure and foster understanding between experts and farmers. As suggested by the results of the qualitative study, the tendency of experts to overlook and even neglect farmers' epistemology and the contextual challenges they face, might undermine farmers' trust in expert opinion and might compromise the uptake of expert-based guidelines

for cattle helminth control. As a consequence, it is only through constructive dialogues between experts, as those responsible for developing guidelines, and farmers, that opportunities will be created for these actors to improve cattle helminth control in the UK.

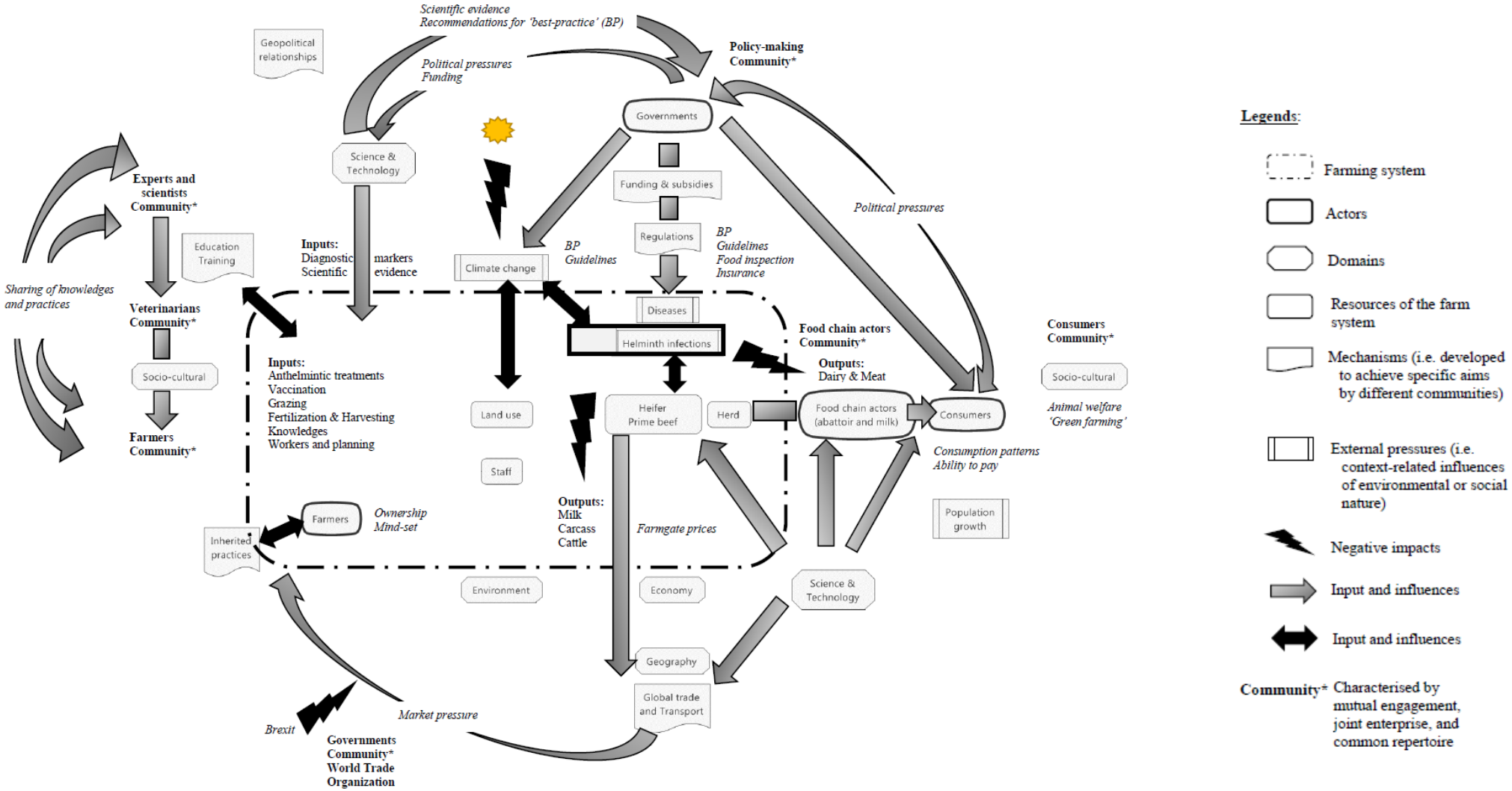
Drawing on a multidisciplinary framework that incorporates both veterinary epidemiology and sociology, this thesis focused on one sub-set of the possible representations of the problem of cattle helminth infections, i.e. at farm level. Therefore, the following sections explore other complementary levels and scales (i.e. actors and domains) that play an important role in cattle helminth control.

2. Complexity in cattle helminth control: developing collective responsibility for challenging infections

The complexity of a system has important implications for the way policy problems are framed and how decision-making processes unfold (Munda, 2004). As previously discussed (Chapters 1. and 6.), the problem of cattle helminth infections is multidimensional and therefore challenging to represent. Firstly, the ecology of the pathogen is complex (e.g. different larval stage and presence of intermediate hosts) and has been changing due to a recent increase in global warming and parasites' resistance to anthelmintic drugs (Skuce et al., 2013). Secondly, the disease is mostly subclinical, requiring intensified efforts to target and implement control strategies amidst uncertainty. In line with the results of this thesis (Chapters 5. and 6.), although helminth infections are frequently associated with significant losses in cattle productivity, causal inferences are difficult to confirm, which can hamper timely and adequate decision-making (Vercruysse and Claerebout, 2001). Thirdly, the issue of cattle helminth infections affects a wide range of actors (e.g. farmers, veterinarians, scientists, consumers, policy makers, and the industry) and domains (e.g. research, economy and policy), which interact with one another and evolve over time (Munda, 2004; DEFRA, 2011). This way, it is necessary for research to engage with this complexity and explore other aspects of the system that are often overlooked. These are related to the political and social context in which farms are embedded (e.g. legal obligations, systems of planning, taxes and tariffs, consumers perception, and technological development); and the awareness, knowledges and concerns of other actors and

communities of practice beyond farmers' communities (e.g. government, food industry, drug industry, and consumers). Moreover, besides being able to identify these different elements of the network in the context of cattle helminth control, it is important to consider how actors interact in a whole-system perspective, considering the drivers behind and the consequences of their actions (Munda, 2004) (Figure 8-1).

Figure 8-1: A representation of the system of cattle helminth control in the UK



As illustrated by Figure 8-1, the representation of the system of cattle helminth control reflects what F.W. Geels calls a 'socio-technical system' (Geels, 2004); that is, a system (i.e. resources and material aspects) composed of, maintained and changed by different actors through their interactions, as well as by the rules and institutions that orientate actors perceptions and activities. As previously mentioned in Chapter 6, actors can form communities of practice, defined by their *mutual engagement* (i.e. engagement in actions, development of shared practices), *joint enterprise* (i.e. actors are working together towards a common goal, not necessarily institutionally defined) and *common repertoire* (i.e. actors share a common history and culture created over the years by shared practices and interactions) (Oreszczyn et al., 2010). Like farmers, veterinarians and other experts (Chapter 6.), policy-makers, food chain actors, and consumers are also part of a broader sharing-practice community (Figure 8-1). In this context, since socio-technical systems are shaped by and only exist because of actors' activities (Geels, 2004), if cattle helminth infections are to be better understood and controlled in the UK, it is appropriate to think that all activities embedded in the system should be integrated in the analysis, including actors' perceptions, concerns and interests.

The interactions and processes of knowledge and sharing-practice between different communities can be complicated by the existence of different *repertoires* and technical languages (e.g. those of experts and farmers (Chapter 6.)) (Oreszczyn et al., 2010). Therefore, it is important for actors to manage their differences and potentially benefit from them (i.e. *joint enterprise*) when focusing on a common goal such as cattle helminth control. Over the last decades, many discussions and debates between the Government and stakeholders (e.g. animal keepers and their representative organisations, veterinarians, scientists, and industry) on the responsibility and the cost sharing in the field of animal health have taken place in the UK. These have resulted in different types of partnerships and initiatives being established (e.g. Bovine Tuberculosis (bTB) Programme, Animal Health & Welfare Strategy, and Animal Welfare Act 2006) (DEFRA, 2011). In the case of helminth control, experts have started to engage with farmers on the topic (Heasman et al., 2012; Morgan et al., 2012; McMahon et al., 2013; Wilson et al., 2015; Moore et al., 2016), which culminated with the publication of several guidelines and training modules for achieving sustainable helminth control in livestock (SCOPS, 2003; COWS, 2010; DEFRA, 2011). However, as discussed in Chapter 6, the approaches adopted in these cases have often been disconnected from farmers' context and realities, without opportunities for farmers to have a real input in the governance of helminth

infections control. Consequently, this body of research has informed the implementation of rigid mechanisms based on one-way flow of information from experts to farmers. Often, these do not allow for *mutual* learning between communities, something that ultimately hinder the adoption of expert-based recommendations by farmers.

By engaging and discussing with farmers regularly throughout the project, this thesis sought to integrate their perspectives and values into the research. Also, a follow-up meeting with the farmers involved in the project is planned for the coming year to discuss the main outcomes of the research. These kinds of approaches could contribute to foster farmers' trust in experts and the credibility of scientific expertise itself, as well as allow experts to better understand farmers' local realities, which is crucial for developing adequate advice. However, it is important to note that these are time and resource-demanding research activities which requires financial support from both policy-making and industry communities. As such, cattle helminth infections must become a priority in the agenda of animal health policy (i.e. as it is the case of bTB and Transmissible Spongiform Encephalopathies (TSEs)) (DEFRA, 2011).

3. Towards renewed practices and new approaches for the governance of cattle helminth control

Raising concerns over anthelmintic resistance have made experts, industries and policy-making communities rethink and re-evaluate practices of cattle helminth control in the UK (COWS, 2010). In this context, the role of science in developing vaccines and diagnostic tests against cattle helminths may be determinant for the sustainable control of infections. However, despite major advances in identifying potential vaccine molecules, no products are yet available in the market (Vercauteren et al., 2004; Molina-Hernández et al., 2015; Gonzalez-Hernandez et al., 2016). Moreover, the report of an ELISA cut-off value that would maximize the benefit of helminth testing in cattle, i.e. the economic and social consequences of both misdiagnosis and disease prevalence, is still lacking (Ridge and Vizard, 1993) and suggests that further investigation is needed to improve the characteristics (i.e. cut-off value) and current use of cattle helminth ELISA tests in the field (Ridge and Vizard, 1993; Charlier et al., 2008). As a

consequence, there is a need to identify easier and more rapidly available ways to take actions against cattle helminth infections in the UK.

As described in the general introduction of this thesis (Chapter 1., section 5.), in order to ensure the sustainability of their businesses, cattle farmers have become compelled to improve the economic efficiency of their production. In this regard, they consider the use of anthelmintic treatments as a good and relatively simple 'insurance policy', especially in the case of young animals (Chapter 6.). Although not including worms as a main priority of their farming activities (as opposed to managing mastitis or bovine tuberculosis), farmers explained during the interviews that they did not wish to take the risk of not controlling helminths on their young-stock given that they could financially regret it. The current project, however, suggests that farmers might want to consider other targeted control practices, based on specific grazing management practices at particular times of the season and age of their young-stock, which could be more sustainable and efficient in decreasing the early exposure of cattle to *O. ostertagi* on pasture without the need of anthelmintic drugs. Moreover, reflecting on the results of the current project, farmers could potentially benefit from integrating the risk of helminth exposure across seasons in the selection of their calving system (i.e. all-year-round and spring and autumn block calving). Taking into account the wide range of negative impacts helminths can have on heifer productivity (i.e. grow rate, age at first calving, milk yield, and offspring) (Chapter 5.), such practices could, at a lower cost (i.e. without the cost of anthelmintic drugs and with better use of cattle feed), increase animal health, welfare and farm net income.

Another way of limiting the use of anthelmintic drugs has been proposed by new regulatory frameworks that support a change in drugs' prescription. Recently, the British Veterinary Association (BVA) attempted to change the Veterinary Medicine regulation so that anthelmintic drugs could only be dispensed by veterinarians or pharmacists on veterinary prescription, as it is already the case in other European countries, such as Denmark and the Netherlands (Anonymous, 2013; Easton et al., 2016). The BVA appears to assume that veterinarians are the only actors capable of making responsible and sensible decisions on the use of anthelmintic drugs. Given the lack of evidence that veterinarians are necessarily more capable than other Suitably Qualified People (SQP) in prescribing anthelmintic drugs, no further action has yet been taken by the Animal Medicines Training Regulatory Authority (AMTRA) (Easton et al., 2016). In any case, each actor prescribing anthelmintic treatments to farmers (i.e. veterinarians, and SQP) should be responsible for actions that may affect the

sustainability of cattle helminth control, such as over or inadequate prescriptions of treatments. To date, very little is known on the attitudes of these actors towards cattle helminth control (Easton et al., 2016). Also, the few studies available suffer from the same limitations as the mainstream literature on farmers' attitudes discussed earlier in Chapter 6, i.e. they rely on instrumental theoretical frameworks and methods that do not leave room for reassessment of research variables and assumptions. Therefore, there is a need to further investigate how actors responsible for prescribing anthelmintic drugs make decisions around the frequency and choice of treatment.

Since farmers have access to a wide range of information transmitted by different channels, such as the press, the internet, and through engagement with veterinarians and drug sellers (Chapter 6.), a key aspect of cattle helminth control is the development of responsible communication, based on the right of farmers to have access to transparent, precise and comprehensive information on the matter. During the interviews, a significant number of farmers suggested that current literature on cattle helminth infections was either too scarce or out-of-date, especially when compared to sheep and other diseases. Moreover, farmers explained that they could not afford losing time and energy in trying to access or decipher information that was either inadequate or inappropriately communicated (i.e. too long or using unintelligible language). This suggests that investments and efforts should be mobilised at different levels (e.g. research, industries, and governments) to guarantee the quality and adequacy of current communication tools and contents on cattle helminth infections and control. This includes a need to detect, monitor and manage conflicts of interest (e.g. when a drug seller or a veterinarian are only interested in selling more drugs), so that the objectives of farmers in relation to helminth control are not adversely affected (Chapter 6.).

Having examined various levers for action towards renewed practices that could be targeted by policies, it is necessary to ensure the sustainability of these recommendations within the system of cattle helminth control. This means evaluating the impacts of the suggested practices considering the *multiple identities* involved in the system (i.e. non-equivalent actors and observations in terms of levels and scales at which the system can be analysed) (Munda, 2004). To achieve this objective, it is necessary for actors included in the system to collaboratively define (1) *what is important* for each of them, taking into account they have different interests and values; and (2) *what is relevant* for the representation of the system in the context of cattle helminth control, e.g. economic and environmental benefits, animal welfare and human health

(Munda, 2004). In this regard, the use of participatory research methods, such as the one included in this thesis, and other deliberative activities can be very useful (Bergold and Thomas, 2012). These methods allow different actors to, firstly, bring in their perspectives while evaluating the context of cattle helminth control and, secondly, to foster constructive dialogues while making decisions on it. Conflicting views of different actors could therefore be integrated into decision-making processes so that a 'compromise solution' through mutual agreement can be reached (Munda, 2004). This way, by taking on board a diversity of concerns and interests, recommendations for best-practice in cattle helminth control are likely to be more adequate, acceptable and lead to more sustainable practices.

