**INTERPRETIVE SUMMARY**

**A longitudinal study of gastrointestinal parasites in English dairy farms. Practices and factors associated with first lactation heifer exposure to *Ostertagia ostertagi* on pasture.** Bellet*.* *Ostertagia ostertagi* is an important cause of lost production, health and welfare in cattle that often leads dairy farmers to apply blanket anthelmintic treatments to their young-stock. Analysis of practices and risk factors associated with heifers’ individual milk antibody levels confirmed that more sustainable alternatives to anthelmintic drugs exist to reduce heifer exposure to *Ostertagia ostertagi* during first years of grazing. However, these can often compete with other farm resources and priorities. Overall our results provide guidance towards acceptable strategies for cattle helminth control before existing methods fail in England and socio-ecological impacts of cattle helminth infections worsen.

**DAIRY HEIFER EXPOSURE TO *O. OSTERTAGI***

**A longitudinal study of gastrointestinal parasites in English dairy farms. Practices and factors associated with first lactation heifer exposure to *Ostertagia ostertagi* on pasture**

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**ABSTRACT**

The gastrointestinal nematode *Ostertagia ostertagi* (*O. ostertagi*) is an important cause of lost production, health and welfare in cattle. Detailed records were obtained over a 5-yr period (2010/2015) by questionnaires and qualitative interviews to investigate the practices adopted by dairy farmers to control cattle helminth infections and the factors associated with heifer exposure to *O. ostertagi* on pasture. In total, 1,454 heifers’ individual milk samples were collected over a 1-yr period (2014/2015) in 43 dairy farms in England and tested for *O. ostertagi* antibodyby ELISA. Multilevel linear regression models were used to investigate the association between individual milk optical density ratio (ODR) against *O. ostertagi* and heifer management from birth to time of sampling. Farm’s and heifer’s median ODR against *O. ostertagi* were 0.98 (interquartile range, 0.76-1.02) and 0.64 (interquartile range, 0.42-0.84), respectively. The majority of heifers (88%) received an anthelmintic treatment prior to sampling in this study. After controlling for the effect of anthelmintic treatments, heifer’s individual milk ODR against *O. ostertagi* significantly increased with high stocking-rate at first grazing and co-grazing with adult cows prior to calving. Conversely, heifer’s individual milk ODR against *O. ostertagi* significantly decreased when heifers had co-grazed with sheep and pasture grass had frequently been mowed. Overall, these results provide evidence to support targeting grazing management toward limiting the use of anthelmintics in dairy young-stock to enable sustainable control of cattle helminth infections in England. However, to be accepted and adopted by farmers, these best practices would need to take into account farmers’ perspectives and contextual challenges.

**Key words:** Dairy heifer, *Ostertagia ostertagi*, individual milkELISA, sustainable control

**INTRODUCTION**

*Ostertagia ostertagi* (***O. ostertagi***)infections are one of the main concerns in the cattle industry in England ([Bellet et al., 2016](#_ENREF_5); [Berk et al., 2016](#_ENREF_8)). Extensive negative impacts of cattle helminths are reported, including loss in milk production, decreased growth performances, impaired reproduction and poor welfare ([Sanchez et al., 2002a](#_ENREF_36); [Charlier et al., 2014](#_ENREF_13); [Bellet et al., 2016](#_ENREF_5)). Moreover, cattle infected with helminths produce more greenhouse gases ([Rushton and Bruce, 2016](#_ENREF_35)). Since cattle helminth infections are mainly subclinical, their control is often difficult ([Charlier et al., 2014](#_ENREF_13)) and mostly relies on the indiscriminate use of anthelmintic drugs ([Vercruysse and Claerebout, 2001](#_ENREF_43)). In the United Kingdom (**UK**), concerns over cattle anthelmintic resistance have led to the development of the Control Of Worms Sustainably guidelines ([COWS, 2010](#_ENREF_16)), but their adoption by cattle farmers in England is still unsatisfactory ([Heasman et al., 2012](#_ENREF_19)). While there is some information available on the use of management practices by sheep farmers for helminth control in England ([Morgan et al., 2012](#_ENREF_29)), there is scant data on the same for the dairy farmers.

