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.

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15 A longitudinal study of gastrointestinal parasites in English dairy farms. Practices and 16 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* antibody by 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 milk ELISA, sustainable control

INTRODUCTION

Ostertagia ostertagi (***O. ostertagi***) infections are one of the main concerns in the cattle industry in England (Bellet et al., 2016; Berk et al., 2016). 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; Charlier et al., 2014; Bellet et al., 2016). Moreover, cattle infected with helminths produce more greenhouse gases (Rushton and Bruce, 2016). Since cattle helminth infections are mainly subclinical, their control is often difficult (Charlier et al., 2014) and mostly relies on the indiscriminate use of anthelmintic drugs (Vercruysse and Claerebout, 2001). In the United Kingdom (**UK**), concerns over cattle anthelmintic resistance have led to the development of the Control Of Worms Sustainably guidelines (COWS, 2010), but their adoption by cattle farmers in England is still unsatisfactory (Heasman et al., 2012). While there is some information available on the use of management practices by sheep farmers for helminth control in England (Morgan et al., 2012), 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). 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; Wilson et al., 2015). 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; AHDB, 2015). 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). 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; Bennema et al., 2009; Vanderstichel et al., 2012). 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; Merlin et al., 2016). 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). 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). 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; Blanco-Penedo et al., 2012).

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.

96 The average size of herds sampled was 150 cows, of which 46 were first lactation heifers.
97 Farms were selected in order to allow the representation of different levels of heifer exposure
98 to helminths and heifer management. Farm selection criteria included heifers calving all-year-
99 round or at least during two different seasons in a year, home rearing of heifers (i.e. not contract
100 reared), compliance on data recording, agreeing with the study protocol and sharing farm
101 records.

102 ***Study heifers.***

103 Heifers' IM samples were obtained from samples routinely collected and stored by
104 QMMS. All heifers entering in first lactation from the beginning of March 2014 to the end of
105 March 2015 were eligible for the study. A total of 1,500 heifer samples were selected by
106 stratified random sampling with the season and the farm as the strata (Dohoo et al., 2009). The
107 selection of the samples was conducted in two steps (October 2014 and June 2015). We aimed
108 to obtain 375 heifer samples per season and 35 per farm. A flowchart of the selection process
109 of the samples is presented Figure 1. Inclusion criteria were DIM (i.e. between 30-90 DIM to
110 limit the confounding effect of milk production factors on antibody levels (Sanchez et al.,
111 2004)), presence of QMMS' sample records on milk yield, fat, protein and SCC and absence
112 of heifer grazing in 2015. In the case where multiple samples had been collected from a heifer,
113 only the sample with the lowest DIM was kept to be tested.

114 ***Data collection***

115 The study was approved by the ethics committee of the School of Veterinary Medicine
116 and Science (**SVMS**), University of Nottingham, UK and participating farmers were asked to
117 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.

142 The questions referred to the period between 2011 and 2013 for calves, and between 2012 and
143 2013 for bulling and in-calf heifers. For each year and category, questions were split into three
144 time periods to facilitate the data collection: (1) from the time of animal turn-out to the 1st of
145 June; (2) from after the 1st of June to the 1st of August; and (3) from after the 1st of August to
146 the time of animal housing. Animal grazing seasons, defined by the interval between turn-out
147 and housing, were confirmed by farmers for each year (i.e. 2011, 2012, and 2013). For each
148 category and time period, questions were asked about numbers of heifer groups, ages of heifers
149 within each group, movements of heifers between groups and number of pastures grazed per
150 group. For each pasture grazed, farmers were asked to provide details on time of entry and exit
151 of heifers, size of pasture, previous grazing on pasture, co-grazing, mowing, fertilisation, and
152 individual anthelmintic treatments. Given the complexity of some of the rotational grazing
153 management systems, information was checked against detailed maps of the farms' grazing
154 fields.

155 ***Telephone interviews (prospective information on heifer general and grazing***
156 ***management).*** At the end of the farm visit, farmers were asked to record the same information
157 for the on-going grazing season (i.e. 2014) and for their upcoming housing management (i.e.
158 2014-2015). These data were collected three-monthly by telephone until March 2015.