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Appendix 1: QMMS farmers' invitation letter (dairy longitudinal study)

The University of Nottingham
UNITED KINGDOM - CHINA - MALAYSIA

QMMS

INFORMATION Letter- Scientific Project on worm control in dairy heifers

Nottingham, March 2014

Dear Sir/Madam,

The University of Nottingham would like to invite you to participate in an important study on worms in dairy heifers, which it is conducting in collaboration with Quality Milk Management Services. The study aims and objectives are described below. Please feel free to contact us with any questions if you would like further information.

Gastrointestinal nematodes infections (worms) are known to cause important production losses in dairy cows. Our objectives are to work towards a better understanding of the control of these infections, understanding the relationships that exist between dairy cow management practices, individual parasites burdens and the prediction of animal's production performance. We would also like to understand your views on the usefulness of different worming strategies.

Your participation would consist of:

- allowing us to visit your farm and ask you some questions (this will take no more than 1 hour and will be recorded - all your data and recordings will be anonymised);

- filling in a short questionnaire about your heifers management and health;

- allowing us to collate individual milk samples from your heifers and two bulk tank milk samples from your herd (free of charge and, for the individual milk samples, from the milk samples you submit to QMMS as part of your routine recording) between March 2014 to March 2015. These will be tested for antibodies to worms and fluke by QMMS – you will be informed of the results of these tests which will give you useful information on infection status;

- allowing us to use the production and health data collated from your heifers to see if the level of worm antibodies affects their production, health or fertility;

- allowing us to access and use the data from your farm which would be provided by QMMS.

All information will be kept confidential and secure, in accordance with the 1998 data protection act and it will not be used for any other purpose.

The University of Nottingham
UNITED KINGDOM - CHINA - MALAYSIA

QMMS

INFORMATION Letter- Scientific Project on worm control in dairy heifers

A meeting with all the participants will be organised at the end of the study to share the main study results.

If you would like to participate, please contact us via email camille.bellet@nottingham.ac.uk or phone 07910449947 or 01159516564. We will thus in a second phase be able to contact you to provide you with more details. If you have further questions please do not hesitate to contact us using either above email or the phone.

We very much look forward to hear from you; your participation would be greatly appreciated. We believe the study will be of great benefit to improve the understanding of the control of worms in your herd.

Appendix 2: Planning of the farm visits and sending of the postal questionnaire (dairy longitudinal study)

The University of Nottingham
UNITED KINGDOM - CHINA - MALAYSIA

QMMS

Scientific Project on worm control in dairy heifers-Postal Questionnaire

Nottingham, 31 March 2014

Re: Visit to your farm on … at/between … to

Dear Mr Surname,

Further to our telephone conversation I will visit your farm on…… and as discussed I would like to interview the person making the most decisions for the herd. In the interview I would like to ask some questions on the grazing management of your calves and heifers for the years 2012 and 2013 and general worm control practices. The interview will last around 1½ hours. Everything we discuss in the interview will be stored securely and used anonymously.

You will find enclosed with this letter a short questionnaire, which we ask you to complete (the same person who will be interviewed); the questionnaire includes general farm and management details for the years 2012 and 2013.

I would be very grateful if you could please fill this in before the interview and I will collect this at the time of the visit.

I very much look forward to visiting you and again greatly appreciate your participation in this study. If you have further questions please do not hesitate to contact us using either the email camille.bellet@nottingham.ac.uk or phone 07910449947.

Yours sincerely,

Camille Bellet (PhD Student)
Faculty of Medicine and Health Science
The University of Nottingham
Sutton Bonington LE12 5RD
United Kingdom
Tel: 07910449947

Email : camille.bellet@nottingham.ac.uk

Researchers involved: Dr Jasmeet Kaler, Prof Martin Green, Dr Andrew Bradley

Appendix 3: Postal questionnaire (dairy longitudinal study)

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

QMMS

Project on worm control in dairy heifers

Date (DD/MM/YYYY):

Name of the farm:

The purpose of the given questionnaire is to gather information on dairy farmers' management practices for calves and heifers for the years 2012 and 2013, focussing on nutrition, housing and general health.
The questions related to the grazing management practices of your animals for the same years will be asked to you separately at the time of the interview, during the farm visit.

SECTION 1: FARMER AND FARM DETAILS

In this section, you will be asked general questions on you farm and your production system. Please tick and complete all that apply.

FARMER'S DESCRIPTION

1. Your name: Mr/Mrs/Miss/Ms/Other: _____ *(Please circle and specify if needed)*

Forename: _____

Surename: _____

2. Your age: _____ years

3. Are you the primary manager of the farm? *(Please circle)*

Yes No

4. If no, what is your position within the farm? _____

For the questions 5. 6. 7, please check if the information is correct and complete if needed

5. Address of the farm: House No/Name & Road: Treble House Farm, Back Lane

Town: Kingston Seymour, Clevedon

County: Somerset North

Postcode: BS21 6UY

6. Contact details: _____ *(Office)* 07546 584386 *(Mobile)*

7. Email details: treblehousefarm@yahoo.co.uk

YOUR FARM

8. What is the type of the enterprise you have?

□ Dairy □ Beef □ Sheep □ Horses □ Other, please specify: _____

9. When was your dairy farm built? _____ *(YYYY)*

10. When did your dairy activities start? _____ *(YYYY)*

11. How would you describe your dairy farming between 2012 and today?

□ No □ Organic □ Conventional □ Integrated □ Other, please specify: _____

12. In relation to the previous categories has it changed between 2012 and today? *(Please circle)*

Yes No If Yes, please specify: _____

13. What is the size of your dairy farm today? _____ *Ha*

14. What is the type of soil used as grazing pasture in your farm?

□ Sandy and light silty □ Medium □ Heavy □ Chalk and limestone □ Peaty

□ Other, please specify: _____

15. What is your dairy farming workforce today? Number of total farming workforce: _____

Number of full-time employees: _____

Number of part-time employees: _____

Number of casual workers: _____

YOUR DAIRY PRODUCTION

16. What are the breeds of your herd today?

□ Ayrshire □ Brown Swiss □ Belted Galloway □ British Blonde □ British Charolais □ British Limousin

□ Devon □ Dexter □ Holstein-Friesian □ British Friesian □ Belgium Blue □ Guernsey

□ Jersey □ Montbeliarde □ Shorthorn □ Red Poll □ Shetland □ Welsh Black

□ Whitebred Shorthorn □ Other, please specify: _____

17. What is the size of your dairy herd today?

Number of preweaned calves: _____

Number of calves from weaning to 12 months: _____

Number of heifers from over 12 months to in-calf (bulling heifers): _____

Number of heifers from in-calf to not-yet-calved: _____

Number of dairy heifers (1st lactation): _____

Number of dairy cows (≥2 lactations): _____

Number of adult bulls: _____

18. What has been your dairy farm breeding system between 2012 and today?

□ Natural breeding with bull from your farm □ With a bull from another farm

□ Artificial Insemination □ Artificial Insemination + Natural services

19. What have been your dairy farm methods of oestrus detection between 2012 and today?

□ Visual observation □ Heat mount detectors □ Tailhead markers (paint, chalk, crayon, paste)

□ Chin-ball markers □ Vasectomised bull □ Electronic heat detection devices (e.g. pedometer, collars…)

□ Other, please specify: _____

20. Have you purchased any new animals between 2012 and today? *(Please circle)*

Yes No If Yes, please specify: _____

If yes, please specify their origins: □ Private □ Market □ Other, please specify: _____

21. What is the approximate mean body weight of your heifers at first calving?

_____ *Kg* ◻ I don't know

SECTION 2: MANAGEMENT INFORMATION – NUTRITION AND HOUSING

In this section, you will be asked questions on your management of your calves and heifers (from over 12 months of age to not-yet-calved) for the years 2012 and 2013. Questions will be asked for the year 2013 (last year) and if any management differed for the year 2012 please specify.

DAIRY PREWEANED CALVES

22. Did your cows calve in individual pens in 2013? *(Please circle)*

Yes No If different in 2012, please specify: _____

23. At what time after birth were your calves separated from the dam in 2013?

◻ 0 hour ◻ <12 hours ◻ 12-24 hours ◻ >24 hours If different in 2012, please specify: _____

24. What was the method of colostrum feeding you used in 2013?

◻ No colostrum fed ◻ Suckling Assisted ◻ Unassisted
◻ Hand fed from bucket or bottle ◻ Hand fed from oesophageal feeder ◻ Other, please specify: _____
If different in 2012, please specify: _____

25. What was the source of colostrum you used in 2013?

◻ Own dam ◻ Pooled, excluding heifers (1st lactation) ◻ Pooled, including heifers (1st lactation)
◻ Stored ◻ Commercial colostrum substitute ◻ Other, please specify: _____
If different in 2012, please specify: _____

26. What was the amount of colostrum you gave in the first 6 hours in 2013?

_____ *Litres*
If different in 2012, please specify: _____ *Litres*

27. What was the amount of colostrum you gave in the first 24 hours in 2013?

_____ *Litres*
If different in 2012, please specify: _____ *Litres*

28. Did you group your preweaned calves in 2013?

Yes No If different in 2012, please specify: _____

29. If you grouped your preweaned calves in 2013 and/or in 2012, at what age were they first grouped?

In 2012: _____ *Weeks* In 2013: _____ *Weeks*

30. If you grouped your preweaned calves in 2013 and/or in 2012, what was the average number of calves per group?

In 2012: _____ *Animals* In 2013: _____ *Animals*

31. What was the type of liquid feeds you used in 2013?

◻ Fresh cow milk ◻ Mastitis or antibiotic milk ◻ Milk replacer ◻ Acidified milk ◻ Other: _____
If different in 2012, please specify: _____

32. In 2013, what was the average age of your calves when you first gave ?

◻ Starter ration: _____ *Days* If different in 2012, please specify: _____ *Days*
◻ Roughages (e.g. hay, straw...): _____ *Days* If different in 2012, please specify: _____ *Days*
◻ Ad-lib water: _____ *Days* If different in 2012, please specify: _____ *Days*

33. What diet supplementation did your preweaned calves get in 2013?

◻ No supplementation ◻ With coccidiostats ◻ With Se ◻ With Ionophores ◻ With concentrate ◻ Other: _____
If different in 2012, please specify: _____

34. What was the average age of your calves at weaning in 2013?

_____*Weeks*

If different in 2012, please specify:_____

34. What was the average age of your calves when they first turned out in the pasture in 2013?

_____*Weeks*

If different in 2012, please specify:_____

35. What was the average weight of your calves when they first turned out in the pasture in 2013?

_____*Kg* □ I don't know

If different in 2012, please specify: _____

WEANED CALVES AND HEIFERS (Not-yet-calved)

36. In 2013, what was the housing system of your:

a. **Calves (weaned to 12 months of age):** □ Housed all year round □ Housed in winter only
If different in 2012, please specify: _____

b. **Heifers over 12 months of age to in-calf (bulling heifers):** □ Housed all year round □ Housed in winter only
If different in 2012, please specify: _____

c. **Heifers in-calf (bulling heifers) to not-yet-calved:** □ Housed all year round □ Housed in winter only
If different in 2012, please specify: _____

37. If you only housed during the winter, please specify the corresponding months for the given year and age group:

a. **Calves weaned to 12 months of age:** In 2012: from _____ to _____
In 2013: from _____ to _____

b. **Heifers over 12 months to in-calf:** In 2012: from _____ to _____
In 2013: from _____ to _____

c. **Heifers in-calf to not-yet-calved:** In 2012: from _____ to _____
In 2013: from _____ to _____

38. During their housing in 2013, did you mix with other animals or animals from other age group your *(Please circle)*:

a. **Calves weaned to 12 months of age:** Yes No Please specify with what: _____
If different in 2012, please specify:_____

b. **Heifers over 12 months to in-calf:** Yes No Please specify with what: _____
If different in 2012, please specify:_____

c. **Heifers in-calf to not-yet-calved:** Yes No Please specify with what: _____
If different in 2012, please specify:_____

38. Did the following groups of age receive any fresh grass during their housing in 2013 *(Please circle)*?

a. **Calves weaned to 12 months of age:** Yes No If different in 2012, please specify:_____

b. **Heifers over 12 months to in-calf:** Yes No If different in 2012, please specify:_____

c. **Heifers in-calf to not-yet-calved:** Yes No If different in 2012, please specify:_____

39. In 2013, what diet supplementation did the following group of age get:

a. **Calves weaned to 12 months of age:**

□ No supplementation □ With coccidiostats □ With Se □ With Ionophores □ With concentrate □ Other: _____

If different in 2012, please specify:_____

b. **Heifers over 12 months to in-calf:**

□ No supplementation □ With coccidiostats □ With Se □ With Ionophores □ With concentrate □ Other: _____

If different in 2012, please specify:_____ _

c. Heifers in-calf to not-yet-calved:

□ No supplementation □ With coccidiostats □ With Se □ With Ionophores □ With concentrate □ Other: _____

If different in 2012, please specify: _____

SECTION 3: HEALTH INFORMATION

In this section, you will be asked questions on the vaccination and the health of your calves and heifers (from over 12 months of age to not-yet-calved) for the year 2012 and 2013.

CALVES VACCINATION

40. Did you vaccinate your calves against pneumonia in 2012 and/or in 2013? *(Please circle)*

Yes No

41. If you vaccinated your calves against pneumonia in 2012 and/or 2013, please specify for each vaccination done:

a. Name of the vaccine: _____	**a. Name of the vaccine:** _____	**a. Name of the vaccine:** _____
b. Done in 2012 (*please circle*): Yes No	**b. Done in 2012** (*please circle*): Yes No	**b. Done in 2012** (*please circle*): Yes No
Please specify the age of the animal: _____	Please specify the age of the animal: _____	Please specify the age of the animal: _____
c. Done in 2013 (*please circle*): Yes No	**c. Done in 2013** (*please circle*): Yes No	**c. Done in 2013** (*please circle*): Yes No
Please specify the age of the animal: _____	Please specify the age of the animal: _____	Please specify the age of the animal: _____

42. Did you vaccinate your calves against lung worm in 2012 and/or in 2013? *(Please circle)*

Yes No

a. Name of the vaccine: _____

b. Done in 2012 (*please circle*): Yes No Please specify the age of the animal: _____

c. Done in 2013 (*please circle*): Yes No Please specify the age of the animal: _____

43. Did you vaccinate your calves against leptospirosis in 2012 and/or in 2013? *(Please circle)*

Yes No

a. Name of the vaccine: _____

b. Done in 2012 (*please circle*): Yes No Please specify the age of the animal: _____

c. Done in 2013 (*please circle*): Yes No Please specify the age of the animal: _____

44. Did you vaccinate your calves against other diseases in 2012 and/or in 2013? *(Please circle)*

Yes No

a. Name of the vaccine: _____	**a. Name of the vaccine:** _____	**a. Name of the vaccine:** _____
b. Done in 2012 (*please circle*): Yes No	**b. Done in 2012** (*please circle*): Yes No	**b. Done in 2012** (*please circle*): Yes No
Please specify the age of the animal: _____	Please specify the age of the animal: _____	Please specify the age of the animal: _____
c. Done in 2013 (*please circle*): Yes No	**c. Done in 2013** (*please circle*): Yes No	**c. Done in 2013** (*please circle*): Yes No
Please specify the age of the animal: _____	Please specif of the animal: _____	Please specify the age of the animal: _____

WORMS INFECTION

45. Have you had a diagnosis for any worms' infection in your animals for the years 2012 and 2013?

Yes No □ I don't know

46. If yes, could you please specify:

a. The year it happens: □ 2012 □ 2013 □ Both years

b. Which group of age were infected:

□ Preweaned calves □ Weaned to 12 months calves □ Heifers from over 12 months to In-calf

□ Heifers from In-calf to Not-yet-calved □ Other group of age, please specify: _____

c. If you confirmed the infection by further analysis: Yes No

If yes, please specify: _____

d. Which parasite(s) was responsible for the infection:

Name(s): _____

_____

□ I don't know

Thank you very much for filling in this questionnaire

Appendix 4: Farmers' consent form (dairy longitudinal study)

The University of Nottingham
UNITED KINGDOM - CHINA - MALAYSIA

QMMS

CONSENT Letter- Scientific Project on dairy heifers' worms control

Name of the researchers: Dr. Jasmeet Kaler, Prof. Martin Green, Camille Bellet (PhD Student) and Dr. Andrew Bradley (QMMS)

Please read the statements below and sign below to give your consent.