In order to implement helminth control, farmers need to use basic epidemiological information ([Vercruysse and Claerebout, 2001](#_ENREF_43)). This includes information on wide range of factors on which exposure of cattle to helminths depends, for example, climate, farm management (e.g. stocking-rate and mowing), and availability of resources ([Charlier et al., 2015](#_ENREF_14); [Wilson et al., 2015](#_ENREF_45)). In dairy farms, this is particularly relevant to heifers, since these are the future of the milking herd and usually the focus of anthelmintic treatments ([COWS, 2010](#_ENREF_16); [AHDB, 2015](#_ENREF_3)). However, estimations of dairy heifer exposure to helminths on pasture are currently unavailable in England. In fact, no survey on the prevalence of helminths in dairy heifers have been conducted in England since the 1980s ([Hong et al., 1981](#_ENREF_20)). Moreover, although the identification of risk factors associated with cattle exposure to *O. ostertagi* has been the focus of much research, there is a lack of similar research focused on heifers. In addition, it remains unknown if and how these risk factors can interplay and vary over the lifetime of the cattle ([Charlier et al., 2005a](#_ENREF_11); [Bennema et al., 2009](#_ENREF_6); [Vanderstichel et al., 2012](#_ENREF_42)). One possible reason for this is the use in previous research of close-ended questionnaires, which restricts the representation of complex systems of management and grazing ([Bennema et al., 2010](#_ENREF_7); [Merlin et al., 2016](#_ENREF_28)). This is especially the case when these approaches are applied to systems such as the ones adopted in England, where cattle graze in rotation ([AHDB, 2013](#_ENREF_2)). Secondly, previous studies mainly relied on bulk tank milk (**BTM**) indicators of cattle exposure to helminths whose antibody levels are difficult to interpret because of the pooled nature of the samples ([Sekiya et al., 2013](#_ENREF_40)). Evidence suggests that since levels of *O. ostertagi* antibody in cows are highly varied within a farm, the use of individual milk (**IM**) samples for this type of research is a better approach ([Charlier et al., 2007](#_ENREF_10); [Blanco-Penedo et al., 2012](#_ENREF_9)).

The goal of the research reported here was to provide a better understanding of strategies to improve the control of helminth infections in heifers in England. To achieve this, we used a longitudinal study (integrating both retrospective and prospective data on individual heifer management, from birth to first lactation) to explore: (1) levels of herd and heifer exposure to helminths, (2) farmers’ practices for cattle helminth control and (3) factors associated with heifer exposure to *O. ostertagi* on pasture.

**MATERIALS AND METHODS**

***Study herds***

Heifers came from a convenience and purposive sample of 43 dairy farms, all members of the Quality Milk Management Services’ (**QMMS**) recording scheme, Somerset, England. The average size of herds sampled was 150 cows, of which 46 were first lactation heifers. Farms were selected in order to allow the representation of different levels of heifer exposure to helminths and heifer management. Farm selection criteria included heifers calving all-year-round or at least during two different seasons in a year, home rearing of heifers (i.e. not contract reared), compliance on data recording, agreeing with the study protocol and sharing farm records.

***Study heifers.***

Heifers’ IM samples were obtained from samples routinely collected and stored by QMMS. All heifers entering in first lactation from the beginning of March 2014 to the end of March 2015 were eligible for the study. A total of 1,500 heifer samples were selected by stratified random sampling with the season and the farm as the strata ([Dohoo et al., 2009](#_ENREF_17)). The selection of the samples was conducted in two steps (October 2014 and June 2015). We aimed to obtain 375 heifer samples per season and 35 per farm. A flowchart of the selection process of the samples is presented Figure 1. Inclusion criteria were DIM (i.e. between 30-90 DIM to limit the confounding effect of milk production factors on antibody levels ([Sanchez et al., 2004](#_ENREF_39))), presence of QMMS’ sample records on milk yield, fat, protein and SCC and absence of heifer grazing in 2015. In the case where multiple samples had been collected from a heifer, only the sample with the lowest DIM was kept to be tested.

***Data collection***

The study was approved by the ethics committee of the School of Veterinary Medicine and Science (**SVMS**), University of Nottingham, UK and participating farmers were asked to sign an informed consent form. Detailed retrospective and prospective information on heifer’s demographic and management was obtained for a 5-yr period from 2010 to 2015. This way, each sampled heifer presented a complete management history from birth to sampling.