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

163 ***Laboratory procedures***

164 ***Pilot study.*** A pilot study was conducted to evaluate the effect of milk samples storage
165 on ELISA results. Eighty-six IM samples from adult cows that had been tested for *O. ostertagi*
166 in 2012 and then stored at -20°C were tested again under similar laboratory conditions in March
167 2014. The test used the same ELISA kit and followed manufacturer's instructions. Results were
168 adjusted using a QMMS' internal control before they were compared. Agreement of paired test
169 results was computed using Lin's concordance correlation coefficient (**CCC**) (Lin, 1989).

170 ***ELISA milk testing.*** After collection on farms, composite IM samples were preserved
171 using bronopol/natamycin and kept at ambient temperature until arrival at the laboratory. In
172 the laboratory, the samples were processed, tested for SCC, fat and protein, before being frozen
173 at -20°C (±2°C) until further testing; this was achieved within the first 48h after sample
174 collection on farms. Only IM samples from heifers born after 2010 and having grazed prior to
175 sampling were tested for *O. ostertagi*. In order to limit cross-reactivity between the crude
176 antigen used for *O. ostertagi* ELISA testing and *Fasciola hepatica* (***F. hepatica***) antibodies
177 (Bennema et al., 2009), herd level exposure to *F. hepatica* was determined by antibody-
178 detection ELISA applied on BTM at the end of the grazing season 2014, in each farm (i.e. from
179 October to December 2014). BTM samples were also tested for *O. ostertagi*. IM and BTM
180 samples were defrosted, defatted by centrifugation (2000 x g, 2 min) and their supernatant
181 collected. Samples were tested undiluted without any duplicate sampling and ELISA tests were
182 carried out according to kits manufacturer's instructions. ELISA tests were conducted by the
183 same technician, blinded to the identity of the animal. The *F. hepatica* test used the Pourquier®
184 ELISA *F. hepatica* serum and milk verification test (IDEXX, Montpellier, France), which is
185 based on an "f2" antigen purified from *F. hepatica* extracts. Results were expressed as a percent
186 positivity (**PP**), after assessment of the corrected optical density of the sample at 450 nm and
187 calculation of the percentage of the positive control. The *O. ostertagi* test used the Svanovir®

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

193 ***Data collation and statistical analysis***

194 Computer data entry was conducted using Microsoft Excel and Access (Microsoft,
195 2013). Due to the nature and the complexity of the grazing management information, a
196 systematic process of data entry was performed for each heifer included in this study: (1) farm
197 housing system and heifer's date of birth estimated the year, the month and the age of the heifer
198 at first turn-out; (2) each heifer was then affiliated to a category and a group within that
199 category for the first grazing season; and (3) this was used to infer on heifer specific grazing
200 management until housing for the first grazing season. Taking the previous grazing season as
201 a reference, we could then estimate the age of heifer for the next grazing season and repeat the
202 same process for each grazing season until a heifer was sampled. If heifers were born prior to
203 2010 or were never turned out, they were excluded from the study. Iterative and triangulation
204 processes (Dohoo et al., 2009) between the different data sources (i.e. questionnaire, interviews
205 and QMMS' information management system) were used to enhance the quality of the final
206 grazing management database.

207 Data were collated and initially analyzed using STATA 12.1 (STATA Inc., Texas,
208 USA). Since farmers did not report significant changes in their farming after 2010, a general
209 profile of demographic and management practices (except grazing) was established for each
210 farm. Descriptive and graphical analyses (e.g. scatterplot) were carried out to explore farm's

211 and heifer's data. Pearson correlation coefficient (McDonald, 2014) was calculated between
212 BTM and heifer's IM ODR, considering all heifer samples in a given farm for the defined
213 period of BTM sampling, i.e. October to December 2014. Related correlations interpreted as
214 strong (above ±0.60), moderate (between ±0.40 and ±0.59) or weak (below ±0.39) (McDonald,
215 2014). A P-value≤0.05 was considered significant.