1. I confirm that I have read, understood the information sheet for the above study and have the opportunity to ask questions.
2. I understand that my participation is voluntary; that the information I will gather would be anonymised and published in the peer-reviewed literature.
3. I agree to take part in the above study, that is: to fill a pre-farm visit heifers' management questionnaire, to allow the visit of my farm and a one-hour interview (anonymously audio recorded) and to stay involved during a one-year post-sampling period for the heifers' production follow-up.
4. I allow the researchers to have access and use the data from TotalVet database.

Name of Participant	**Date**	**Signature**
Jasmeet Kaler		Jkaler
Name of Researcher	**Date**	**Signature**

Appendix 5: Grazing management face-to-face interview schedule (dairy longitudinal study): example of the section 1 (calves), period 1 (from the time of turn-out to the 1st of June)

Pasture Management 1- Weaned calves to 12 months	Farmers' Drivers of Action

1. How would you describe your calves' PASTURE GRAZING SYSTEM TODAY?

2. In relation to this description was it different in 2012 and in 2013? If yes, could you please specify and explain why?

3. Do you buy any replacement heifers on your farm? If yes, at what age do you buy them?

A. IN 2012: If we come back to what practices you had on weaned calves to 12 months in 2012 (not last year, but the year before):

From the month of turn-out : _____ to 1st of June

1. When they were first turned-out, could you tell me which animals had previously grazed on that same pasture?

2. When they first turned-out, could you tell me if manure had been previously spread on the pasture? How much (%)?

3. Could you tell me what was your calves' stocking rate on the pasture (number/ha)?

4. How long were your calves grazing per day?

5. Were the animals co-grazing with other animals in the pasture? If yes, could you precise which ones?

6. Did you give any diet supplementation to this group? Could you specify what (Coccidiostats, Se, Ionophore, Concentrate…)?

7. Did the calves change pasture during this period? If yes, could you specify how many times? Could you explain why?

8. If your calves changed pasture during this period, please could you estimate the date they moved and the type of pasture they went on?

Change	Date of change	Type of pasture (*Fresh/Grazed/ Mowed*)	If previously grazed, by what?	If mowed, how much (%)?	Percentage of manure spread on it?	Other
1						
2						
3						

9. Did you have any new animals introduced in the group of weaned calves to 12 months during this period-could you please specify?

10. If new calves were bought from outside joined the group, did you apply any quarantine? If yes, for how long? If no, could you explain why?

11. Did you give any anthelmintic treatments to this group during this period?

Product name	Form of the treatment	Start of the treatment	Frequency of the treatment	Dosing of the treatment

12. Regarding the treatments you just detailed to me, could you please say:
    a. What make you decide to start an anthelmintic treatment?

b. How do you dose your products?

c. Why do you specifically use these products?

d. Do you feel that you understand what these products are doing? Did anyone explain it to you?

e. Do you have any preferences on these products and forms? Could you detail on that?

Appendix 6: Cover letter and instructions for the spring bulk tank milk sampling (dairy longitudinal study): example of the first sampling

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

QMMS

Scientific Project on worm control in dairy heifers

Nottingham, June 2014

Re: Spring Bulk Tank Milk Sampling and 2014 Grazing season telephone follow-up

Dear Mr X,

As discussed with you at the farm visit, you will find enclosed with this letter a tube for you to take a sample of your bulk tank milk. The tube is provided with a document detailing sampling protocol, along with a cotton wool wad, a zip lock bag, and a self-addressed jiffy envelope, for you to send it back as soon as possible to QMMS. As previously discussed, the sample will thus be tested for both Ostertagia and Fluke, as part of the survey of your herd's worm burden.
Please note that the sample must be sent by the 30th of June. This is crucial for us to accurately monitor, for this grazing season, the spring worm burden of your herd. We will send you another tube next autumn and all the sample results will be provided to you after the end of the study.

As previously mentioned, you will also find enclosed a short questionnaire. The aim of this questionnaire is to gather information on the grazing season practices you will have for your calves and heifers over this year (2014). As discussed, I will telephone you every two to three months from end of June 2014 to end of April 2015 (end of the heifers' individual milk collection) and gather this information.
Please note that you don't have to fill this questionnaire in, but might find it useful to record the requested information.
The questions in this follow–up questionnaire are similar to the ones I asked during our first interview and grouped by three age categories.

I very much again appreciate your participation and help with this study. If you have further questions please do not hesitate to contact us using either the email camille.bellet@nottingham.ac.uk or phone 0746 289 4477.

Yours sincerely,

Camille Bellet (PhD Student)
Faculty of Medicine and Health Science
The University of Nottingham
Sutton Bonington LE12 5RD
United Kingdom
Tel: 07462894477

Email : camille.bellet@nottingham.ac.uk

Researchers involved: Dr Jasmeet Kaler, Prof Martin Green, Dr Andrew Bradley

Scientific Project on worm control in dairy heifers

Bulk Milk Tank Sampling Protocol

1. Please take a bulk milk sample when the tank contains milk from a complete day of milking (*i.e.* after 1 or 2 days if daily or every other day collection respectively) and after the milk has been stirred continuously for 5 minutes.

2. Please record the approximate number of litres in your tank in the table provided on the reverse of this page- **If you have only one tank, fill only the box "tube ID 1"; If you have >1 bulk tank, fill both tube ID 1 and tube ID 2, respectively.**

3. Please put the milk sample in the container supplied.
   NOTE: The container has a tiny pellet of the milk preservative Bronopol, **Please do not remove this**.

4. Screw top on container securely and invert several times, thoroughly mixing preservative pellet with milk.

5. Place the container inside the cotton wool wad and into the zip lock bag provided. Please place this bag, along with the completed Contact Information form on the reverse of this page, into the pre-addressed jiffy bag provided.

6. Ensure the jiffy bag is **securely sealed** and not likely to open up whilst in the post.

7. Please post sample **as soon as possible** – no stamp is required.
   NOTE: Keep jiffy bag and sample refrigerated until the sample is posted (**max 3 days**).

   Please complete the Contact Information details on the reverse of this page to ensure your sample is tested and you receive your results. Failure to complete the required Contact Information on the reverse of this page will at best delay the test and issue of results and at worst result in the sample being destroyed with no test completed or result given.

   For enquiries please call QMMS Customer services on 01749 871171.

   PLEASE NOTE: SAMPLES SHOULD BE SUBMITTED **BEFORE END of JUNE**.

   PLEASE NOTE: SAMPLE RESULTS WILL BE PROVIDED **AFTER THE END OF THE STUDY**.

Fields marked * indicate required information and must be completed IN FULL.

Please complete the details in BLOCK CAPITALS.

Tank / Tube ID	Milk Volume	Tank / Tube ID	Milk Volume
1		2	

Farm information

*Title (Mr/Mrs/Ms)		Farm Name	
*First Name		*Farm Address	
*Surname			
Telephone		*Town	
*Mobile		*County	
		*Postcode	
*E-mail Address			
Results will be e-mailed to you unless this postal request box is ticked ☐			

SQP information (if relevant)

*Title (Mr/Mrs/Ms)		*Company Name	
*First Name		*Company Address	
*Surname			
Telephone		*Town	
*Mobile		*County	
		*Postcode	
*E-mail Address			
Results will be e-mailed to you unless this postal request box is ticked ☐			

Vet information (please source this information directly with the farmer)

*Title (Mr/Mrs/Ms)		*Practice Name	
*First Name		*Practice Address	
*Surname			
Telephone		*Town	
*Mobile		*County	
		*Postcode	
*E-mail Address			
Results will be e-mailed to you unless this postal request box is ticked ☐			

▫ Please tick this box if you wish your vet and or SQP to be informed of the study results.

Appendix 7: Description of heifer grazing management variables by grazing season ($N_{Heifers}$=1,454; N_{Farms}=41)

	First grazing season ($N_{Heifers}$=1,454)		Second grazing season ($N_{Heifers}$=1,121)		Third grazing season ($N_{Heifers}$=206)	
Variable	**N (%)**	**Median (p25-p75)[a] Mean (SE)[a]**	**N (%)**	**Median (p25-p75)[a] Mean (SE)[a]**	**N (%)**	**Median (p25-p75)[a] Mean (SE)[a]**
Month of turn-out	1,453 (99.9)		1,121 (100.0)		206 (100)	
Season of turn-out	1,453 (99.9)		1,121 (100.0)		206 (100.0)	
Spring	920 (63.2)		1,060 (94.6)		198 (96.1)	
Summer	519 (35.7)		61 (5.4)		8 (3.9)	
Autumn	14 (1.0)					
Age at turn-out (m)	1,454 (100)	9.5 (6.9-13.6)	1,121 (100.0)	18.0 (12.5-21.5)	206 (100.0)	28.1 (25.7-29.9)
<6	280 (19.3)		≤15 139 (12.4)		≤25 40 (19.4)	
>6	1173 (80.7)		>15 982 (88.2)		>25 166 (80.6)	
Time grazing (d)	1,452 (99.9)	165 (124-199)	1,121 (100)	186 (153-211)	206 (100.0)	149 (89-182)
Pasture grazed (N)	1,429 (98.3)	3 (2-6)	1,117 (99.6)	3 (2-7)	206 (100.0)	3 (1-6)
≤10	1,236 (85.0)					
>10	193 (13.3)					
Average pasture size (ac)	1,429 (98.3)	12.8 (7.1-20.0)	1,117 (99.6)	17.0 (10.0-22.3)	206 (100.0)	12.6 (8.6-19.8)
Minimum pasture size (ac)	1,429 (98.3)	7.0 (4.0-12.0)	1,117 (99.6)	10.0 (6.0-17.0)	206 (100.0)	8.0 (6.0-15.0)
			≤6 291 (26.0)			
			>6 826 (73.7)			
Maximum pasture size (ac)	1,429 (98.3)	19.8 (12.0-30.0)	1,117 (99.6)	20.0 (15.0-32.0)	206 (100.0)	19.0 (12.4-22.0)
≤30	1,109 (76.3)		808 (72.1)		≤10 31 (15.0)	
>30	320 (22.0)		309 (27.6)		>10 175 (85.0)	
Average time on pasture (w)	1,453 (99.9)	6.8 (3.1-10.7)	1,121 (100.0)	6.8 (3.0-12.4)	206 (100.0)	4.0 (2.5-6.9)
			≤12 834 (74.4)		≤4.5 121 (58.7)	
			>12 287 (25.6)		>4.5 85 (41.3)	
Minimum time on pasture (w)	1,453 (99.9)	3.0 (1.5-6.0)	1,121 (100.0)	2.8 (1.5-8.0)	206 (100.0)	2.0 (1.5-4.9)
≤10	1,226 (84.3)		≤8 842 (75.1)		≤4.5 151 (73.3)	
10-20	143 (9.8)		>8 279 (24.9)		>4.5 55 (26.7)	
>20	84 (5.8)					

Maximum time on pasture (w)	1,453 (99.9)	10.0 (4.4-16.0)		1,121 (100.0)	10.5 (4.5-17.0)		206 (100.0)	6.0 (3.0-9.6)
Average stocking rate (an/ac)	1,429 (98.3)	2.8 (1.7-4.4)		1,117 (99.6)	2.1 (1.4- 3.4)		206 (100.0)	2.8 (1.6-4.0)
			<3	737 (65.7)		<1.5	43 (20.9)	
			≥3	380 (33.9)		≥1.5	163 (79.1)	
Minimum stocking rate (an/ac)	1,429 (98.3)	1.7 (1.0-2.3)		1,117 (99.6)	1.2 (0.8-1.7)		206 (100.0)	1.6 (1.0-2.0)
			<3	1,034 (92.2)		<1	48 (23.3)	
			≥3	83 (7.4)		≥1	158 (76.7)	
Maximum stocking rate (an/ac)	1,429 (98.3)	4.0 (2.4-7.3)		1,117 (99.6)	3.5 (1.9-4.9)		206 (100.0)	3.7 (2.5-5.8)
<3	438 (30.1)			473 (42.2)				
≥3	991 (68.2)			644 (57.4)				
Pasture contamination (cows)	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	965 (66.4)			623 (55.6)			59 (28.6)	
Yes	488 (33.6)			498 (44.4)			147 (71.4)	
Pasture contamination (sheep)	1,451 (99.8)			1,121 (100.0)			206 (100.0)	
No	781 (53.7)			639 (57.0)			152 (73.8)	
Yes	670 (46.1)			482 (43.0)			54 (26.2)	
Pasture contamination (YS)[(b)]	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	21 (1.4)			7 (1.0)			9 (04.4)	
Yes	1,432 (98.4)			1,114 (99.4)			197 (95.6)	
Co-grazing (cows)	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	1,436 (98.8)			953 (85.0)			149 (72.3)	
Yes	17 (1.2)			168 (15.0)			57 (27.7)	
Co-grazing (sheep)	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	1,408 (96.9)			1,025 (91.4)			192 (93.2)	
Yes	45 (3.1)			96 (8.6)			14 (6.8)	
Co-grazing (YS)[(b)]	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	833 (57.2)			644 (57.4)			137 (66.5)	
Yes	620 (42.6)			478 (42.6)			69 (33.5)	
Co-grazing (bull)	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	1,268 (87.3)			571 (50.9)			142 (68.9)	
Yes	185 (12.7)			551 (49.2)			64 (31.1)	
Field mowed (N)	1,391 (95.7)	2.0 (0.7-5.0)		1,108 (98.8)	1 (0-3)		205 (99.5)	0 (0-2)