 ***Postal questionnaires (retrospective information on heifer general management).*** Retrospective information on demographic (i.e. farm and heifer) and general young-stock management (i.e. housing, feeding and vaccination) was gathered for each heifer and farm, using close-ended questionnaires. Information was collected for the years 2010 to 2013, assuming that first lactation heifers could calve from 30 months onwards in Great Britain (AHDB, 2014). Questions were grouped into sections according to topics (e.g. demographic, housing, and vaccination) and animal category (e.g. pre-weaned calves, weaned calves, and bulling heifers). Questions were asked for the year 2013 and, in the case of any change from the previous years (i.e. 2010 to 2012), farmers were asked to specify this change. The questionnaire was pilot-tested prior to its distribution on three colleagues of the dairy herd health research group at the SVMS, University of Nottingham, UK. Collected data were validated with farmers during a subsequent farm visit.

 ***Farm visit (retrospective information on heifer grazing management).*** Forty-three face-to-face semi-structured interviews (**SSI**) were conducted by the lead author (CB) during a farm visit between April and May 2014 to collect retrospective data on each heifer grazing management for the years 2011 to 2013. The interviews were audio-recorded and followed a pilot-tested interview schedule. Only managers with day to day responsibility for the dairy herd were interviewed. The interview schedule was divided into three different sections that referred to three different animal categories, i.e. (1) calves (i.e. defined as animals from weaned to bulling age); (2) bulling heifers (i.e. defined as animals from bulling age to in-calf); and (3) in-calf heifers (i.e. defined as animals from in-calf to not-yet-calved). The definition of these terms was developed beforehand and discussed with farmers in order to avoid any misunderstanding. The questions referred to the period between 2011 and 2013 for calves, and between 2012 and 2013 for bulling and in-calf heifers. For each year and category, questions were split into three time periods to facilitate the data collection: (1) from the time of animal turn-out to the 1st of June; (2) from after the 1st of June to the 1st of August; and (3) from after the 1st of August to the time of animal housing. Animal grazing seasons, defined by the interval between turn-out and housing, were confirmed by farmers for each year (i.e. 2011, 2012, and 2013). For each category and time period, questions were asked about numbers of heifer groups, ages of heifers within each group, movements of heifers between groups and number of pastures grazed per group. For each pasture grazed, farmers were asked to provide details on time of entry and exit of heifers, size of pasture, previous grazing on pasture, co-grazing, mowing, fertilisation, and individual anthelmintic treatments. Given the complexity of some of the rotational grazing management systems, information was checked against detailed maps of the farms’ grazing fields.

***Telephone interviews (prospective information on heifer general and grazing management).*** At the end of the farm visit, farmers were asked to record the same information for the on-going grazing season (i.e. 2014) and for their upcoming housing management (i.e. 2014-2015). These data were collected three-monthly by telephone until March 2015.

***QMMS’ information management system*.** Parameters of heifer’s milk sample, i.e. date of sampling, date of first calving, breed, DIM, milk yield and SCC, were extracted from QMMS’ information management system and processed using the dairy herd data analysis program, TotalVet (QMMS Ltd/SUM-IT Computer Systems).

***Laboratory procedures***

 ***Pilot study.*** A pilot study was conducted to evaluate the effect of milk samples storage on ELISA results. Eighty-six IM samples from adult cows that had been tested for *O. ostertagi* in 2012 and then stored at -20°C were tested again under similar laboratory conditions in March 2014. The test used the same ELISA kit and followed manufacturer’s instructions. Results were adjusted using a QMMS’ internal control before they were compared. Agreement of paired test results was computed using Lin’s concordance correlation coefficient (**CCC**) ([Lin, 1989](#_ENREF_25)).