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

226 $$y_{ij} = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + \upsilon_{0j} + e_{ij}$$

227 Where: subscripts *i* and *j* denote the *i*th heifer of the *j*th farm, respectively. y_{ij} = heifer's IM
228 ODR, β_0 = intercept value, β_1 = vector of coefficients for x_{ij}, x_{ij} = vector of covariates
229 associated with each heifer, β_2 = vector of coefficients for x_j, x_j = vector of covariates
230 associated with each farm, u_{0j} and e_{ij} were random effects to account for residual variation
231 between farms and heifers, respectively; both assumed to be normally distributed. Associations
232 between heifer's IM ODR and collected variables were evaluated using a stepwise approach
233 with elimination of non-significant effects (p-value>0.05) and observation of overall

234 significance of factors. Based on Wald tests, all significant main effects at p-value≤0.05 were
235 left in the model. Information on known confounding variables, as identified from previous
236 literature (Klesius, 1993; Kloosterman et al., 1993; Sanchez et al., 2004), was collected and
237 these variables were also retained in the final model. Confounding variables included were:
238 herd size, BTM ODR, BTM PP, breed, record season, DIM, milk yield and log (SCC). We
239 explored interactions among predictors that were found to be significant in main effects model.
240 This was done by two ways: descriptive plots of the variables with outcome and including
241 statistical two-way interactions between predictors and checking the significance of the main
242 effects and the interaction term (Dohoo et al., 2009). Model goodness-of-fit was assessed by
243 examination of QQ plots and kurtosis of residual distributions (Dohoo et al., 2009). Collinearity
244 was explored by calculating the variance inflation factor (**VIF**) of the variables included in the
245 model (Dohoo et al., 2009; Rasbash et al., 2012).

246

247 **RESULTS**

248 ***Pilot study***

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

252 ***Study population***

253 Of the 43 dairy farmers included in the study, two withdrew shortly after the farm visit,
254 resulting in a study participation rate of 95%. Main characteristics of the 41 farm participants
255 are presented Table 1. Most of the farms (80%) were clustered around south-west counties,

256 including counties of Somerset (N=18), Wiltshire (N=9), Devon (N=3), Cornwall (N=2), and
257 Gloucestershire (N=1). A total of 1,454 heifer's IM samples were included in the analysis with
258 350 collected in spring (i.e. between April and June), 357 in summer (i.e. between July and
259 September), 373 in autumn (i.e. between October and December) and 375 in winter (i.e.
260 January and March). The median number (interquartile range (**q25-q75**)) of heifers sampled
261 per farm was 34 (25-44). Sampled heifers were predominantly Holstein Friesian with 83%
262 purebreds (N=1,207) and 8% crossbreds (N=117). Most heifers were born in 2012 (N=1,013;
263 70%) and 2011 (N=384; 26%); the rest were born in 2013 (N=45; 3%) and 2010 (N=12; 1%).
264 The median ages (q25-q75) of heifers at first turn-out and first calving were 9.5 (6.9-13.6) 27.3
265 (25.0-30.6) months, respectively. Most heifers (59%) had two grazing seasons prior to
266 sampling; others had one (17%) or more than two (24%). In total, 85 % and 44% of the farmers
267 systematically dewormed their young-stock and adult cows, respectively. Out of the sampled
268 heifers, 88% from 39 farms (95%) had received at least one anthelmintic treatment prior to
269 sampling. Farmers predominantly used pour-on (N=27; 77%) and long-acting forms of
270 anthelmintics (N=23; 66%) in young-stock. Most common anthelmintic class used in young-
271 stock was macrocyclic lactones (**ML**) (N=31; 89%), in particular ivermectin compound (N=23;
272 66%). Around half of the farms (N=17) exclusively relied on one anthelmintic compound to
273 treat their young-stock against parasites. Moreover, 37%, 29% and 5% of the farmers had
274 treated their heifers more than 3 times in a given grazing season (**Gr_i**) prior to sampling
275 (treatment range: Gr_1, 4-10; Gr_2, 4-5; and Gr_3, 5-5).

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

277 The median PP and ODR estimated in BTM at the end of the grazing season 2014 in
278 the study farms were 20.30 (q25-q75, 4.38-89.33) and 0.98 (q25-q75, 0.76-1.02), respectively.
279 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) that cattle immunity against *O. ostertagi* develops slowly, only after long and repeated exposure to parasites on pasture (Klesius, 1988).