	≤5.5	1,102 (75.8)							
	>5.5	289 (19.9)							
Field fertilized (N)		1,356 (93.3)	3.0 (1.0-7.0)		1,100 (98.1)	3 (1-7)		205 (99.5)	2 (0-7)
							≤7	180 (87.4)	
							>7	25 (12.1)	
Field with manure (N)		1,369 (94.2)	1.0 (0.0-3.0)		1,114 (99.4)	1 (0-3)		206 (100.0)	1 (0-2)
Anthelmintic treatments		1,453 (99.9)			1,089 (97.1)			199 (96.6)	
	No	187 (12.9)			386 (34.4)			130 (63.1)	
	Yes	1,266 (87.1)			703 (62.7)			69 (33.5)	
Anthelmintic treatments (N)		1,453 (99.9)	2 (1- 2)		1,096 (97.8)	1 (0-2)		199 (96.6)	0 (0-1)
				<2	782 (69.8)				
				≥2	314 (28.0)				
Anthelmintic form (N)		1,433 (98.6)	1.1(0.02)		1,096 (97.8)	0.7 (0.02)		190 (92.2)	0 (0-1)
	0	187 (13.1)			386 (34.4)			119 (57.8)	
	1	911 (63.6)			600 (53.5)			68 (33.0)	
	2	335 (23.4)			110 (9.8)			3 (1.5)	
Anthelmintic class (N)		1,453 (99.9)	1.4 (0.02)		1,096 (97.8)	1 (0- 1)		190 (92.2)	0 (0-1)
	0	187 (12.9)		≤3	1,054 (94.0)				
	1	659 (45.4)		>3	42 (3.7)				
	2	500 (34.4)							
	3	107 (7.4)							
Pour-on		1,433 (98.6)			1,096 (97.8)			190 (92.2)	
	No	599 (41.2)			534 (47.6)			134 (65.0)	
	Yes	834 (57.4)			562 (50.1)			56 (27.2)	
Drench		1,433 (98.6)			1,096 (97.8)			190 (92.2)	
	No	1,361 (95.0)			998 (89.0)			177 (85.9)	
	Yes	72 (5.0)			98 (8.7)			13 (6.3)	
Injection		1,433 (98.6)			1,096 (97.8)			190 (92.2)	
	No	1,045 (72.9)			964 (86.0)			188 (91.3)	
	Yes	388 (27.1)			132 (11.8)			2 (1.0)	
Bolus		1,433 (98.6)			1,096 (97.8)			190 (92.2)	
	No	1,146 (80.0)			1,068 (95.3)			190 (92.2)	

	Yes	287 (20.0)	28 (2.5)	0 (0)
Fenbendazole		1,453 (99.9)	1,096 (97.8)	-
	No	1,255 (86.4)	1,071 (95.5)	
	Yes	198 (13.6)	25 (2.2)	
Triclabendazole		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,402 (96.6)	976 (87.1)	185 (89.8)
	Yes	50 (3.4)	120 (10.7)	14 (6.8)
Oxfendazole		1,452 (99.9)	1,096 (97.8)	-
	No	1,363 (93.9)	1,091 (97.3)	
	Yes	89 (6.1)	5 (0.4)	
Levamisole		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,305 (89.9)	1,014 (90.5)	194 (13.4)
	Yes	147 (10.1)	82 (7.3)	5 (0.3)
Ivermectin		1,453 (99.9)	1,096 (97.8)	199 (96.6)
	No	706 (48.6)	697 (62.2)	181 (91.0)
	Yes	747 (51.4)	399 (35.6)	18 (8.7)
Moxidectin		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,104 (76.0)	905 (80.7)	182 (88.3)
	Yes	348 (24.0)	191 (17.0)	17 (8.3)
Eprinomectin		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,436 (98.9)	977 (87.2)	173 (84.0)
	Yes	16 (1.1)	119 (10.6)	26 (12.6)
Doramectin		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,431 (98.6)	1,076 (96.0)	196 (95.1)
	Yes	21 (1.4)	20 (1.8)	3 (1.5)
Closantel		1,452 (99.9)	1,096 (97.8)	199 (96.6)
	No	1,222 (84.2)	1,025 (91.4)	196 (95.1)
	Yes	230 (15.8)	71 (6.3)	3 (1.5)
Clorsulon		1,452 (99.9)	1,096 (97.8)	-
	No	1,395 (96.1)	1,095 (97.7)	
	Yes	57 (3.9)	1 (0.1)	
Nitroxynil		1,452 (99.9)	1,096 (97.8)	-

No	1,387 (95.5)			1,064 (94.9)				
Yes	65 (4.5)			32 (2.9)				
Oxyclozanide	1,452 (99.9)			1,096 (97.8)			199 (96.6)	
No	1,447 (99.7)			1,088 (97.1)			198 (96.1)	
Yes	5 (0.3)			8 (0.7)			1 (0.5)	
Treatment (turn-out)	1,453 (99.9)			1,096 (97.8)			186 (90.3)	
No	930 (64.0)			932 (83.1)			165 (80.1)	
Yes	523 (36.0)			164 (14.6)			21 (10.2)	
Treatment (grazing)	1,453 (99.9)			1,096 (97.8)			186 (90.3)	
No	844 (58.1)			669 (59.7)			146 (70.9)	
Yes	609 (41.9)			427 (38.1)			40 (19.4)	
Treatment (housing)	1,453 (99.9)			1,096 (97.8)			186 (90.3)	
No	521 (35.9)			644 (57.4)			150 (72.8)	
Yes	931 (64.1)			452 (40.3)			36 (17.5)	
No housing	1 (0.1)			-			-	
Diet supplementation	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
No	326 (22.4)			719 (64.1)			175 (85.0)	
Yes	1,127 (77.6)			402 (35.9)			31 (15.0)	
Month of housing	1,453 (99.9)			1,121 (100.0)			206 (100.0)	
Season of housing	1,453 (99.9)			1,455 (100.0)			206 (100.0)	
Spring	4 (0.3)			16 (1.4)			18 (8.7)	
Summer	105 (7.2)			179 (16.0)			74 (35.9)	
Autumn	1,156 (79.6)			747 (66.6)			109 (52.9)	
Winter	187 (12.9)			179 (16.0)			5 (2.4)	
No housing	1 (0.1)			-			-	
Age at housing (m)	1,453 (99.9)	15.2 (12.0-19.1)		1,121 (100.0)	25.2 (23.1-27.9)		206 (100.0)	32.5 (29.3-35.0)
≤12	367 (25.2)		≤20	69 (6.2)		≤34	134 (65.0)	
>12	1,085 (74.6)		>20	1,052 (93.8)		>34	72 (35.0)	
No housing	1 (0.1)							

[(a)]SE=standard error; p25-p75=25th-75th percentiles;

[(b)]YS= young-stock

Appendix 8: Univariable multilevel linear regression models of association between heifer individual milk ODR and demographic and management variables as fixed effects ($N_{Heifers}$= 1,454; N_{Farms}= 41)

- *Farmer and farm demographics, including variables on production system, calving system, housing, vaccination and cows anthelmintic treatment*

Variable		N (%)	Median (p25-p75)[(a)]	β	95% CI[(a)]
Farmer gender		1,454 (100.0)			
	Male	1,278 (87.9)		ref	-
	Female	176 (12.1)		0.25*	0.17;0.34
Prime manager		1,454 (100.0)			
	No	1,316 (90.5)		ref	-
	Yes	138 (9.5)		0.11	-0.08;0.30
Farmer age (y)		1,454 (100.0)	46 (39-53)		
	19-35	238 (16.4)		ref	-
	36-48	770 (53.0)		-0.08	-0.23;0.07
	51-63	446 (30.7)		0.10	-0.04;0.25
Farmer age at start of his dairy activity (y)		1,454 (100.0)	17 (14-22)		
	<20	1,019 (70.1)		ref	-
	≥20	435 (29.9)		0.21*	0.13;0.30
Dairy family		1,454 (100.0)			
	No	138 (9.5)		ref	-
	Yes	1,316 (90.5)		-0.02	-0.16;0.21
Farmer education in agro-farming		1,454 (100.0)			
	No	558 (38.4)		ref	-
	Yes	803 (55.2)		-0.15*	-0.24;-0.05
	Training	93 (6.4)		-0.19*	-0.38;-0.01
Age of the dairy facilities (y)		1,454 (100.0)	65 (47-108)		
	≤50	365 (25.1)		ref	-
	>50	1,089 (74.9)		-0.12*	-0.21;-0.02
System of production		1,454 (100.0)			
	Conventional	1,162 (79.9)		ref	-
	Integrate	164 (11.3)		0.09	-0.03;0.20
	Organic	128 (8.8)		0.32*	0.24;0.39
Type of cattle production		1,454 (100.0)			
	Dairy only	917 (63.1)		ref	-
	Dairy and beef	537 (36.9)		-0.10	-0.21;0.01
Sheep production		4 (100.0)			
	No	1,236 (85.0)		ref	-
	Yes	218 (15.0)		-0.04	-0.18;0.11
Other type of production		1,454 (100.0)			
	No	1,284 (88.3)		ref	-
	Yes	170 (11.7)		-0.09	-0.24;0.06

Staff (N)		1,454 (100.0)	5 (4-8)	-0.02*	-0.03;-0.01
Full-time staff (N)		1,454 (100.0)	4 (2-4)	-0.01	-0.03;0.02
Part-time staff (N)		1,454 (100.0)	2 (1-4)	-0.02*	-0.04;-4E-3
Casual staff (N)		1,454 (100.0)	0 (0-1)	-0.10*	-0.17;-0.03
Size of the milking herd (march 2014)		1,454 (100.0)	230 (122-420)	-5E-4*	-8E-4;-2E-4[(a)]
Preweaned calves (march 2014) (N)		1,454 (100.0)	30 (12-60)	-3E-3*	-5E-3;-1E-3
Calves (march 2014) (N)		1,454 (100.0)	60 (42-131)	-2E-3*	-2E-3;-7E-4
Bulling heifers (march 2014) (N)		1,454 (100.0)	49 (27-65)		
	≤50	910 (62.6)		ref	-
	>50	544 (37.4)		-0.21*	-0.32;-0.10
In-calf heifers (march 2014) (N)		1,454 (100.0)	46 (32-100)	-2E-3*	-4E-3;-4E-5
First lactation heifers (march 2014) (N)		1,454 (100.0)	72 (42-150)	-9E-4*	-2E-3;-1E-4
Mature bull (march 2014) (N)		1,454 (100.0)	1 (1-1)	0.01	-0.05;0.07
Heifers sent away during their grazing		1,454 (100.0)			
	No	1,130 (77.7)		ref	-
	Yes	324 (22.3)		-0.18*	-0.30;-0.05
Age at first turn-out (m) (questionnaire)		1,454 (100.0)	5 (4-8)	1E-3	-3E-3;5E-3
Minimal age at first turn-out (m) (interview)		1,454 (100.0)	9.5 (6.9-13.6)	-1E-3	-5E-3;3E-3
Farm altitude (m)		1,454 (100.0)	50 (19-117)	2E-3*	9E-4;2E-3
Total grazing surface (ha)		1,454 (100.0)	200 (150-370)	5E-4*	3E-4;7E-4
Type of soils on farm (N)		1,454 (100.0)	1 (1-2)	0.12	0.07;0.16
Heavy soil		1,454 (100.0)			
	No	833 (57.3)		ref	-
	Yes	621 (42.7)		-0.21*	-0.30;-0.11
Medium soil		1,454 (100.0)			
	No	710 (48.8)		ref	-
	Yes	744 (51.2)		0.16*	0.06;0.25
Sandy silty soil		1,454 (100.0)			
	No	1,123 (77.2)		ref	-
	Yes	331 (22.8)		0.22*	0.14;0.31
Clay limestone soil		1,454 (100.0)			
	No	1,192 (82.0)		ref	-
	Yes	262 (18.0)		0.19*	0.10;0.28
Peaty soil		1,454 (100.0)			
	No	1,333 (91.7)		ref	-
	Yes	121 (8.3)		-0.04	-0.23;0.15
Clay soil		1,454 (100.0)			
	No	1,330 (91.5)		ref	-
	Yes	124 (8.5)		-0.02	-0.20;0.17
Scrub soil		1,454 (100.0)			
	No	1,442 (99.2)		ref	-
	Yes	12 (0.8)		0.30*	-0.07;0.67
Calving system		1,454 (100.0)			
	All year round	1045 (71.9)		ref	-
	Block	278 (19.1)		0.17*	0.08; 0.27
	More than 3 months	131 (9.0)		0.01	-0.14; 0.16
Cow breeding system		1,454 (100.0)			
	AI only	925 (63.6)		ref	-
	AI and natural breeding	529 (36.4)		-0.09	-0.20; 0.02
Heifer breeding system		1,454 (100.0)			

AI only	338 (23.2)		ref	-
Natural breeding only	111 (7.6)		0.16	-8E-3;0.32
AI and natural breeding	1,005 (69.1)		0.19*	0.06;0.31
Age targeted by farmers at first breeding (m)	1,454 (100.0)	15 (14-17)	0.06*	0.04;0.08
Age at first breeding (m) (TotalVet)	1,158 (79.6)	16 (14-18)	0.01*	3E-3;0.01
Breeding place	1,454 (100.0)			
In	510 (35.1)		ref	-
Seasonal	944 (64.9)		0.24*	0.13;0.35
Winter/Summer breeding system	1,454 (100.0)			
Same	1,269 (87.2)		ref	-
Different	185 (12.7)		-0.02	-0.17;0.13
Visual detection	1,454 (100.0)			
No	441 (30.3)		ref	-
Yes	1,013 (69.7)		0.11	-0.015;0.22
Heat mount detectors	1,454 (100.0)			
No	1,089 (74.9)		ref	-
Yes	365 (25.1)		0.24*	0.16;0.32
Tail head markers	1,454 (100.0)			
No	649 (44.6)		ref	-
Yes	805 (55.4)		-0.18*	-0.27;-0.09
Vasectomised bull	1,454 (100.0)			
No	1,442 (99.2)		ref	-
Yes	12 (0.8)		-0.07	-0.45;0.31
Electronic heat detection devices	1,454 (100.0)			
No	673 (46.3)		ref	-
Yes	781 (53.7)		-0.21*	-0.30;-0.12
Insemination at first breeding (TotalVet) (N)	1,255 (86.3)	1 (1-2)	-0.01	-0.02;0.01
Exchange of bull between cows and heifers	1,204 (82.8)			
No	614 (42.2)		ref	-
Yes	590 (40.6)		-0.16*	-0.25;-0.06
Pregnancy diagnosis (PD) test	1,180 (81.2)			
No	167 (11.5)		ref	-
Yes	1,013 (69.7)		-0.01	-0.09;0.08
PD test at first breeding (TotalVet) (N)	1,161 (79.8)	1 (1-1)	0.01	-0.02;0.04
Calves housing	1,454 (100.0)			
Winter only	1,201 (82.6)		ref	-
All year round	215 (14.8)		-0.23*	-0.38;-0.07
Kale	38 (2.6)		-0.26	-0.55;0.04
Calves mixed with other group of age	1,454 (100.0)			
No	683 (47.0)		ref	-
Young-stock	166 (11.4)		-0.07	-0.24;0.11
Beef	600 (41.3)		0.17*	0.08;0.26
Vary	5 (0.3)		0.12	-0.27;0.51
Male and female calves housed together	1,454 (100.0)			
No	673 (46.3)		ref	-
Yes	780 (53.6)		0.18*	0.09;0.28
Vary	1 (0.1)		-0.01	-0.59;0.57
Calves concentrate at housing	1,454 (100.0)			
No	475 (32.7)		ref	-
Yes	979 (67.3)		-0.16*	-0.25;-0.07