***ELISA milk testing.*** After collection on farms, composite IM samples were preserved using bronopol/natamycin and kept at ambient temperature until arrival at the laboratory. In the laboratory, the samples were processed, tested for SCC, fat and protein, before being frozen at -20°C (±2°C) until further testing; this was achieved within the first 48h after sample collection on farms. Only IM samples from heifers born after 2010 and having grazed prior to sampling were tested for *O. ostertagi*.In order to limit cross-reactivity between the crude antigen used for *O. ostertagi* ELISA testing and *Fasciola hepatica* (***F. hepatica***)antibodies ([Bennema et al., 2009](#_ENREF_6)), herd level exposure to *F. hepatica* was determined by antibody-detection ELISA applied on BTM at the end of the grazing season 2014, in each farm (i.e. from October to December 2014). BTM samples were also tested for *O. ostertagi*. IM and BTM samples were defrosted, defatted by centrifugation (2000 x g, 2 min) and their supernatant collected. Samples were tested undiluted without any duplicate sampling and ELISA tests were carried out according to kits manufacturer’s instructions. ELISA tests were conducted by the same technician, blinded to the identity of the animal. The *F. hepatica* test used the Pourquier® ELISA *F. hepatica* serum and milk verification test (IDEXX, Montpellier, France), which is based on an “f2” antigen purified from *F. hepatica* extracts. Results were expressed as a percent positivity (**PP**), after assessment of the corrected optical density of the sample at 450 nm and calculation of the percentage of the positive control. The *O. ostertagi* test used the Svanovir® kit sourced from Svanova Ltd. (Sweden), which is an indirect ELISA based on crude saline-extracts of *O. ostertagi* adult worm as antigens ([Keus et al., 1981](#_ENREF_21); [Sanchez et al., 2002c](#_ENREF_38)). Results were expressed as an Optical Density Ratio (**ODR**) of the sample to guarantee test repeatability ([Sanchez et al., 2002c](#_ENREF_38)), after the measure the OD of both sample and positive and negative controls at 405 nm.

***Data collation and statistical analysis***

 Computer data entry was conducted using Microsoft Excel and Access (Microsoft, 2013). Due to the nature and the complexity of the grazing management information, a systematic process of data entry was performed for each heifer included in this study: (1) farm housing system and heifer’s date of birth estimated the year, the month and the age of the heifer at first turn-out; (2) each heifer was then affiliated to a category and a group within that category for the first grazing season; and (3) this was used to infer on heifer specific grazing management until housing for the first grazing season. Taking the previous grazing season as a reference, we could then estimate the age of heifer for the next grazing season and repeat the same process for each grazing season until a heifer was sampled. If heifers were born prior to 2010 or were never turned out, they were excluded from the study. Iterative and triangulation processes ([Dohoo et al., 2009](#_ENREF_17)) between the different data sources (i.e. questionnaire, interviews and QMMS’ information management system) were used to enhance the quality of the final grazing management database.

Data were collated and initially analyzed using STATA 12.1 (STATA Inc., Texas, USA). Since farmers did not report significant changes in their farming after 2010, a general profile of demographic and management practices (except grazing) was established for each farm. Descriptive and graphical analyses (e.g. scatterplot) were carried out to explore farm’s and heifer’s data. Pearson correlation coefficient (McDonald, 2014) was calculated between BTM and heifer’s IM ODR, considering all heifer samples in a given farm for the defined period of BTM sampling, i.e. October to December 2014. Related correlations interpreted as strong (above ±0.60), moderate (between ±0.40 and ±0.59) or weak (below ±0.39) (McDonald, 2014). A P-value≤0.05 was considered significant.

A multilevel linear regression (random effects) model ([Dohoo et al., 2009](#_ENREF_17)) was used to investigate the association between heifer’s IM ODR and collected and constructed variables on cow, farm and heifer management. Constructed variables consisted in providing the time sequence of heifer exposure to the factor of interest from birth to time of sampling (e.g. heifer treatment protocol and co-grazing with adult cows). The model incorporated two hierarchical levels given that several heifers originated from the same farm: level 1 (*i*), the heifer-level, level 2 (*j*), the farm-level. The outcome variable was heifer’s IM ODR. All collected variables were firstly tested in a univariable multilevel linear regression model. The model was developed using a reweighted generalised iterative least squares algorithm in MLwiN 2.30 ([Rasbash et al., 2012](#_ENREF_32)) and took the form:



Where: subscripts *i* and *j* denote the *i*th heifer of the *j*th farm, respectively. $y\_{ij}$ = heifer’s IM ODR, $β\_{0}$ = intercept value, $β\_{1}$ = vector of coefficients for $x\_{ij}$, $x\_{ij}$ = vector of covariates associated with each heifer, $β\_{2}$ = vector of coefficients for $x\_{j}$, $x\_{j}$ = vector of covariates associated with each farm, $u\_{0j}$ and $e\_{ij}$ were random effects to account for residual variation between farms and heifers, respectively; both assumed to be normally distributed. Associations between heifer’s IM ODR and collected variables were evaluated using a stepwise approach with elimination of non-significant effects (p-value>0.05) and observation of overall significance of factors. Based on Wald tests, all significant main effects at p-value≤0.05 were left in the model. Information on known confounding variables, as identified from previous literature ([Klesius, 1993](#_ENREF_23); [Kloosterman et al., 1993](#_ENREF_24); [Sanchez et al., 2004](#_ENREF_39)), was collected and these variables were also retained in the final model. Confounding variables included were: herd size, BTM ODR, BTM PP, breed, record season, DIM, milk yield and log (SCC). We explored interactions among predictors that were found to be significant in main effects model. This was done by two ways: descriptive plots of the variables with outcome and including statistical two-way interactions between predictors and checking the significance of the main effects and the interaction term (Dohoo et al., 2009). Model goodness-of-fit was assessed by examination of QQ plots and kurtosis of residual distributions (Dohoo et al., 2009). Collinearity was explored by calculating the variance inflation factor (**VIF**) of the variables included in the model ([Dohoo et al., 2009](#_ENREF_17); [Rasbash et al., 2012](#_ENREF_32)).

**RESULTS**

***Pilot study***

The CCC with 95% CI between the 2012 and 2014 mean ODR of cow’s IM samples were substantial and ranged from 0.87 (0.82-0.92) (no ODR adjustment) to 0.89 (0.84-0.93) (ODR adjustment).

***Study population***

Of the 43 dairy farmers included in the study, two withdrew shortly after the farm visit, resulting in a study participation rate of 95%. Main characteristics of the 41 farm participants are presented Table 1. Most of the farms (80%) were clustered around south-west counties, including counties of Somerset (N=18), Wiltshire (N=9), Devon (N=3), Cornwall (N=2), and Gloucestershire (N=1). A total of 1,454 heifer’s IM samples were included in the analysis with 350 collected in spring (i.e. between April and June), 357 in summer (i.e. between July and September), 373 in autumn (i.e. between October and December) and 375 in winter (i.e. January and March). The median number (interquartile range (**q25-q75**)) of heifers sampled per farm was 34 (25-44). Sampled heifers were predominantly Holstein Friesian with 83% purebreds (N=1,207) and 8% crossbreds (N=117). Most heifers were born in 2012 (N=1,013; 70%) and 2011 (N=384; 26%); the rest were born in 2013 (N=45; 3%) and 2010 (N=12; 1%). The median ages (q25-q75) of heifers at first turn-out and first calving were 9.5 (6.9-13.6) 27.3 (25.0-30.6) months, respectively. Most heifers (59%) had two grazing seasons prior to sampling; others had one (17%) or more than two (24%). In total, 85 % and 44% of the farmers systematically dewormed their young-stock and adult cows, respectively. Out of the sampled heifers, 88% from 39 farms (95%) had received at least one anthelmintic treatment prior to sampling. Farmers predominantly used pour-on (N=27; 77%) and long-acting forms of anthelmintics (N=23; 66%) in young-stock. Most common anthelmintic class used in young-stock was macrocyclic lactones (**ML**) (N=31; 89%), in particular ivermectin compound (N=23; 66%). Around half of the farms (N=17) exclusively relied on one anthelmintic compound to treat their young-stock against parasites. Moreover, 37%, 29% and 5% of the farmers had treated their heifers more than 3 times in a given grazing season (**Gr*i***) prior to sampling (treatment range: Gr1, 4-10; Gr2, 4-5; andGr3, 5-5).

***Farm and heifer exposure to Ostertagia ostertagi and Fasciola hepatica***

The median PP and ODR estimated in BTM at the end of the grazing season 2014 in the study farms were 20.30 (q25-q75, 4.38-89.33) and 0.98 (q25-q75, 0.76-1.02), respectively. Tested heifers were on average in their 47 (q25-q75, 38-58) DIM at sampling. Heifer’s median IM ODR was 0.64 (q25-q75, 0.42-0.84). From October to December, correlation between heifer’s IM and BTM ODR was moderate (r=0.54 (0.17-0.77)).