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). 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; COWS, 2010) and that sheep behaviour can influence ingestion of infective larvae by cattle (ADAS, 2011). 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). For this reason, 'best-practice' guidelines often focus on young-stock when providing advice for cattle helminth control in the UK (COWS, 2010). 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). 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; Waller, 2006). 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). 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

375 (COWS, 2010). Farmers' use of anthelmintics remained however high in this study. As
376 evidence of this, a majority of farms (95%) had treated heifers (88%) against helminths prior
377 to sampling and 37% used anthelmintics more than 4 times on heifers' first year of grazing
378 although rotating and mowing grass (COWS, 2010). It is likely that farmers' aversion to
379 production loss, lack of complete understanding of what impact helminths have on production
380 and inability to adopt 'clean grazing' influenced such practices (COWS, 2010; Taylor, 2010).
381 In fact, 34% and 98% of first-grazing heifers co-grazed with cows and older young-stock,
382 respectively. Moreover, the convenience, safety and ease of use of some anthelmintics can
383 influence farmer's decision-making on helminth control (Taylor, 2010; Wilson et al., 2015).
384 As evidence of this, most farmers included in this study used pour-on, long-lasting
385 anthelmintics and ML, often formulated as pour-on (Taylor, 2010). Although concerns over
386 helminth resistance to anthelmintics, especially ML, have been increasing in the UK (Coles,
387 2005; COWS, 2010), this finding also indicates that the issue of anthelmintic resistance might
388 be of even more significant concern given prevalence of such practices (Charlier et al., 2015).
389 In line with previous research (Wilson et al., 2015; O'Kane et al., 2016), our results suggest
390 that farm labour and farmer conscientiousness (e.g. decision-making based on the risk for
391 heifers to be exposed or the build-up of cattle immunity) may influence farmers' decisions on
392 cattle helminth control. Cattle helminth control cannot be considered separately from the rest
393 of the farm-system management since it can compete with other farm resources such as number
394 of staff, finance and skills (Morley and Donald, 1980). The systematic approach adopted by
395 conscientious farmers may also facilitate adoption of sustainable cattle helminth control.
396 Moreover, conscientious farmers are more likely to take the time to search for information and
397 to remain updated on the most efficient practices (O'Kane et al., 2016).

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; Roeber et al., 2013). 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; Charlier et al., 2005b) 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; Charlier et al., 2007) 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; Sanchez et al., 2004; Liua et al., 2009). 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) and the interpretation of factors associated with ODR often challenging (Roeber et al.,

2013). 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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446 **REFERENCES**

447 ADAS, 2011. Impact of grazing management on cattle and sheep parasites. ADAS report, 26
448 pp.

449 AHDB, 2013. The structure of the GB dairy farming industry - What drives change? AHDB
450 dairy report, 39pp.

451 AHDB, 2014. Fertility workshop. AHDB website. https://dairy.ahdb.org.uk/news/dairyleader-
452 articles/november-2014/fertility-workshop/#.WSqv7ca1s2w. Accessed 28th May 2015.

453 AHDB, 2015. Managing replacement heifers for Better Returns AHDB Beef and Lamb report,
454 20pp.

455 Armour, J., 1980. The epidemiology of helminth disease in farm animals. Vet. Parasitol. 6, 7-
456 46.

457 Bellet, C., Green, M.J., Vickers, M., Forbes, A., Berry, E., Kaler, J., 2016. *Ostertagia spp.*,
458 rumen fluke and liver fluke single- and poly-infections in cattle: An abattoir study of
459 prevalence and production impacts in England and Wales. Prev. Vet. Med. 132, 98-
460 106.

461 Bennema, S., Vercruysse, J., Claerebout, E., Schnieder, T., Strube, C., Ducheyne, E.,
462 Hendrickx, G., Charlier, J., 2009. The use of bulk-tank milk ELISAs to assess the
463 spatial distribution of *Fasciola hepatica*, *Ostertagia ostertagi* and *Dictyocaulus*
464 *viviparus* in dairy cattle in Flanders (Belgium). Vet. Parasitol. 165, 51-57.

465 Bennema, S.C., Vercruysse, J., Morgan, E., Stafford, K., Hoglund, J., Demeler, J., von Samson-
466 Himmelstjerna, G., Charlier, J., 2010. Epidemiology and risk factors for exposure to
467 gastrointestinal nematodes in dairy herds in northwestern Europe. Vet. Parasitol. 173,
468 247-254.