Coccidiostat for calves at housing	1,454 (100)		
No	1,159 (79.7)	ref	-
Yes	295 (20.3)	-0.14	-0.28;3E-3
Bulling heifers housing	1,454 (100.0)		
Winter only	1,114 (76.6)	ref	-
All year round	303 (20.8)	-0.37*	-0.50;-0.24
Kale	37 (2.5)	-0.29*	-0.57;-0.01
Bulling heifers mixed with other group of age	1,454 (100.0)		
No	333 (22.9)	ref	-
Young-stock	357 (24.6)	0.21*	0.08;0.34
Beef	29 (2.0)	0.28*	-0.06;0.62
Bull	426 (29.3)	0.05	-0.10;0.19
Vary	309 (21.3)	0.22*	0.09;0.35
Bulling heifers DS[(b)] at housing	1,454 (100.0)		
No	581 (40.0)	ref	-
Yes	873 (60.0)	-0.15*	-0.24;-0.06
In-calf heifers housing	1,454 (100.0)		
Winter only	1,417 (97.5)	ref	-
Kale	37 (2.5)	-0.27	-0.63;0.08
In-calf heifers mixed with other group of age	1,454 (100.0)		
No	136 (9.4)	ref	-
Milking cows	350 (24.1)	0.12	-0.11;0.35
Dry cows	760 (52.3)	0.27*	0.06;0.47
Vary	208 (14.3)	0.22	-2E-3;0.44
In-calf heifers DS[(b)] at housing	1,454 (100.0)		
No	907 (62.4)	ref	-
Yes	547 (37.6)	-0.09	-0.19;0.02
Cows housing	1,454 (100.0)		
Winter only	906 (62.3)	ref	-
All year round	289 (19.9)	-0.29*	-0.44;-0.13
Only low yield out	259 (17.8)	-0.24*	-0.38;-0.10
Rispoval RS+PI3 Intranasal	1,409 (96.9)		
No	1,185 (81.5)	ref	-
Yes	224 (15.4)	-0.23*	-0.38;-0.07
Bovilis Bovipast RSP	1,453 (99.9)		
No	1,391 (95.7)	ref	-
Yes	62 (4.3)	0.03	-0.12;0.17
Rispoval 4	1,429 (98.3)		
No	1,304 (89.7)	ref	-
Yes	125 (8.6)	-0.06	-0.18;0.05
Rispoval Pasteurella	1,454 (100.0)		
No	1,426 (98.1)	ref	-
Yes	28 (1.9)	0.03	-0.09;0.14
BVD	1,454 (100.0)		
No	372 (25.6)	ref	-
Yes	1,082 (74.4)	0.12*	5E-3;0.154
BVD first vaccine in calves	1,454 (100.0)		
No	951 (65.4)	ref	-
Yes	499 (34.3)	-0.18*	-0.29;-0.07
BVD first vaccine in bulling heifers	1,454 (100.0)		

	No	874 (60.2)	ref	-
	Yes	578 (39.8)	0.20*	0.11;0.28
BVD first vaccine in in-calf heifers		1,452 (99.9)		
	No	1,451 (99.8)	ref	-
	Yes	1 (0.1)	-0.14	-0.73;0.46
IBR		1,454 (100.0)		
	No	581 (40.0)	ref	-
	Yes	873 (60.0)	-0.13*	-0.23;-0.03
IBR first vaccine in calves		1,451 (99.8)		
	No	1,289 (88.7)	ref	-
	Yes	162 (11.1)	0.03	-0.14;0.20
IBR first vaccine in bulling heifers		1,452 (99.9)		
	No	1,076 (74.0)	ref	-
	Yes	376 (25.9)	-0.18*	-0.30;-0.05
IBR first vaccine in in-calf heifers		1,452 (99.9)		
	No	1,316 (90.5)	ref	-
	Yes	136 (9.4)	-0.06	-0.27;0.15
Clostridial		1,392 (95.7)		
	No	1,094 (75.2)	ref	-
	Yes	298 (20.5)	0.19*	0.10;0.28
Leptospirosis		1,454 (100.0)		
	No	327 (22.5)	ref	-
	Yes	1,127 (77.5)	0.14*	0.03;0.25
Leptospirosis first vaccine in calves		1,451 (99.8)		
	No	909 (62.5)	ref	-
	Yes	542 (37.2)	0.14*	0.05;0.23
Leptospirosis first vaccine in bulling heifers		1,452 (99.9)		
	No	954 (65.6)	ref	-
	Yes	498 (34.3)	-0.07	-0.18;0.04
Leptospirosis first vaccine in in-calf heifers		1,452 (99.9)		
	No	1,417 (97.5)	ref	-
	Yes	35 (2.4)	-0.09	-0.40;0.22
Leptospirosis first vaccine in cows		1,454 (100.0)		
	No	1,405 (96.6)	ref	-
	Yes	49 (3.4)	-0.02	-0.28;0.24
Huskvac		1,452 (99.9)		
	No	946 (65.0)	ref	-
	Yes	506 (34.8)	0.16*	0.07;0.25
Huskvac first vaccine in calves		1,448 (99.6)		
	No	1,090 (75.0)	ref	-
	Yes	358 (24.6)	0.19*	0.10;0.28
Huskvac first vaccine in bulling heifers		1,452 (99.9)		
	No	1,369 (94.2)	ref	-
	Yes	83 (5.7)	0.15*	0.05;0.24
Huskvac first vaccine in in-calf heifers		1,452 (99.9)		
	No	1,391 (95.7)	ref	-
	Yes	61 (4.2)	-0.20*	-0.30;-0.10
Salmonella		1,454 (100.0)		
	No	1,327 (91.3)	ref	-
	Yes	127 (8.7)	-0.10	-0.34;0.13

Cow anthelminthic treatment		1,454 (100.0)	0 (0-1)		
	No	851 (58.5)		ref	-
	Always done	353 (24.3)		0.07	-0.03;0.17
	Depend	250 (17.2)		-0.04	-0.24;0.06
Cow anthelminthic form (N)		1,396 (96.0)	0 (0-1)		
	0	851 (58.5)		ref	-
	1	365 (25.1)		0.06	-0.04;0.15
	2	180 (12.4)		-0.02	-0.18;0.15
Cow anthelminthic class (N)		1,334 (91.7)	0 (0-1)		
	0	851 (58.5)		ref	-
	1	394 (27.1)		0.06*	-0.04;0.16
	2	89 (6.1)		-0.06	-0.27;0.16
Pour-on in cows		1,396 (96.0)			
	No	1,240 (85.3)		ref	-
	Yes	156 (10.7)		-0.10	-0.26;0.07
Drench in cows		1,396 (96.0)			
	No	1,081 (74.3)		ref	-
	Yes	315 (21.7)		0.10*	0.01;0.20
Injection in cows		1,396 (96.0)			
	No	1,142 (78.5)		ref	-
	Yes	254 (17.5)		-0.09	-0.23;0.06
Eprinomectin in cows		1,334 (91.7)			
	No	1,269 (87.3)		ref	-
	Yes	65 (4.5)		-0.18	-0.39;0.03
Oxyclozanide in cows		1,334 (91.7)			
	No	1,139 (78.3)		ref	-
	Yes	195 (13.4)		0.01	-0.15;0.17
Nitroxynil in cows		1,334 (91.7)			
	No	1,233 (84.8)		ref	-
	Yes	101 (6.9)		-0.01	-0.22;0.20
Triclabendazole in cows		1,334 (91.7)			
	No	1,277 (87.8)		ref	-
	Yes	57 (3.9)		-0.03	-0.25;0.19
Ivermectin in cows		1,334 (91.7)			
	No	1,243 (85.5)		ref	-
	Yes	91 (6.3)		-0.19	-0.44;0.06
Albendazole in cows		1,334 (91.7)			
	No	1,271 (87.4)		ref	-
	Yes	63 (4.3)		0.15*	0.03;0.26

[(a)]CI=confidence interval; p25-p75=25th-75th percentiles; $E^x = 10^x$; *=significant (P-value≤0.05);

[(b)]DS= diet supplementation

Appendices

- *Pre-weaned calves management variables*

Variable		N (%)	Median (p25-p75)[(a)]	β	95% CI[(a)]
Season of birth		1,454 (100.0)			
	Spring	318 (21.9)		ref	-
	Summer	375 (25.8)		0.05*	7E-3;0.09[(a)]
	Autumn	410 (28.2)		0.03	-0.01;0.06
	Winter	351 (24.1)		0.01	-0.03;0.05
Year of birth		1,454 (100.0)			
	2010	12 (0.8)		ref	-
	2011	384 (26.4)		0.04	-0.11;0.20
	2012	1,013 (69.7)		-0.01	-0.16;0.14
	2013	45 (13.1)		-0.07	-0.24;0.10
Place of birth		1,407 (96.8)			
	In	1,198 (82.4)		ref	-
	Out	209 (14.4)		0.01	-0.05;0.08
Cows calving in individual pen		1,454 (100.0)			
	No	1,204 (82.8)		ref	-
	Yes	182 (12.5)		-0.09	-0.25;0.06
	Vary	68 (4.7)		-0.40*	-0.64;-0.17
Time of separation from the dam (h)		1,454 (100.0)			
	0	38 (2.6)		ref	-
	<12	775 (53.3)		-0.07	-0.40;0.26
	12-24	336 (23.1)		0.15	-0.18;0.48
	>24	305 (21.0)		-0.01	-0.34;0.33
Unassisted colostrum feeding		1,454 (100.0)			
	No	1,077 (74.1)		ref	-
	Yes	377 (25.9)		0.21*	0.12;0.29
Suckling assisted colostrum feeding		1,454 (100.0)			
	No	1,112 (76.5)		ref	-
	Yes	342 (23.5)		0.19*	0.10;0.27
Bucket-bottle colostrum feeding		1,454 (100.0)			
	No	551 (37.9)		ref	-
	Yes	903 (62.1)		0.14*	0.03;0.24
Oesophageal colostrum feeding		1,454 (100.0)			
	No	471 (32.4)		ref	-
	Yes	983 (67.6)		0.01	-0.09;0.12
Own dam colostrum		1,454 (100.0)			
	No	456 (31.4)		ref	-
	Yes	998 (68.6)		0.16*	0.06;0.26
Pooled colostrum (first lactation)		1,454 (100.0)			
	No	856 (58.9)		ref	-
	Yes	598 (41.1)		0.12*	0.03;0.21
Pooled colostrum (no first lactation)		1,454 (100.0)			
	No	1,323 (91.0)		ref	-
	Yes	131 (9.0)		-0.17	-0.38;0.04
Stored colostrum		1,454 (100.0)			
	No	1,156 (79.5)		ref	-
	Yes	298 (20.5)		-0.06	-0.19;0.07
Commercial colostrum substitute		1,454 (100.0)			
	No	1,387 (95.4)		ref	-

Yes	67 (4.6)		-0.09	-0.35;0.16
Colostrum tested on farm	1,454 (100.0)			
No	953 (65.5)		ref	-
Yes	384 (26.4)		-0.24*	-0.36;-0.12
Vary	117 (8.0)		-0.10	-0.34;0.13
Johne's disease tested on farm	1,454 (100.0)			
No	435 (29.9)		ref	-
Yes	1,019 (70.1)		-0.01	-0.07;0.06
Johne's tests since 2010 (N)	1,454 (100.0)	2 (0-3)	-0.03*	-0.04;-5E-3
Volume colostrum (first 6h) (l)	1,454 (100.0)			
1.5-2.5	413 (28.4)		ref	-
2.75-3.5	624 (42.9)		-0.14*	-0.24;-0.04
4-6	260 (17.9)		-0.17*	-0.30;-0.03
Unknown	157 (10.8)		0.17*	0.07;0.26
Volume colostrum (first 24h) (l)	1,454 (100.0)			
3-5	484 (33.3)		ref	-
5.5-6	535 (36.8)		-0.31*	-0.40;-0.22
7-8	358 (24.6)		-0.18*	-0.29;-0.08
Unknown	77 (5.3)		-0.02	-0.17;0.12
Pre-weaned calves (PWC) grouped	1,454 (100.0)			
No	1,155 (79.4)		ref	-
Yes	299 (20.6)		0.09	-0.03;0.20
Age of PWC at grouping (d)	1,155 (79.4)	7 (0-14)	-3E-3	-7E-3;9E-4
PWC per group (N)	1,155 (79.4)	8 (5-10)	2E-3	-2E-3;6E-3
Fresh cow milk	1,454 (100.0)			
No	761 (52.3)		ref	-
Yes	693 (47.7)		0.23*	0.15;0.32
Mastitis or antibiotic milk	1,454 (100.0)			
No	872 (60.0)		ref	-
Yes	582 (40.0)		0.20*	0.11;0.29
Milk replacer	1,454 (100.0)			
No	361 (24.8)		ref	-
Yes	1,093 (75.2)		-0.23*	-0.31;-0.15
Age at first ration (d)	1,454 (100.0)	7 (3-7)	-0.01	-0.02;3E-3
Age at first roughage (d)	1,454 (100.0)	6 (2-10)	-3E-4	-6E-3;6E-3
Age at first *ad libidum* water (d)	1,454 (100.0)	6 (2-10)	-1E-3	-3E-3;1E-3
DS[(b)] PWC	1,454 (100.0)			
No	369 (25.4)		ref	-
Yes	1,085 (74.6)		-0.19*	-0.28;-0.10
PWC concentrate	1,454 (100)			
No	710 (48.8)		ref	-
Yes	744 (51.2)		-0.10	-0.20;-4E-3
PWC coccidiostat	1,454 (100)			
No	1032 (71.0)		ref	-
Yes	422 (29.0)		-0.22*	-0.34;-0.09
Age at weaning (w)	1,454 (100.0)	8 (8-10)	0.05*	0.03;0.07

[(a)]CI=confidence interval; p25-p75=25th-75th percentiles; $E^x = 10^x$; *=significant (P-value≤0.05);

[(b)]PWC= pre-weaned calve; DS= diet supplementation

Appendices

- *Heifer grazing management variables by grazing season (for number and percentage of observations, see Appendix 7)*

	First grazing season (N=1,454)			Second grazing season (N=1,121)			Third grazing season (N=206)	
Variable	**β**	**95% CI[a]**		**β**	**95% CI[a]**		**β**	**95% CI[a]**
Month of turn-out	-0.03*	-0.04;-0.02		1E-4	-3E-4;5E-4[a]		-2E-4	-6E-4;2E-4
Season of turn-out								
Spring	ref	-		ref	-		ref	-
Summer	-0.08*	-0.11;-0.04		-0.09*	-0.18;1E-3		0.01	-0.19;0.21
Autumn	-0.10	-0.23;0.04		-	-		-	-
Age at turn-out (m)								
<6	ref	-	≤15	ref	-	≤25	ref	-
>6	-0.05*	-0.09;-0.01	>15	0.08*	0.03;0.13	>25	0.13*	0.03;0.22
Time of grazing (d)	6E-4*	2E-4;1E-3		1E-4	-1E-4;3E-4		-1E-4	-3E-4;1E-4
Pasture grazed (N)				4E-5	-4E-4;5E-4		-2E-4	-6E-4;2E-4
≤10	ref	-						
>10	0.06	-3E-3;0.12						
Average pasture size (ac)	2E-3*	4E-5;4E-3		2E-4	-2E-4;5E-4		-2E-4	-6E-4;2E-4
Minimum pasture size (ac)	1E-3	-1E-3;3E-3					-2E-4	-6E-4;2E-4
			≤6	ref	-			
			>6	0.09*	0.03;0.14			
Maximum pasture size (ac)	2E-3*	1E-3;3E-3		2E-4*	4E-6;4E-4			
≤30						≤10	ref	-
>30						>10	-0.09*	-0.20;-0.02
Average time on pasture (w)	4E-3*	5E-4;8E-3						
			≤12	ref	-	≤4.5	ref	-
			>12	0.05*	1E-3;0.09	>4.5	-0.05	-0.13;0.03
Minimum time on pasture (w)								
≤10	ref	-	≤8	ref	-	≤4.5	ref	-
10-20	-0.01	-0.08;0.05	>8	0.05*	2E-3;0.10	>4.5	-0.08*	-0.16;0.01
>20	0.08*	5E-3;0.15						
Maximum time on pasture (w)	0.01*	3E-3;9E-3		1E-4	-3E-4;5E-4		-2E-4	-6E-4;2E-4
Average stocking rate (an/ac)	-2E-3	-8E-3;5E-3						
			<3	ref	-	<1.5	ref	-
			≥3	-0.05*	-0.10;-0.01	≥1.5	-0.14*	-0.24;-0.04
Minimum stocking rate (an/ac)	0.01	-8E-3;0.03						
			<3	ref	-	<1	ref	-
			≥3	-0.11*	-0.18;-0.03	≥1	-0.10*	-0.20;-0.01
Maximum stocking rate (an/ac)							-2E-4	-6E-4; 2E-4
<3	ref	-		ref	-			
≥3	0.09*	0.04;0.15		-0.08*	-0.13;-0.03			
Pasture contamination (cows)								
No	ref	-		ref	-		ref	-
Yes	0.04	-0.01;0.09		-1E-3	-0.05;0.05		-0.04	-0.13;0.05
Pasture contamination (sheep)								
No	ref	-		ref	-		ref	-
Yes	0.09*	0.02; 0.15		0.11*	0.06; 0.16		-0.05	-0.15;0.04
Pasture contamination (YS)[b]								