***Multilevel Linear regression model for heifer exposure to Ostertagia ostertagi on pasture***

Table 2 shows the results from the final multilevel linear regression model. There were no significant differences in heifer’s IM ODR according to the seasons and the stage of lactation (i.e. DIM). Moreover, there was no significant interactions between both time and anthelmintic treatment, and the final predictors of the model. Heifer’s IM ODR significantly decreased with increasing milk yield at sampling [Coefficient (**β**) (95% confidence interval (**CI**)) = -0.004 (-0.006 to -0.002)] but significantly increased with higher SCC in milk [β (95% CI): 0.030 (0.010 to 0.050)]. Compared to dairy crossbred, dairy purebred heifers had significantly higher IM ODR [β (95% CI): 0.112 (0.058 to 0.165)]. Heifer’s IM ODR significantly decreased with an increasing number of dairy staff [β (95% CI): -0.010 (-0.020 to -0.002E-1)] and when young-stock were sent in another farm for grazing [β (95% CI): -0.096 (-0.147 to -0.044)] but increased with increasing age at weaning on-farm [β (95% CI): 0.015 (0.004 to 0.026)].Compared to heifers always turned out in the ‘spring only’, heifers turned out either in the ‘spring/summer’ or in the ‘spring/autumn’ had a significant decrease in IM ODR by -0.076 units (95% CI: -0.113 to -0.039). There was a significant association between the contamination of heifer’s pasture and heifer’s IM ODR. First, compared to heifers that did not co-graze with mature cows, heifers that co-grazed for more than 14 days with mature cows (i.e. either dry or milking or both) had significantly higher IM ODR (β from 0.067 to 0.120). Second, heifers that went on pasture previously grazed by sheep during the first two grazing season had a significant increase in IM ODR (β from 0.073 to 0.174). Third, heifers that co-grazed with sheep at least during their third grazing season had a significant decrease in IM ODR by -0.196 units (95% CI: -0.387 to -0.004). Heifers that had higher minimum stocking rate during their first grazing season had significantly higher IM ODR [β (95% CI): 0.041 (0.024 to 0.058)] and heifers that grazed more mowed pastures during their second grazing season had significantly lower IM ODR [β (95% CI): -0.003 (-0.006 to -0.003E-1)]. After controlling for number of treatment application, heifers that were treated with long-acting anthelmintic treatments at turn-out or pour-on exclusively had significantly lower IM ODR (β from -0.108 to -0.219). Similarly, heifers that were treated, with a combination of pour-on and injection during the grazing season and at housing, had significantly lower IM ODR, compared to non-treated heifers [β (95% CI): -0.248 (-0.400 to -0.095)]. Final model residuals indicated a good overall fit; QQ plot indicated residuals were normally distributed. VIF of variables were <10.

**DISCUSSION**

This is the first longitudinal study using records of past anthelmintic treatments in heifers along with detailed grazing history and management practices to holistically investigate effects of these on heifer’s IM antibody levels against *O. ostertagi*. The study design and methods offered a reliable and valid approach to collect a wide range of data and address research questions that are particularly complex. First, it gave opportunities to engage with farmers, whose participation remained particularly high (95%), which is of significant value in a longitudinal study (Goldstein et al., 2015). Second, the use of interviews allowed to better understand local realities that are crucial for robustness of data analysis and interpretation. Despite the fact that this study used a convenience sample of dairy farms members of QMMS, exposure to helminth and management history highly varied between heifers. Moreover, affiliation of farms to QMMS Ltd. may have foster active participation of farmers and collection of consistent and high-quality data on heifer management. The use of a stratified random sampling approach for the selection of heifers within farm ensure that all strata were represented in the sample and may have increased the precision of our results (Dohoo et al., 2009). Although possibly not generalisable to the entire population of English dairy farms, the underlying biological associations of risk factors reported in this study are likely to be valid for all-year-round dairy calving heifers in England. Our results suggest that grazing management factors not only have a significant impact on exposure to *O. ostertagi* irrespective of anthelmintic use, but also that their impact on exposure may vary depending on their timing in the grazing history. We will discuss our main results below.