469 Berk, Z., Bishop, S.C., Forbes, A.B., Kyriazakisa, I., 2016. A simulation model to investigate
470 interactions between first season grazing calves and *Ostertagia ostertagi*. Vet.
471 Parasitol. 226, 198-209.
472 Blanco-Penedo, I., Hoglund, J., Fall, N., Emanuelson, U., 2012. Exposure to pasture borne
473 nematodes affects individual milk yield in Swedish dairy herds. Vet. Parasitol. 188, 93-
474 98.
475 Charlier, J., Camuset, P., Claerebout, E., Courtay, B., Vercruysse, J., 2007. A longitudinal
476 survey of anti-*Ostertagia ostertagi* antibody levels in individual and bulk tank milk in
477 two dairy herds in Normandy. Res. Vet. Sci. 83, 194-197.
478 Charlier, J., Claerebout, E., De Muelenaere, E., Vercruysse, J., 2005a. Associations between
479 dairy herd management factors and bulk tank milk antibody levels against *Ostertagia*
480 *ostertagi*. Vet. Parasitol. 133, 91-100.
481 Charlier, J., Duchateau, L., Claerebout, E., Vercruysse, J., 2005b. Assessment of the
482 repeatability of a milk *Ostertagia ostertagi* ELISA and effects of sample preparation.
483 Prev. Vet. Med. 68, 277-288.
484 Charlier, J., Van der Voort, M., Kenyon, F., Skuce, P.J., Vercruysse, J., 2014. Chasing
485 helminths and their economic impact on farmed ruminants. Trends Parasitol. 30, 361-
486 367.
487 Charlier, J., Velde, F.V., van der Voort, M., Van Meensel, J., Lauwers, L., Cauberghe, V.,
488 Vercruysse, J., Claerebout, E., 2015. ECONOHEALTH: Placing helminth infections of
489 livestock in an economic and social context. Vet. Parasitol. 212, 62-67.
490 Coles, G.C., 2005. Anthelmintic resistance--looking to the future: a UK perspective. Res. Vet.
491 Sci. 78, 99-108.
492 COWS, 2010. Integrated parasite control on cattle farms. COWS technical guide, 13 pp.

493 Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research. 2nd Edition.
494 VER Inc. Canada, 865 pp.
495 Fox, M.T., 1997. Pathophysiology of infection with gastrointestinal nematodes in domestic
496 ruminants: recent developments. Vet. Parasitol. 72, 285-297; discussion 297-308.
497 Goldstein, H., Lynn, P., Muniz-Terrera, G., Hardy, R., O'Muircheartaigh, C., Skinner, C.,
498 Lehtonen, R., 2015. Population sampling in longitudinal survey. Longit. Life Course
499 Stud. 6, 447-475
500 Heasman, L., Potter, T., Nanjiani, I., Burden, D., Taylor, M.A., 2012. Farmer practices and
501 attitudes towards anthelmintic use in cattle in the United Kingdom. UK: Westpoint
502 Veterinary Group.
503 Hong, C., Lancaster, M.B., Michel, J.F., 1981. Worm burdens of dairy heifers in England and
504 Wales. Vet. Rec. 109, 12-14.
505 Keus, A., Kloosterman, A., Van den Brink, R., 1981. Detection of antibodies to *Cooperia spp.*
506 and Ostertagia spp. in calves with the enzyme-linked immunosorbent assay (ELISA).
507 Vet. Parasitol. 8, 229-236.
508 Klesius, P.H., 1988. Immunity to *Ostertagia ostertagi*. Vet. Parasitol. 27, 159-167.
509 Klesius, P.H., 1993. Regulation of immunity to *Ostertagia ostertagi*. Vet. Parasitol. 46, 63-79.
510 Kloosterman, A., Verhoeff, J., Ploeger, H.W., Lam, T.J., 1993. Antibodies against nematodes
511 in serum, milk and bulk milk samples as possible estimators of infection in dairy cows.
512 Vet. Parasitol. 47, 267-278.
513 Lin, L.I., 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics
514 45, 255-268.