	No	ref	-		ref	-		ref	-
	Yes	0.10	-0.06;0.26		0.02	-0.24;0.27		-0.12	-0.40;0.15
Co-grazing (cows)									
	No	ref	-		ref	-		ref	-
	Yes	0.04	-0.09;0.17		0.05	-0.02;0.13		-0.03	-0.14;0.07
Co-grazing (sheep)									
	No	ref	-		ref	-		ref	-
	Yes	0.02	-0.10;0.14		0.06	-0.04;0.16		-0.18*	-0.32;-0.03
Co-grazing (YS)[(b)]									
	No	ref	-		ref	-		ref	-
	Yes	0.01	-0.03;0.05		-1E-3	-0.05;0.05		-0.05	-0.13;0.04
Co-grazing (bull)									
	No	ref	-		ref	-		ref	-
	Yes	-0.01	-0.06;0.04		-0.01	-0.05;0.04		0.04	-0.05;0.13
Field mowed (N)					1E-4	-3E-4;5E-4		-2E-4	-6E-4;2E-4
	≤5.5	ref	-						
	>5.5	-0.06*	-0.11;-0.02						
Field fertilized (N)		1E-3	-3E-3;5E-3		-1E-4	-5E-4;3E-4			
							≤7	ref	-
							>7	0.17*	0.05;0.28
Field with manure (N)		-2E-3	-8E-3;4E-3		2E-5	-4E-4;5E-4		-2E-4	-6E-4;2E-4
Anthelmintic treatments									
	No	ref	-		ref	-		ref	-
	Yes	-0.10*	-0.16;-0.03		-0.05*	-0.09;-0.01		4E-3	-0.08;0.09
Anthelmintic treatments (N)		-6E-3	-0.02;0.01					-2E-4	-6E-4;2E-4
				<2	ref	-			
				≥2	-0.07*	-0.12;-0.03			
Anthelmintic form (N)									
	0	ref	-		ref	-		ref	-
	1	0.12*	0.03;0.21		-0.04	-0.08; 0.01		-0.02	-0.11;0.07
	2	0.02	-0.05;0.09		-0.09*	-0.15;-0.03		0.17	-0.12;0.46
Anthelmintic class (N)								-1E-4	-5E-4;3E-4
	0	ref	-						
	1	-0.09*	-0.15;-0.02	≤3	ref	-			
	2	-0.13*	-0.21;-0.05	>3	-0.36*	-0.71;-0.02			
	3								
Pour-on									
	No	ref	-		ref	-		ref	-
	Yes	-0.06	-0.12;5E-3		-0.07*	-0.12;-0.03		-1E-3	-0.09;0.09
Drench									
	No	ref	-		ref	-		ref	-
	Yes	-0.02	-0.15;0.11		0.06	-0.04;0.15		-0.05	-0.20;0.11
Injection									
	No	ref	-		ref	-		ref	-
	Yes	-0.02	-0.08;0.05		-0.08*	-0.14;-0.02		0.22	-0.13;0.56
Bolus								-	-
	No	ref	-		ref	-			
	Yes	-0.09	-0.20;0.01		0.09	-0.04;0.22			
Fenbendazole								-	-
	No	ref	-		ref	-			
	Yes	-0.15*	-0.29;-7E-3		0.11	-0.04;0.26			

Triclabendazole							
	No	ref	-	ref	-	ref	-
	Yes	0.04	-0.12;0.20	-0.01	-0.12;0.10	0.05	-0.10;0.20
Oxfendazole						-	-
	No	ref	-	ref	-		
	Yes	-0.03	-0.19;0.13	-0.07	-0.31;0.16		
Levamisole							
	No	ref	-	ref	-	ref	-
	Yes	1E-3	-0.10;0.10	-2E-3	-0.09;0.08	0.05	-0.18;0.28
Ivermectin							
	No	ref	-	ref	-	ref	-
	Yes	-0.01	-0.06;0.05	-0.02	-0.07;0.02	-0.03	-0.16;0.10
Moxidectin							
	No	ref	-	ref	-	ref	-
	Yes	-0.05	-0.13;0.03	-0.11*	-0.19;-0.03	-3E-3	-0.14;0.13
Eprinomectin							
	No	ref	-	ref	-	ref	-
	Yes	-0.02	-0.15;0.11	-0.11*	-0.18;-0.05	0.06	-0.08;0.19
Doramectin							
	No	ref	-	ref	-	ref	-
	Yes	-0.16	-0.45;0.13	-0.21	-0.57;0.15	0.14	-0.17;0.44
Closantel							
	No	ref	-	ref	-	ref	-
	Yes	-0.13*	-0.25;-0.02	-0.12*	-0.20;-0.04	0.24	-0.05;0.52
Clorsulon						-	-
	No	ref	-	ref	-		
	Yes	0.03	-0.09;0.15	0.05	-0.44;0.55		
Nitroxynil						-	-
	No	ref	-	ref	-		
	Yes	-0.03	-0.29;0.23	-0.03	-0.16;0.10		
Oxyclozanide							
	No	ref	-	ref	-	ref	-
	Yes	0.08	-0.16;0.32	-0.05	-0.24;0.15	-0.21	-0.70;0.29
Treatment (turn-out)							
	No	ref	-	ref	-	ref	-
	Yes	-0.13*	-0.19;-0.06	-0.09	-0.18;0.01	0.03	-0.10;0.16
Treatment (grazing)							
	No	ref	-	ref	-	ref	-
	Yes	-4E-3	-0.06;0.05	-0.06*	-0.11;-0.02	-0.04	-0.13;0.06
Treatment (housing)							
	No	ref	-	ref	-	ref	-
	Yes	0.01	-0.04;0.06	-0.05*	-0.09;-0.01	0.05	-0.06;0.16
	No housing	-0.13	-0.73;0.47				
Diet supplementation							
	No	ref	-	ref	-	ref	-
	Yes	0.03	-0.02;0.07	0.05*	8E-5;0.11	-0.02	-0.13;0.08
Month of housing		2E-4	-5E-3;5E-3	1E-4	-3E-4;5E-4	-2E-4	-6E-4;2E-4
Season of housing							
	Spring	ref	-	ref	-	ref	-
	Summer	-0.23	-0.48;0.02	0.23*	0.10;-0.37	-0.06	-0.08;0.19
	Autumn	-0.23	-0.48;0.02	0.22*	0.09;0.35	2E-3	-0.13;0.13

	Winter	-0.16*	-0.42;0.09		0.19	0.05;0.32		0.04	-0.21;0.30
	No housing	-0.34	-0.98;0.30						
Age at housing (m)									
	≤12	ref	-	≤20	ref	-	≤34	ref	-
	>12	0.04*	7E-3;0.08	>20	0.11*	0.03;0.18	>34	0.08*	2E-3;0.17
	No housing	-0.12	-0.71;0.49						

[(a)]CI=confidence interval; $E^x = 10^x$; *=significant (P-value≤0.05);

[(a)]YS= young-stock

Appendices

- *Heifer grazing management variables from first turn-out to sampling*

Variable		N (%)	Median (p25-p75)[a]	β	95% CI[a]
Total grazing season(s) (N)		1,453 (99.9)	2 (2-2)		
	1	250 (17.2)		ref	-
	2	852 (58.6)		0.08*	0.03;0.13
	3	340 (23.4)		0.08*	0.01;0.14
	4	11 (0.8)		0.09	-0.09;0.27
Coverage of Gr_1 (%)[b]		1,453 (99.9)	100 (100-100)	0.02	-0.09;0.13
Coverage of Gr_2 (%)[b]		1,454 (100.0)	94 (30-100)	0.03	-0.02;0.08
Coverage of Gr_3 (%)[b]		1,454 (100.0)	0 (0-0)	-0.06*	-0.11;-2E-4[a]
Coverage of Gr_4 (%)[b]		1,454 (100.0)	0 (0-0)	-0.06	-0.40;0.28
Total time of grazing (d)		1,453 (99.9)	332 (227-404)	2E-4*	4E-6;4E-4
Total time of co-grazing (cows) (d)		1,454 (100)			
	0	750 (51.6)		ref	-
	Milking and Dry >14	248 (17.1)		0.14*	0.08;0.20
	Dry ≤14	100 (6.9)		-0.02	-0.11;0.07
	Dry >14	104 (7.2)		0.17*	0.08;0.25
	Milking ≤14	59 (4.1)		0.12*	0.04;0.19
	Milking >14	193 (13.3)		0.10*	0.05;0.16
Season of turn-out		1,453 (99.9)			
	Spring only	916 (63.0)		ref	-
	Summer only	174 (12.0)		-0.08*	-0.14;-0.02
	Spring and summer	349 (24.0)		-0.08*	-0.12;-0.04
	Spring and autumn	14 (1.0)		-0.10	-0.23;0.04
Season of housing		1,453 (99.9)			
	Autumn only	769 (52.9)		ref	-
	Spring only	4 (0.3)		0.22	-0.02;0.47
	Summer only	99 (6.8)		-0.02	-0.08;0.04
	Winter only	43 (3.0)		0.06	-0.04;0.16
	Spring and summer	2 (0.1)		0.04	-0.30;0.39

	Spring and autumn	27 (1.9)		-0.17*	-0.27;-0.07
	Summer and autumn	210 (14.4)		0.03	-7E-3;0.08
	Summer and winter	3 (0.2)		0.30	-0.01;0.59
	Autumn and winter	271 (18.6)		3E-3	-0.05;0.06
	All	25 (1.7)		0.11	-7E-3;0.22
Total pasture grazed (N)		1,453 (99.9)	7 (3-15)		
	≤8	869 (59.8)		ref	-
	>8	584 (40.1)		-0.05*	-0.09;-4E-3
Average size of the pasture (ac)		1,425 (98.0)	15.0 (9.8-20.9)	2E-4	-6E-4;1E-3
Average of the minimum size of the pasture (ac)		1,425 (98.0)	8.6 (6.0-14.0)	1E-4	-1E-3;1E-3
Minimum size of the pasture (ac)		1,425 (98.0)	6.0 (4.0-10.0)	-7E-4	-2E-3;3E-4
Average of the maximum size of the pasture (ac)		1,425 (98.0)	20.0 (14.0-28.3)	2E-3*	7E-4;2E-3
Maximum size of the pasture (ac)		1,425 (98.0)	27.0 (15.0-36.0)	7E-4*	1E-4;1E-3
Average time spent on pasture (w)		1,454 (100.0)	6.5 (3.4-11.3)	-5E-4	-2E-3;9E-4
Average of the minimum time spent on pasture (w)		1,454 (100.0)	3.0 (1.8-8.8)	2E-3	-2E-3;5E-3
Minimum time spent on pasture (w)		1,454 (100.0)	2.0 (1.0-4.0)	-5E-4	-2E-3;9E-4
Average of the maximum time spent on pasture (w)		1,454 (100.0)	10.5 (6.3-15.1)	5E-3*	1E-3;9E-3
Maximum time spent on pasture (w)		1,454 (100.0)	13.0 (7.0-19.6)	-4E-4	-2E-3;8E-4
Average pasture stocking rate (an/ac)		1,425 (98.0)	2.5 (1.6-4.2)	-1E-3*	-3E-3;-3E-5
Average of the minimum pasture stocking rate (an/ac)		1,425 (98.0)	1.4 (1.0-2.0)	-6E-3	-0.02;0.01
Minimum pasture stocking rate (an/ac)		1,425 (98.0)	1.1 (0.8-1.7)	-1E-3	-02E-3;3E-4
Average of the maximum pasture stocking rate (an/ac)		1,425 (98.0)	3.8 (2.4-6.2)	-0.01*	-0.02;-3E-3
Maximum pasture stocking rate (an/ac)		1,425 (98.0)	4.4 (2.8-7.5)	-1E-3*	-3E-3;-3E-5
Pasture contamination (cows)		1,453 (99.9)			
	No	742 (51.0)		ref	-
	Gr_1 only	166 (11.4)		0.01	-0.07;0.08
	Gr_2 only	155 (10.7)		-0.07	-0.16;0.02
	Gr_3 only	38 (2.6)		-0.13*	-0.25;7E-3
	Gr_1 and Gr_2	254 (17.5)		0.01	-0.07;0.09
	Gr_1 and Gr_3	9 (0.6)		-0.08	-0.26;0.10
	Gr_2 and Gr_3	30 (2.1)		-0.12	-0.25;0.01
	All	59 (4.1)		-0.03	-0.14;0.07

Pasture contamination (sheep)		1,451 (99.8)			
	No	746 (51.3)		ref	-
	Gr_1 only	218 (15.0)		0.08	-2E-3;0.15
	Gr_2 only	28 (1.9)		0.14*	0.03;0.25
	Gr_1 and Gr_2	405 (27.9)		0.15*	0.08;0.23
	Gr_3/Gr_1 and Gr_3/Gr_2 and Gr_3	9 (0.6)		-0.05	-0.23;0.14
	All	45 (3.1)		0.09	-0.02;0.19
Co-grazing (cows)		1,453 (99.9)			
	No	1,254 (86.2)		ref	-
	Gr_1 only	10 (0.7)		0.17	-0.01;0.35
	Gr_2 only	126 (8.7)		0.12*	0.03;0.21
	Gr_3 only	21 (1.4)		0.14	-9E-3;0.29
	Gr_1 and Gr_2	7 (0.5)		0.06	-0.15;0.27
	Gr_2 and Gr_3	35 (2.4)		0.01*	-0.14;0.15
Co-grazing (sheep)		1,453 (99.9)			
	No	1,350 (92.8)		ref	-
	Gr_2 only	51 (3.5)		0.06	-0.05;0.17
	Gr_1 and Gr_2	38 (2.6)		0.05	-0.10;0.21
	Gr_3/Gr_1 and Gr_3/Gr_2 and Gr_3/ all	14 (1.1)		-0.14	-0.31;0.04
Pasture mowed (N)		1,452 (99.9)	3 (1-8)	-0.01*	-7E-3;-3E-3
Pasture fertilized (N)		1,452 (99.9)	6 (2-15)	-4E-3*	-6E-3;-2E-3
Pasture with manure (N)		1,452 (99.9)	2 (1-6)	-0.01*	-0.01;-2E-3
Anthelmintic treatments		1,428 (98.2)	2 (1-4)		
	No	153 (10.5)		ref	-
	Yes	1,275 (87.7)		-0.20	-0.27;-0.12
Anthelmintic form (N)		1,391 (95.7)			
	0	143 (9.8)	1 (1-2)	ref	-
	1	767 (52.8)		-0.18*	-0.26;-0.09
	≥2	481 (33.1)		-0.23*	-0.32;-0.14
Anthelmintic class (N)		1,425 (98.0)	2 (1-2)	-0.06*	-0.09;-0.03
Treatment protocol		1,392 (95.7)			
	No treatment	164 (11.3)		ref	-