After controlling for the effect of anthelmintic treatments, heifer’s IM ODR significantly increased in the case of an early start of the grazing season (spring). This result supports previous findings ([Bennema et al., 2010](#_ENREF_7)) that cattle immunity against *O. ostertagi* develops slowly, only after long and repeated exposure to parasites on pasture ([Klesius, 1988](#_ENREF_22)).

Our results also corroborated evidence suggesting that heifer co-grazing with adult cows significantly increases heifer exposure to *O. ostertagi*. In reality, our result suggest that such an association depends on the timing in pregnancy when heifers co-graze with adult cows (i.e. prior to calving). Higher susceptibility of cattle to infections prior to calving has been reported in previous research and could be a reason for such observation ([Armour, 1980](#_ENREF_4)). By contrast, though this was poorly represented in our study, we observed that mixing heifers with sheep significantly decreased heifer exposure to *O. ostertagi*. Possible explanations of this could be that sheep can act as dead-end hosts for *O. ostertagi* ([Waller, 2006](#_ENREF_44); [COWS, 2010](#_ENREF_16)) and that sheep behaviour can influence ingestion of infective larvae by cattle ([ADAS, 2011](#_ENREF_1)). Although our study suggests that sequential grazing of heifers with sheep may significantly increase heifer exposure to *O. ostertagi*, we believe this was due to some test cross-reactivity between the crude antigens used for the ELISA and antibodies against other nematodes common to both cattle and sheep (Roberts, 1942; Bennema et al. 2009).

 To date, cattle risks of disease and production losses due to *O. ostertagi* have been mainly associated with a lack of host immunity against *O. ostertagi* ([Fox, 1997](#_ENREF_18)). For this reason, ‘best-practice’ guidelines often focus on young-stock when providing advice for cattle helminth control in the UK ([COWS, 2010](#_ENREF_16)). As these mainstream recommendations highlight, young-stock exposure to *O. ostertagi* is positively associated with young-stock stocking-rate, something we observed in the current study but only for first grazing heifers. Evidence suggests that naive animals are more likely to be infected when grazing highly-stocked, contaminated pastures ([Armour, 1980](#_ENREF_4)). Moreover, aligned with what is suggested in these guidelines, higher frequencies of grass mowing in heifer’s pastures significantly decreased the level of heifer’s IM ODR, irrespective of time of turn-out and stocking-rate. It is possible that the adverse microclimates or mechanical removal of *O. ostertagi* larvae following mowing caused the death of infective larvae on pasture ([Armour, 1980](#_ENREF_4); [Waller, 2006](#_ENREF_44)). Moreover, mowed pastures are likely to be less intensively grazed and/or not grazed in the early season, reducing pasture larval contamination.

Most of the study farmers controlled helminth infections in their young-stock, as shown by the difference of systematic treatments applied in young and adult cattle. Farmers integrated, to some extent, several ‘best-practice’ recommendations included in COWS guidelines for cattle helminth control, into their grazing management of heifers. For instance, heifers were on average turned out older than six months of age, i.e. when guidelines suggest that the risks of disease and production losses due to helminths are lower ([COWS, 2010](#_ENREF_16)). Moreover, study farmers decreased the frequency of their anthelmintic use over time, possibly in line with COWS recommendations and the progressive build-up of host immunity against helminths ([COWS, 2010](#_ENREF_16)). Farmers’ use of anthelmintics remained however high in this study. As evidence of this, a majority of farms (95%) had treated heifers (88%) against helminths prior to sampling and 37% used anthelmintics more than 4 times on heifers’ first year of grazing although rotating and mowing grass ([COWS, 2010](#_ENREF_16)). It is likely that farmers’ aversion to production loss, lack of complete understanding of what impact helminths have on production and inability to adopt ‘clean grazing’ influenced such practices ([COWS, 2010](#_ENREF_16); [Taylor, 2010](#_ENREF_41)). In fact, 34% and 98% of first-grazing heifers co-grazed with cows and older young-stock, respectively. Moreover, the convenience, safety and ease of use of some anthelmintics can influence farmer’s decision-making on helminth control ([Taylor, 2010](#_ENREF_41); [Wilson et al., 2015](#_ENREF_45)). As evidence of this, most farmers included in this study used pour-on, long-lasting anthelmintics and ML, often formulated as pour-on ([Taylor, 2010](#_ENREF_41)). Although concerns over helminth resistance to anthelmintics, especially ML, have been increasing in the UK ([Coles, 2005](#_ENREF_15); [COWS, 2010](#_ENREF_16)), this finding also indicates that the issue of anthelmintic resistance might be of even more significant concern given prevalence of such practices ([Charlier et al., 2015](#_ENREF_14)). In line with previous research ([Wilson et al., 2015](#_ENREF_45); [O'Kane et al., 2016](#_ENREF_31)), our results suggest that farm labour and farmer conscientiousness (e.g. decision-making based on the risk for heifers to be exposed or the build-up of cattle immunity) may influence farmers’ decisions on cattle helminth control. Cattle helminth control cannot be considered separately from the rest of the farm-system management since it can compete with other farm resources such as number of staff, finance and skills ([Morley and Donald, 1980](#_ENREF_30)). The systematic approach adopted by conscientious farmers may also facilitate adoption of sustainable 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 ([O'Kane et al., 2016](#_ENREF_31)).