515 Liua, G.L., Wanga, J.Q., Bua, D.P., Chenga, J.B., Zhanga, C.G., Weia, H.Y., Zhoua, L.Y.,
516 Zhoua, Z.F., Hua, H., Donga, X.L., 2009. Factors affecting the transfer of
517 immunoglobulin G1 into the milk of Holstein cows. Vet. J. 182, 79-85.
518 McDonald, J.H., 2014. Handbook of Biological Statistics (3rd ed.). . Sparky House Publishing,
519 Baltimore, Maryland, 180-185.
520 Merlin, A., Chauvin, A., Madouasse, A., Froger, S., Bareille, N., Chartier, C., 2016. Explaining
521 variability in first grazing season heifer growth combining individually measured
522 parasitological and clinical indicators with exposure to gastrointestinal nematode
523 infection based on grazing management practice. Vet. Parasitol. 225, 61-69.
524 Morgan, E.R., Hosking, B.C., Burston, S., Carder, K.M., Hyslop, A.C., Pritchard, L.J.,
525 Whitmarsh, A.K., Coles, G.C., 2012. A survey of helminth control practices on sheep
526 farms in Great Britain and Ireland. Vet. J. 192, 390-397.
527 Morley, F.H.W., Donald, A.D., 1980. Farm management and systems of helminth control. Vet.
528 Parasitol. 6, 105-134.
529 O'Kane, H., Ferguson, E., Kaler, J., Green, L.E., 2016. Associations between sheep farmer
530 attitudes, beliefs, emotions and personality, and their barriers to uptake of best practice:
531 The example of footrot. Prev. Vet. Med., 11pp.
532 Rasbash, J., Steele, F., Browne, W.J., Goldstein, H., 2012. A user's guide to MLwiN version
533 2.26. Centre for Multilevel Modelling, University of Bristol, 306pp.
534 Rinaldi, M., Geldhof, P. Immunologically based control strategies for ostertagiosis in cattle:
535 where do we stand? Parasitol. Immunol. 34(5), 254-264.
536 Roberts, F.H.S., 1942. The host specificity of sheep and cattle helminths, with particular
537 reference to the use of cattle in cleansing sheep pastures Aust. Vet. J. 18, 10-19.

538 Roeber, F., Jex, A.R., Gasser, R.B., 2013. Advances in the diagnosis of key gastrointestinal
539 nematode infections of livestock, with an emphasis on small ruminants. Biotech. Adv.
540 31, 1135-1152.
541 Rushton, J., Bruce, M., 2016. Using a One Health approach to assess the impact of parasitic
542 disease in livestock: how does it add value? Parasitology 4, 1-11.
543 Sanchez, J., Nodtvedt, A., Dohoo, I., DesCoteaux, L., 2002a. The effect of eprinomectin
544 treatment at calving on reproduction parameters in adult dairy cows in Canada. Prev.
545 Vet. Med. 56, 165-177.
546 Sanchez, J., Dohoo, I., Nodtvedt, A., Keefe, G., Markham, F., Leslie, K., DesCoteaux, L.,
547 Campbell, J., 2002b. A longitudinal study of gastrointestinal parasites in Canadian
548 dairy farms. The value of an indirect *Ostertagia ostertagi* ELISA as a monitoring tool.
549 Vet. Parasitol. 107, 209-226.
550 Sanchez, J., Dohoo, I.R., Markham, F., Leslie, K., Conboy, G., 2002c. Evaluation of the
551 repeatability of a crude adult indirect *Ostertagia ostertagi* ELISA and methods of
552 expressing test results. Vet. Parasitol. 109, 75-90.
553 Sanchez, J., Markham, F., Dohoo, I., Sheppard, J., Keefe, G., Leslie, K., 2004. Milk antibodies
554 against *Ostertagia ostertagi*: relationships with milk IgG and production parameters in
555 lactating dairy cattle. Vet. Parasitol. 120, 319-330.
556 Sekiya, M., Zintl, A., Doherty, M.L., 2013. Bulk milk ELISA and the diagnosis of parasite
557 infections in dairy herds: a review. Irish Vet. J. 66, 14.
558 Taylor, M.A., 2010. COWS. Sustainable Worm Control Strategies for Cattle. A Technical
559 Manual for Veterinary Surgeons and Advisors. AHDB.