	Long-acting (turn-out)	402 (27.6)	-0.28*	-0.37;-0.19
	Drench (turn-out)	8 (0.6)	0.08	-0.15;0.32
	Injection (turn-out)	43 (3.0)	-0.13*	-0.25;-0.01
	Pour-on (turn-out)	201 (13.8)	-0.19*	-0.29;-0.10
	Pour-on (grazing)	301 (20.7)	-0.20*	-0.29;-0.12
	Pour-on (housing)	120 (8.3)	-0.11	-0.24;0.01
	Drench (grazing and housing)	11 (0.8)	-0.11	-0.35;0.12
	Drench and pour-on (housing)	8 (0.6)	-0.11	-0.37;0.15
	Injection and pour-on (housing)	14 (1.0)	-0.15	-0.32;0.03
	Drench and injection (grazing and housing)	38 (2.6)	-0.17	-0.45;0.11
	Drench and pour-on (grazing and housing)	12 (0.8)	0.06	-0.19;0.30
	Injection and pour-on (grazing and housing)	70 (4.8)	-0.20*	-0.34;-0.06
Fenbendazole		1,423 (97.9)		
	No	1,210 (83.2)	ref	-
	Yes	213 (14.6)	-0.15*	-0.28;-0.02
Triclabendazole		1,423 (97.9)		
	No	1,271 (87.4)	ref	-
	Yes	152 (10.5)	-2E-3	-0.17;0.17
Oxfendazole		1,423 (97.9)		
	No	1,328 (91.3)	ref	-
	Yes	95 (6.5)	-0.13	-0.33;0.08
Levamisole		1,423 (97.9)		
	No	1,225 (84.3)	ref	-
	Yes	198 (13.6)	0.07	-0.04;0.18
Ivermectin		1,423 (97.9)		
	No	605 (41.6)	ref	-
	Yes	818 (56.3)	-0.10*	-0.17;-0.02
Moxidectin		1,423 (97.9)		
	No	1,029 (70.8)	ref	-
	Yes	394 (27.1)	-0.09*	-0.16;-0.02
Eprinomectin		1,423 (97.9)		
	No	1,264 (86.9)	ref	-

	Yes	159 (10.9)	-0.09*	-0.15;-0.02
Doramectin		1,434 (97.9)		
	No	1,402 (96.4)	ref	-
	Yes	21 (1.4)	-0.16	-0.45;0.13
Closantel		1,423 (97.9)		
	No	1,175 (80.8)	ref	-
	Yes	248 (17.1)	-0.13*	-0.22;-0.03
Clorsulon		1,423 (97.9)		
	No	1,373 (94.4)	ref	-
	Yes	50 (3.4)	0.04	-0.06;0.15
Nitroxynil		1,423 (97.9)		
	No	1,365 (93.9)	ref	-
	Yes	58 (4.0)	-0.02	-0.28;0.24
Oxyclozanide		1,423 (97.9)		
	No	1,409 (96.9)	ref	-
	Yes	14 (1.0)	-0.02	-0.19;0.16

[(a)]CI=confidence interval; p25-p75=25th-75th percentiles; $E^x = 10^x$; *=significant (P-value≤0.05);

[(b)]Gr_i= grazing season *i*

Appendices

- *Farms bulk tank milk antibody levels against O. ostertagi and F. hepatica*

Variable	N (%)	Median (p25-p75)[a]	β	95% CI[a]
BTM ODR[b] (first sampling)	1,454 (100.0)	0.94 (0.77-1.02)	0.73*	0.53;0.92
BTM ODR[b] (second sampling)	1,454 (100.0)	0.95 (0.76-1.01)	0.67*	0.48;0.86
BTM PP[b] (first sampling)	1,454 (100.0)	29.08 (4.22-61.15)	2E-3*	1E-3;3E-3[a]
BTM PP[b] (second sampling)	1,454 (100.0)	22.80 (5.97-103.90)	1E-3*	1E-3;2E-3

[a]CI=confidence interval; p25-p75=25th-75th percentiles; $E^x = 10^x$; *=significant (P-value≤0.05);

[b]ODR= *O. ostertagi;* PP= *F. hepatica*

Appendices

- *Heifer demographics and milk parameters*

Variable		N (%)	Median (p25-p75)[(a)]	β	95% CI[(a)]
Season of sampling		1,454 (100.0)			
	Spring	350 (24.1)		ref	-
	Summer	357 (24.6)		-0.02	-0.06;0.02
	Autumn	373 (25.6)		-0.02	-0.06;0.02
	Winter	374 (25.7)		-0.04*	-0.08;-3E-3
Sample storage time by freezing (d)		1,454 (100.0)	211 (155-260)	2E-5	-1E-4;2E-4[(a)]
Dairy breed		1,454 (100.0)			
	Purebred	1,254 (86.2)		ref	-
	Crossbred	200 (13.8)		-0.08*	-0.13;-0.02
Days in milk		1,454 (100.0)	47 (38-57)	-1E-3*	-2E-3;-2E-5
Milk yield (start lactation to sampling) (kg)		1,454 (100.0)	1351 (1046-1711)	-5E-5*	-7E-5;-3E-5
Protein (start lactation to sampling) (%)		1,454 (100.0)	3.18 (3.02-3.34)	0.02	-0.03;0.07
Fat (start lactation to sampling) (%)		1,454 (100.0)	3.77 (3.16-4.36)	0.01	-7E-3;0.02
Protein yield (start lactation to sampling) (kg)		1,454 (100.0)	42.67 (33.36-54.33)	-1E-3*	-2E-3;-5E-4
Fat yield (start lactation to sampling) (kg)		1,454 (100.0)	49.90 (36.60-66.50)	-6E-4	-1E-3;4E-5
Milk yield at the time of the sampling (kg)		1,454 (100.0)	29.6 (25.4-34.1)	-4E-3*	-06E-3;-2E-3
Protein at the time of the sampling (%)		1,452 (99.9)	3.16 (2.99-3.33)	0.04	-6E-3;0.02
Fat at the time of the sampling (%)		1,452 (99.9)	3.70 (3.03-4.36)	0.01	-6E-3;-2E-3
Somatic cell count (*1,000 cells/mL)		1,451 (99.8)	31 (17-69)	3E-5	9E-6;7E-5
Mastitis status		1,454 (100.0)			
	Uninfected	1,084 (74.6)		ref	-
	Chronic	48 (3.3)		0.09*	0.01;0.16
	Infected	13 (0.9)		0.01	-0.13;0.15
	New (dry)	4 (0.3)		-0.09	-0.33;0.16
	New (milk)	75 (5.2)		0.07*	0.02;0.13
	Recovered	87 (6.0)		0.02	-0.04;0.07
	Uncertain	143 (9.8)		0.04	-7E-3;0.08
Age at first calving (m)		1,454 (100.0)	27.3 (25.0- 30.6)	3E-3	-9E-4;7E-3
Body weight at first calving (kg)		742 (51.0)	575 (550- 600)	-1E-3	-3E-3;1E-3
Offspring at first calving (N)		1,385 (95.3)			
	0	9 (0.6)		ref	-
	1	1,362 (93.7)		-0.03	-0.19;0.14
	2	14 (1.0)		-0.04	-0.25;0.17
Sex offspring at first calving		1,249 (85.9)			
	Male	488 (33.6)		ref	-
	Female	759 (52.2)		-0.01	-0.03;0.02
	Both	2 (0.1)		-0.20	-0.55;0.14
Death/still birth offspring at first calving		1,385 (95.3)			
	No	1,243 (85.5)		ref	-
	Yes	142 (9.8)		0.05*	7E-3;0.10

[(a)]CI=confidence interval; p25-p75=25th-75th percentiles; $E^x = 10^x$; *=significant (P-value≤0.05)

Appendix 9: Univariable multilevel linear regression models of association between heifer milk/protein/fat yields at sampling and 305 day and demographic variables as fixed effects

- *At the time of the sampling*

Outcome		**Milk yield (kg) (model 1)**			**Protein yield (kg) (model 2)**		**Fat yield (kg) (model 3)**	
Variables	**Categories**	**N (%)**	**β**	**95% C.I[(a)]**	**β**	**95% C.I**	**β**	**95% C.I**
***O. ostertagi* individual milk ODR[(b)]**		1,454 (100)	-0.31*	-0.44;-0.18	-8E-3*	-0.01;-4E-3[(a)]	-8E-3	-0.02;3E-4
Dairy breed		1,454 (100)						
	Purebred	1,254 (86.2)	*Baseline*		*Baseline*		*Baseline*	
	Crossbred	200 (13.8)	-0.85	-2.23;0.54	-0.02	-0.06;3E-3	0.05	-0.04;0.13
Season at sampling		1,454 (100)						
	Spring	350 (24.1)	*Baseline*		*Baseline*		*Baseline*	
	Summer	357 (24.6)	-0.85	-1.84;0.14	-0.03	-0.06;3E-3	-0.02	-0.07;0.04
	Autumn	373 (25.7)	-0.26	-1.26;0.74	-0.01	-0.02;0.04	0.01	-0.05;0.07
	Winter	374 (25.7)	-0.18	-1.17;0.81	3E-3	-0.03;0.04	0.09*	0.03;0.14
Age at sampling (m)		1,454 (100)	0.11*	0.01;0.22	4E-3*	7E-4;7E-3	5E-3	-1E-3;0.01
DIM at sampling		1,454 (100)	5E-3	-0.02;0.03	1E-3*	2E-5;2E-3	2E-5	-1E-3;1E-3
$DIM^{-0.05}$		1,454 (100)	-3.62	-33.83;26.58	-	-	-	-
Milk yield at sampling (kg)		1,454 (100)	-	-	0.03*	2.8E-2;2.9E-2	0.03*	3.0E-2;3.4E-2
Log(SCC) (x1,000c/mL)		1,451 (99.8)	-0.84*	-1.50;-0.19	-0.02	-0.04;0.01	0.04*	2E-3;0.08
Size of the dairy herd		1,454 (100)	0.01*	4E-3;0.02	3E-4*	1E-4;5E-4	1E-3*	3E-4;1E-4
Bulk tank milk ODR[(b)] (second sampling)		1,454 (100)	-1.08*	-1.51;-0.66	-0.03*	-0.04;-0.02	-0.06*	-0.09;0.02
Bulk tank milk PP[(b)] (second sampling)		1,454 (100)	-2E-3*	-3E-3;-3E-4	-3E-5	-1E-5;1E-5	-1E-4*	-2E-4;1E-5

[(a)]CI=confidence interval; *=significant (P-value≤0.05); $E^x = 10^x$; [(b)]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendices

- *At day 305*

Outcome		Milk yield (kg) (model 1)			Protein yield (kg) (model 2)		Fat yield (kg) (model 3)	
Variables	**Categories**	**N (%)**	**β**	**95% C.I[a]**	**β**	**95% C.I**	**β**	**95% C.I**
***O. ostertagi* individual milk ODR[b]**		1,005 (100)	25.85	-5.67;57.37	-1.03*	-2.00;-0.05	-0.59	-2.05;0.87
Dairy breed		1,005 (100)						
	Purebred	884 (88.0)	*Baseline*		*Baseline*		*Baseline*	
	Crossbred	121 (12.0)	-219.27	-578.35;139.82	-6.65	-17.75;4.45	1.61	-15.09;18.31
Season at sampling		1,005 (100)						
	Spring	236 (23.5)	*Baseline*		*Baseline*		*Baseline*	
	Summer	239 (23.8)	381.28*	140.44;622.12	14.54*	7.11;21.97	25.18*	14.13;36.23
	Autumn	263 (26.2)	556.56*	316.00;797.11	17.41*	9.99;24.82	23.12*	12.08;34.16
	Winter	267 (26.6)	505.93*	269.83;742.02	14.61*	7.33;21.89	27.24*	16.41;38.07
Age at sampling (m)		1,005 (100)	53.80*	26.52;81.09	1.91*	1.07;2.75	2.24*	0.97;3.50
Related yield at sampling (kg)		1,005 (100)	125.50*	115.26;135.73	2.28*	1.85;2.70	-6.28	-41.02;28.46
Milk yield at day 305		1,005 (100)	-		0.03*	2.8E-2;2.9E-2	0.03*	2.7E-2;3.1E-2[a]
Length of the first lactation (d)		1,005 (100)	1.96*	0.84;3.08	0.07*	0.03;0.10	0.06*	0.01;0.11
Size of the dairy herd		1,005 (100)	3.49*	1.92;5.07	0.11*	0.06;0.16	0.24*	0.15;0.32
Cow anthelmintic treatment after sampling		1,005 (100)						
	No	577 (57.4)	*Baseline*		*Baseline*		*Baseline*	
	Yes	428 (42.6)	-409.44	-1,000.23;1181.35	-10.23	-28.96;8.50	-1.53	-34.38;31.32
Cow grazing after sampling		1,005 (100)						
	No	230 (22.9)	*Baseline*		*Baseline*		*Baseline*	
	Yes	775 (77.1)	-2,095.66*	-2,991.92;-1,199.40	-62.95*	-91.87;-34.03	-123.16*	-173.30;-73.02
Bulk tank milk ODR[b] (second sampling)		1,005 (100)	-322.33*	-461.23;-183.44	-10.43*	-14.78;-6.08	-20.03*	-27.72;-12.33
Bulk tank milk PP[b] (second sampling)		1,005 (100)	-0.39*	-0.76;-0.03	-0.01	-0.02;2E-3	-0.03*	-0.05;-7E-3

[a]CI=confidence interval; *=significant (P-value≤0.05); $E^x = 10^x$; [b]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendix 10: Univariable multilevel linear regression models of association between heifer last antibody titres against Johne's disease in the first lactation and demographic variables as fixed effects

Variables	Categories	N (%)	β	95% C.I[a]
***O. ostertagi* individual milk ODR[b]**		1,135 (100)	0.33*	0.03;0.64
Dairy breed		1,135 (100)		
	Purebred	1,000 (88.1)	*Baseline*	
	Crossbred	135 (11.9)	-0.74	-3.69;2.22
Season at sampling		1,135 (100)		
	Spring	275 (24.2)	*Baseline*	
	Summer	272 (24.0)	0.97	-1.54;3.47
	Autumn	293 (25.8)	-0.26	-2.73;2.20
	Winter	295 (26.0)	0.55	-1.90;2.99
Age at sampling (m)		1,135 (100)	-0.06	-0.29;0.18
DIM at the time of Johne's test		1,135 (100)	5E-3	-3E-3;0.01[a]
Interval between individual ELISA and Johne's test		1,135 (100)	3E-3	-5E-3;0.01
Yield at day 305 (kg)		1,135 (100)	-2E-4	-8E-4;4E-4
Size of the dairy herd		1,135 (100)	4E-3*	8E-5;8E-3
Cow anthelmintic treatments after sampling		1,135 (100)		
	No	694 (61.1)	*Baseline*	
	Yes	441 (38.9)	-0.44	-2.72;1.83
Cow grazing after sampling		1,135 (100)		
	No	325 (28.6)	*Baseline*	
	Yes	810 (71.4)	-1.26	-3.94;1.42
Bulk tank milk ODR[b] (second sampling)		1,135 (100)	-0.32	-0.84;0.20
Bulk tank milk PP[b] (second sampling)		1,135 (100)	-1E-3	-3E-3;5E-4