The accurate diagnosis of *O. ostertagi* infections is crucial to understand patterns of infection under field conditions. This depends on the tool used for the diagnosis and the interpretation of the results ([Dohoo et al., 2009](#_ENREF_17); [Roeber et al., 2013](#_ENREF_34)). The high reproducibility of the Svanovir® *O. ostertagi* ELISA kit observed in the current study supports previous findings of research done with adult cows ([Sanchez et al., 2002c](#_ENREF_38); [Charlier et al., 2005b](#_ENREF_12)) and confirms that this kit is a very good candidate for conducting extensive longitudinal studies of *O. ostertagi* infections in cattle. Moreover, the only moderate correlation observed between heifer’s IM and BTM ODR corroborates earlier research ([Sanchez et al., 2002b](#_ENREF_37); [Charlier et al., 2007](#_ENREF_10)) and suggests that IM should be the preferred choice when exploring *O. ostertagi* infection in young-stock. Nevertheless, as we observed in this study, it is important to note that several individual parameters, especially milk yield, SCC and breed, are likely to influence ODR interpretations possibly due to effects of dilution, test cross-reactivity, genetic traits and physiology ([Kloosterman et al., 1993](#_ENREF_24); [Sanchez et al., 2004](#_ENREF_39); [Liua et al., 2009](#_ENREF_26)). Therefore these individual parameters should always be taken into account when interpreting ODR from heifer’s IM samples. It is also very important to mention that our overall understanding of mechanisms of host–parasite interactions and how immune responses are induced by *O. ostertagi* is still limited (Rinadi and Geldhof, 2012). For example, detection of milk antibodies does not allow to differentiate between past and current infections and between different levels of infection severity. This might be a reason why no significant association could be observed between heifer’s IM ODR and time of grazing when the total time of heifer grazing was added up from birth to sampling and confirms the importance of considering the interplay and variation of factors over the lifetime of cattle when exploring cattle exposure to helminths. Moreover, this also makes raw ODR a result that, on its own, is not informative ([Wright et al., 1993](#_ENREF_46)) and the interpretation of factors associated with ODR often challenging ([Roeber et al., 2013](#_ENREF_34)). Finally, some predictors included in the final model, such as ‘age at weaning’ and ‘size of the herd’, may have acted as surrogate for other variables not captured in this study. As a consequence, there will be a need to conduct further intervention studies in the field to test observed associations.

**CONCLUSIONS**

Our results suggest that heifer’s length of grazing, stocking-rate, mixed grazing with mature cows and sequential grazing with sheep highly influence heifer exposure to *O. ostertagi* in England. Importantly, we observed that effects of such grazing management practices depend on heifer’s susceptibility to parasite infections and if managed with a particular care during the first year of heifer grazing and prior to calving, could help reducing the excessive use of anthelmintics by dairy farmers in the UK. Having examined various levers for action towards renewed grazing management practices that could be targeted by farmers, it is necessary to ensure the cost-effectiveness of these recommendations within the system of cattle farming, considering other cattle parasites and farm’s socio-economic dimensions that can influence cattle helminth control, such as financial resources and specific characteristics of the workforce, including availability of personnel and workers’ skills.

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