560 Vanderstichel, R., Dohoo, I., Sanchez, J., Conboy, G., 2012. Effects of farm management
561 practices and environmental factors on bulk tank milk antibodies against
562 gastrointestinal nematodes in dairy farms across Canada. Prev. Vet. Med. 104, 53-64.
563 Vercruysse, J., Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in
564 cattle: defining the threshold. Vet. Parasitol. 98, 195-214.
565 Waller, P.J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by
566 grazing management and biological control. Anim. Feed Sci. Technol. 126, 277-289.
567 Wilson, L., Rhodes, A.P., Dodunski, G., 2015. Parasite management extension - challenging
568 traditional practice through adoption of a systems approach. NZ Vet. J. 63, 292-300.
569 Wright, P.F., Nilsson, E., Van Rooij, E.M.A., Lelenta, M., Jeggo, M.H., 1993. Standardisation
570 and validation of enzymelinked immunosorbent assay techniques for the detection of
571 antibody in infectious. Rev. sci. tech. Off. int. Epiz. 12, 435-450.

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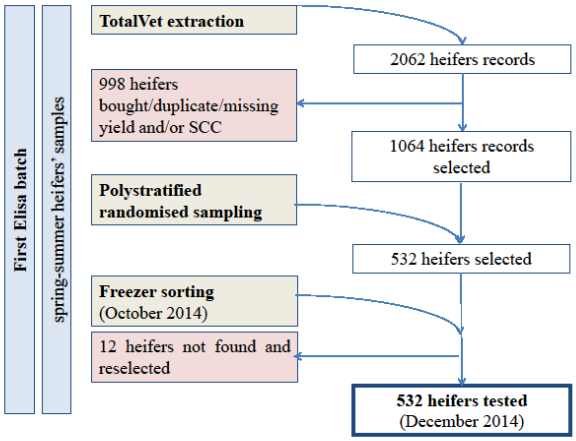
574 Bellet, Figure 1. Illustration of the stratified random sampling used for the selection of the 1,500

575 heifer individual milk samples tested for *Ostertagia ostertagi*



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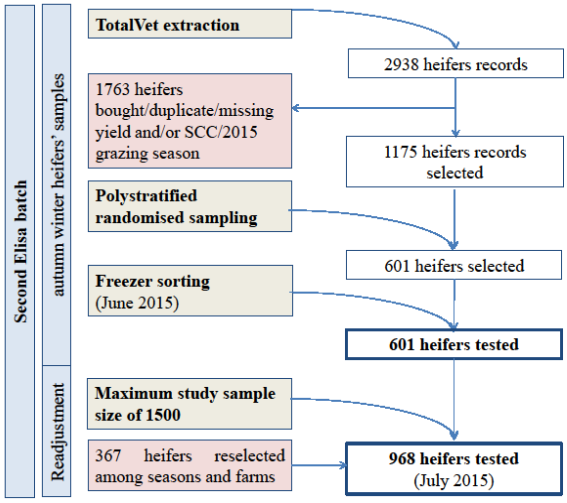

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584 Table 1. Characteristics of the study 41 farms included in the dairy longitudinal study

Variables	n	%
Enterprise		
Conventional (including integrated)	35	85
Organic	6	15
Production		
Pure-dairy	24	59
Mixed (including beef and/or sheep)	17	41
Closed herd		
No	26	63
Yes	15	37
Total dairy staff		
<5	16	39
5-22	25	61
Number of adult dairy cows		
<100	9	22
100-150	12	29
151-890	20	49
Dairy grazing surface (ha)		
<100	15	37
100-560	26	63
Calving system		
All-year-round	14	34
At least over two different seasons	27	66
All-year-round housing		
From weaned to bulling age	5	12
From bulling age to in-calf	5	12
Cows	4	10
Reports of helminth infections since 2010		
No	21	51
Yes	20	49

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586 Table 2. Final multilevel linear regression model of association between heifer individual milk ODR

587 and demographic and management variables as fixed effects ($N_{heifers}$=1,454 and N_{farms}=41)