[a]CI=confidence interval; *=significant (P-value≤0.05); $E^x = 10^x$; [b]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendix 11: Univariable multilevel logistic regression models of association between heifer status on-farm at the end of the study and demographic variables as fixed effects

- *Heifer status on-farm defined as present or absent*

		Present (Reference)	Absent (i.e. culled, sold or dead)		
Variables	**Categories**	**N (%)**	**N (%)**	**O.R**	**95 % C.I[(a)]**
***O. ostertagi* individual milk ODR[(b)]**		1,113 (78.2)	310 (21.8)	1.06*	1.01;.11
Dairy breed		1,113 (78.2)	310 (21.8)		
	Purebred	954 (67.0)	282 (19.8)	*Baseline*	
	Crossbred	159 (11.2)	28 (2.0)	0.66	0.39;1.13
Season at sampling		1,113 (78.2)	310 (21.8)		
	Spring	216 (15.2)	118 (8.3)	*Baseline*	
	Summer	263 (18.5)	87 (6.2)	0.66*	0.47;0.94
	Autumn	317 (22.3)	54 (3.8)	0.42*	0.29;0.60
	Winter	317 (22.3)	51 (3.6)	0.35*	0.24;0.51
Age at sampling (m)		1,113 (78.2)	310 (21.8)	1.02	0.98;1.06
Length of first lactation (d)		1,113 (78.2)	310 (21.8)	1.00	0.99;1.00
Size of the dairy herd		1,113 (78.2)	310 (21.8)	1.00	0.99;1.00
Cow anthelmintic treatments after sampling		1,113 (78.2)	310 (21.8)		
	No	658 (46.2)	178 (12.5)	*Baseline*	
	Yes	455 (32.0)	132 (9.3)	1.17	0.67;2.05
Cow grazing after sampling		1,113 (78.2)	310 (21.8)		
	No	252 (17.7)	91 (6.4)	*Baseline*	
	Yes	861 (60.5)	219 (15.4)	0.86*	0.37;2.00
Bulk tank milk ODR[(b)] (second sampling)		1,113 (78.2)	310 (21.8)	1.02	0.89;1.18
Bulk tank milk PP[(b)] (second sampling)		1,113 (78.2)	310 (21.8)	1.00	0.99;1.00

[(a)]CI=confidence interval; *=significant (P-value≤0.05); $E^x = 10^x$; [(b)]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendices

- *Heifer status on-farm defined as present, absent or dead*

Variables	Categories	Present (Reference) N (%)	Absent (i.e. culled or sold) N (%)	O.R	95 % C.I	Dead N (%)	O.R	95 % C.I[a]
***O. ostertagi* individual milk ODR[b]**		1,113 (78.2)	254 (17.8)	1.00	0.96;1.05	56 (3.9)	1.09	0.991.19
Dairy breed		1,113 (78.2)	254 (17.8)			56 (3.9)		
	Purebred	954 (67.0)	229 (16.1)	*Baseline*		53 (3.7)	*Baseline*	
	Crossbred	159 (11.2)	25 (1.8)	0.66	0.42;1.02	3 (0.2)	0.34	0.10;1.10
Season at sampling		1,113 (78.2)	254 (17.8)			56 (3.9)		
	Spring	216 (15.2)	103 (7.2)	*Baseline*		15 (1.1)	*Baseline*	
	Summer	263 (18.5)	72 (5.1)	0.57*	0.41;0.81	15 (1.1)	0.82	0.39;1.71
	Autumn	317 (22.3)	43 (3.0)	0.28*	0.19;0.42	11 (0.8)	0.50	0.23;1.10
	Winter	317 (22.3)	36 (2.5)	0.24*	0.16;0.36	15 (1.1)	0.68	0.33;1.41
Age at sampling (m)		1,113 (78.2)	254 (17.8)	0.99	0.96;1.03	56 (3.9)	0.99	0.92;1.06
Length of first lactation (d)		1,113 (78.2)	254 (17.8)	0.99	0.96;1.03	56 (3.9)	0.99*	0.98;0.99
Size of the dairy herd		1,113 (78.2)	254 (17.8)	1.00	1.00;1.01	56 (3.9)	1.00	1.00;1.01
Cow anthelmintic treatments after sampling		1,113 (78.2)	254 (17.8)			56 (3.9)		
	No	658 (46.2)	147 (10.3)	*Baseline*		31 (2.2)	*Baseline*	
	Yes	455 (32.0)	107 (7.5)	1.05	0.80;1.38	25 (1.8)	1.17	0.68;2.00
Cow grazing after sampling		1,113 (78.2)	254 (17.8)			56 (3.9)		
	No	252 (17.7)	74 (5.2)	*Baseline*		17 (1.2)	*Baseline*	
	Yes	861 (60.5)	180 (12.6)	0.71*	0.53;0.96	39 (2.7)	0.67	0.38;1.20
Bulk tank milk ODR[b] (second sampling)		1,113 (78.2)	254 (17.8)	0.93*	0.88;0.99	56 (3.9)	1.01	0.89;1.14
Bulk tank milk PP[b] (second sampling)		1,113 (78.2)	254 (17.8)	1.00	0.99;1.00	56 (3.9)	1.00	0.99;1.00

[a]CI=confidence interval; *=significant (P-value≤0.05); [b]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendix 12: Univariable multilevel binomial regression models of association between heifer offspring mortality and demographic variables as fixed effects

Variables	Categories	Alive (Reference) N (%)	Dead N (%)	O.R	95 % C.I[a]
***O. ostertagi* individual milk ODR[b]**		1,243 (89.7)	142 (10.3)	1.06	0.99;1.14
Dairy breed		1,243 (89.7)	142 (10.3)		
	Purebred	1,080 (78.0)	110 (7.9)	*Baseline*	
	Crossbred	163 (11.8)	32 (2.3)	1.34	0.71;2.51
Season at calving		1,243 (89.7)	142 (10.3)		
	Spring	286 (20.6)	27 (1.9)	*Baseline*	
	Summer	293 (21.2)	38 (2.7)	1.13	0.65;1.98
	Autumn	327 (23.6)	54 (3.9)	1.42	0.84;2.40
	Winter	337 (24.3)	23 (1.7)	0.62	0.34;1.13
Age at calving (m)		1,243 (89.7)	142 (10.3)	0.99	0.93;1.04
Milk yield at sampling (kg)		1,243 (89.7)	142 (10.3)	0.96*	0.94;0.99
Log (SCC) at sampling (x1,000 cells/mL)		1,241 (89.6)	141 (10.2)	0.83	0.57;1.20
Size of the dairy herd		1,243 (89.7)	142 (10.3)	1.00	0.99;1.00
Bulk tank milk ODR[b] (second sampling)		1,243 (89.7)	142 (10.3)	0.91	0.79;1.06
Bulk tank milk PP[b] (second sampling)		1,243 (89.7)	142 (10.3)	1.00	0.99;1.00

[a]CI=confidence interval; *=significant (P-value≤0.05); [b]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendix 13: Univariable multilevel discrete survival time models of association between heifer hazard to calve for a second time in an interval *t* and demographic and interval level variables as fixed effects

Variables	Categories	No (Reference) N (%)	Yes N (%)	O.R	95 % C.I[a]
Interval (I_1) (d)		2,291 (65.6)	1,201 (34.4)		
	201-321 (I_1)	1,372 (39.3)	57 (1.63)	*Baseline*	
	322-441 (I_2)	443 (12.7)	929 (26.60)	27.80*	21.18;36.47
	442-561 (I_3)	245 (7.02)	201 (5.76)	16.10*	11.96;21.67
	562-681 (I_4)	231 (47.0)	14 (0.40)	1.63	0.93;2.87
***O. ostertagi* individual milk ODR[b]**		2,291 (65.6)	1,201 (34.4)	0.83	0.68;1.02
Dairy breed		2,291 (65.6)	1,201 (34.4)		
	Purebred	2,033 (58.2)	1,033 (29.6)	*Baseline*	
	Crossbred	258 (7.4)	168 (4.81)	1.18	0.98;1.42
Season at calving		2,291 (65.6)	1,201 (34.4)		
	Spring	496 (14.2)	282 (8.08)	*Baseline*	
	Summer	531 (84.7)	308 (8.82)	1.03	0.87;1.22
	Autumn	627 (18.0)	328 (9.39)	0.94	0.79;1.10
	Winter	637 (18.2)	283 (8.10)	0.84*	0.71;0.99
Age at first calving (m)		2,291 (65.6)	1,201 (34.4)	0.99	0.98;1.01
Length of first lactation (d)		2,291 (65.6)	1,201 (34.4)	1.00	0.99;1.00
Size of the herd		2,291 (65.6)	1,201 (34.4)	1.00	0.99;1.00
Cow anthelmintic treatments after sampling		2,291 (65.6)	1,201 (34.4)		
	No	1,436 (41.1)	733 (21.0)	*Baseline*	
	Yes	855 (24.5)	468 (13.4)	1.07	0.92;1.25
Cow grazing after sampling		2,291 (65.6)	1,201 (34.4)		
	No	582 (16.7)	280 (8.0)	*Baseline*	
	Yes	1,709 (48.9)	921 (26.4)	1.06	0.87;1.29
Bulk tank milk PP[b] (second sampling)		2,291 (65.6)	1,201 (34.4)	1.00	0.99;1.00

[a]CI=confidence interval; *=significant (P-value≤0.05); [b]ODR= *O. ostertagi;* PP= *F.* hepatica. The scale of the ELISA predictors was converted to be interpreted as 0.1 unit increase of the predictor on the outcome

Appendix 14: Schedule of the semi-structured face-to-face interviews conducted with the herd prime manager of the 42 dairy farms participating to the qualitative study

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Worms' control in dairy heifers – Draft interview schedule

*Introduce self

*Our objectives are to work towards a better understanding of the control of worm infections, understanding the relationships that exist between dairy cow management practices, individual parasites burdens and the prediction of animal's production performance..

* Thank farmers regarding the questionnaire they previously had received by post and completed.

*To complete the information they already had provided, there will be two parts in the interview:
-> Part 1: ask you some questions on the grazing management of your calves and heifers for the years 2012 and 2013;
-> Part 2: ask you some questions on general worm control practices.

*There are no right or wrong answers; we would also like to understand your views on the usefulness of different worming strategies

*Assure respondents of confidentiality
*Ask permission to tape record interviews, in order not to miss any information; we will understand if you do not wish to answer any of these questions.

*Consent form to sign

*Have you got any questions before we start?

PART 1 - Epi & qualitative: Data on farmers' young stock management when they are in pasture (Retrospective epi data from the years 2012 and 2013 along with farmers' drivers of actions)

Cf. file: 2014-03-19_Dairy Study_Retrospective Data Capture Form_PastureManagement & Drivers

PART 2 - Purely qualitative:

1. Industries present internal parasites (worms and fluke) as a significant threat to animal health and performance- what do you think about it?

2. If we now come to the particular situation of your farm:
   - Do you have or ever had any problems related to worms on your farm? – Could you please describe these problems to me?
   - What are the worms you are trying to control in your farm? Why these worms in particular?
   - What about the control of those? Are the management practices you talked to me about previously (*Part 1. Interview*) part of your worms' control practices? Do you have any other practices?
   - Could you please tell me for how long you have been controlling worms in your farm / Or: Could you remember when you started controlling worms in your farm?
   - Have your control practices changed over the years? Why? How?
   - Do you perceive the benefits of your efforts to control worms? Does it impact your production?

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- Has it all worked or not?
  -> *If not:* Have you ever checked out if your practices were working or not?
  -> *If yes:* How are you aware of that? Have you done any test of drug efficacy to confirm it?
- Have drugs resistance been part of any of your worries? Did anyone mention it to you?

3. In term of the risk factors related to worms' infection, what would be your best advice to give to other farmers to avoid the impact of worm burden? Could you tell me what are the three most important things to control worms for you?
   -> *If drug resistance was mentioned before*: Would drug resistance get into that?

4. How much time in a week or year would you spend on trying to control worms?
   - In all you have to do in the farms does it feel very demanding for you? Why?
   - Is it taking you too much time? Is it easy for you to get the anthelmintics you need?

5. Do you keep records on the routine measures you take for worms control and on the diseases you know related to worms?
   - Does this make part of a more global herd health planning record system you have in the farm?
   - What is the form of these records? How frequently do you record?
   -> If no record is kept: Have you ever considered keeping records? What make you not keeping them?

6. Do you think controlling worms represent a big cost for farmers?
   - How about you, in term of your farm? What percentage of your overall costs do you think worms' control go into?
   - How do you feel about it? Do you think these costs could be lowered in any way?
   - Overall, are there any schemes offering financial support available for farmers to help for worms' control? Are you able to use these?

7. Thinking about your vet:
   - Do you have a main vet coming to your farm?
   - Do you have other vets coming as well?
   - Are they all coming from the same practice?
   - Do you have a contract established between your vet and you, where vets would come in a routine basis or would your vets come whenever you ask for them?
   - How would you discuss about worms' control? Would it be discussed during their routine visit? Do you have opportunities to discuss those on phone?
   - Could you please describe to me the role your vet is playing in your worms control practices?
   - Regarding the nowadays changing of environment, the new diseases' challenges on farmers' livestock productions, do you think vets are providing the source services farmers really need?
   -> In term of your own vets, how would like the service to be in the future?

8. Is there any other people helping you in your worm control strategies/practices?
   - Who are they?
   - What are their roles?
   - In what way does it differ from the help your vet(s) provide to you?
   - Do you think your vet(s) could provide this same help to you?

9. In term of the other information you get regarding worms' control, where do you pick it up?
   -> *Possible hint:* Farmers' newspapers, Farmers' groups...
   -> Have you ever been in trainings on worms' control? Could you detail on that?

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10. If you had to help vets or dairy industry providing information on dairy farmers' real needs to control worms what would you say?
    -> Is it what you would specifically need for your farm?

11. Are you part of any assurance group? If yes, would you mind telling me which one?

Conclusion-
*Thank farmers for the precious time and information they offered.

*Explain the next steps of the study and the need to capture data on heifers' production performances and worms' control until April 2016, considering that a one-year period would have to be covered from the start of the heifers' milk sampling by QMMS. If they agree with the mode of contact, farmers will receive a phone every two months for the collection of these additional data.

*Explain that two bulk tank milk samples -one in spring and one in autumn- for which we will send a tube will be ask to them; this sample will then be tested by QMMS for Ostertagia and fluke and provide us an idea of the herd level infection status for these two parasites.

*Guarantee that a final meeting will be organised at the end of the study to present and discuss the results with them.
Also, their vets would be informed of the results of the study.