Variable	$N_{Heifers}$ (%)	β	95% CI Lower	Upper
Intercept (SE)		0.736 (0.102)		
Season at heifer sampling	1454 (100.0)			
Spring	350 (24.1)	reference		
Summer	357 (24.6)	-0.029	-0.081	0.022
Autumn	373 (25.6)	0.016	-0.031	0.063
Winter	374 (25.7)	0.010	-0.043	0.063
Heifer's days in milk (d)	1454 (100.0)	-0.001	-0.002	0.003E-2[(a)]
Heifer's milk yield at sampling (kg)	1454 (100.0)	-0.004*	-0.006	-0.002
Heifer's log (SCC) at sampling (x1000 c/mL)	1454 (100.0)	0.030*	0.010	0.050
Farm's herd size	1454 (100.0)	0.001E-1	-0.002E-1	0.003E-1
Heifer's dairy breed	1454 (100.0)			
Purebred	1254 (86.2)	reference		
Crossbred	200 (13.8)	-0.112*	-0.165	-0.058
Heifer's total grazing (d)	1450 (99.7)	0.001E-1	-0.001E-1	0.003E-1
Heifer's number of treatment(s)	1428 (98.2)	0.004	-0.008	0.017
Heifer's protocol of treatment	1392 (95.7)			
No treatment	164 (11.3)	reference		
Long-acting wormer (turn-out)	402 (27.6)	-0.219*	-0.313	-0.125
Drench (turn-out)	8 (0.6)	-0.065	-0.284	0.155
Injection (turn-out)	43 (3.0)	-0.063	-0.167	0.042
Pour-on (turn-out)	201 (13.8)	-0.151*	-0.250	-0.052
Pour-on (grazing)	301 (20.7)	-0.119*	-0.208	-0.030
Pour-on (housing)	120 (8.3)	-0.108*	-0.202	-0.014
Drench (grazing and housing)	11 (0.8)	-0.100	-0.281	0.082
Drench and pour-on (housing)	8 (0.6)	-0.049	-0.252	0.153
Injection and pour-on (housing)	14 (1.0)	-	-	-
Drench and injection (grazing and housing)	38 (2.6)	-0.248*	-0.400	-0.095
Drench and pour-on (grazing and housing)	12 (0.8)	0.138	-0.043	0.319
Injection and pour-on (grazing and housing)	70 (4.8)	-0.342*	-0.472	-0.211
Heifer season of turn-out	1453 (99.9)			
Spring only	916 (63.0)	reference		
Vary	537 (36.9)	-0.076*	-0.113	-0.039
Heifer co-grazing with adult cows (d)	1454 (100)			
0	750 (51.6)	reference		
Milking and dry >14	248 (17.1)	0.093*	0.026	0.161
Dry ≤14	100 (6.9)	0.043	-0.022	0.107
Dry >14	104 (7.2)	0.120*	0.048	0.192
Milking ≤14	59 (4.1)	-0.022	-0.135	0.092
Milking >14	193 (13.3)	0.067*	0.006	0.128
Heifer grazing on sheep pasture	1451 (99.8)			
No	746 (51.3)	reference		
Gr_1 only	218 (15.0)	0.097*	0.032	0.162
Gr_2 only	28 (1.9)	0.174*	0.072	0.276

Gr_1 and Gr_2	405 (27.9)	0.073*	0.016	0.129
Gr_3/Gr_1 and Gr_3 /Gr_2 and Gr_3	9 (0.6)	-0.090	-0.314	0.134
Always	45 (3.1)	0.072	-0.051	0.194
Heifer co-grazing with sheep	1451 (99.8)			
No	1348 (92.7)	reference		
Gr_2 only	51 (3.5)	0.043	-0.061	0.148
Gr_1 and Gr_2	38 (2.6)	-0.030	-0.151	0.091
Gr_3/Gr_1 and Gr_3 /Gr_2 and Gr_3/ always	14 (1.0)	-0.196*	-0.387	-0.004
Heifer minimal stocking rate in Gr_1 (an/ha)	1429 (98.3)	0.041*	0.024	0.058
Number of mowed pasture grazed by heifer in Gr_2	1108 (76.2)	-0.003*	-0.006	-0.003E-1
Farm's bulk tank milk PP	1454 (100.0)	0.002E-1	-0.002E-1	0.006E-1
Farm's number of total dairy staff	1454 (100.0)	-0.010*	-0.020	-0.002E-1
Farm weaning age (w)	1454 (100.0)	0.015*	0.004	0.026
Farm use of another farm for heifer grazing	1454 (100.0)			
No	1130 (77.7)	reference		
Yes	324 (22.5)	-0.096*	-0.147	-0.044
Random effects				
Farm-level		0.001 (0.001)		
Heifer-level		0.052 (0.115)		

588 *= significant (P-value≤0.05). CI= confidence interval. SE= standard error. Ex= 10^x. Gr_i= grazing season *i*. PP=marker for *F.*
589 *hepatica.*
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