Short- and long-term association between individual levels of milk antibody against *Ostertagia ostertagi* and first-lactation heifer's production performances

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Abstract

It is agreed that exposure of adult dairy cattle to helminths on pasture can negatively affect production performances as milking herd. Young animals, especially replacement heifers, represent the future of a dairy farm and are among the most vulnerable to helminth infections in a dairy herd. For this reason, dairy farmers tend to frequently treat heifers against helminths, although the impact of helminths on heifers' production performances is still poorly understood. Using different epidemiological and serological tools, this study examines the relationship between heifer exposure to helminths on pasture and production performances over time. During a one-year period, 1,454 individual milk samples were collected from first-lactation heifers in England and tested for *Ostertagia ostertagi* (*O. ostertagi*) antibodies. After controlling for other confounders, increasing milk antibody levels against *O. ostertagi* were significantly associated with decreased milk yield at sampling but not at day 305 of heifer lactation. We did not observe any relationship between milk antibody levels against *O. ostertagi* in heifers and yields in fat and protein. However, heifers with a high level of milk antibodies against *O. ostertagi* were more likely to produce dead calf at first calving and present a delay in second calving. Moreover, these heifers had significantly higher levels of milk antibodies against *Mycobacterium paratuberculosis* (*M. paratuberculosis*) during their first lactation and were more likely to die before the end of the study. We argue that epidemiological approaches can be useful but must be complemented by other methodologies to better understand the impact of helminth infections in dairy heifers. In order to address the complex dynamics of helminth infections in dairy cattle production we require more comprehensive approaches that include triangulation between data sources and interdisciplinary studies.

Keywords: England; dairy heifer; *Ostertagia ostertagi*; individual milk ELISA*;* epidemiology; impact.

1. Introduction

Worldwide, there is an increasing demand for food, especially meat and milk (FAO, 2009). Alongside this demand, and due to growing concerns around food production sustainability (i.e. the need for increased food production with less waste and environmental impact) and other issues such as animal welfare, high expectations are put on livestock systems not only for increasing production and efficiency but also for complying with sustainability and ethical standards (Rushton and Bruce, 2016). According to recent reports, the global production of animal-source food is reduced by 20 % due to diseases (Vallat, 2009). Cattle helminth infections represent one of the growing concerns for the cattle industry around the world (Skuce et al., 2013). Intensification of cattle production as well as changes in climate and management practices have affected the distribution of helminth infections in cattle (Rushton and Bruce, 2016). In fact, in recent years, the incidence of chronic diseases due to cattle helminth infections has increased along with evidence of parasite resistance to cattle anthelmintic drugs (Stafford and Coles, 1999; Pritchard et al., 2005; Skuce et al., 2013).

In temperate areas such as England, there is a general agreement that cattle helminths, particularly *Ostertagia ostertagi* (*O. ostertagi*), are of major importance in terms of their economic impact on the dairy livestock system (Skuce et al., 2013; Charlier et al., 2014; Sargison, 2014). However, to date there is no systematic and agreed approach to assess the costs associated with cattle helminth infections (Rushton and Bruce, 2016). In this context, there is a need for better understanding the biological processes underlying cattle helminth infections, in particular *O. ostertagi*, under real farm conditions.

A number of studies have been conducted on farms to understand the effects of helminth infections on cattle milk production and reproductive performances (Sanchez et al., 2004a). Some of these studies have shown that effective treatments for subclinical helminth infections

61 are associated with increasing milk production (Sanchez et al., 2004a; Charlier et al., 2007b;
62 Verschave et al., 2014). A meta-analysis of published literature estimated that, after controlling
63 for study bias, anthelmintic treatments were associated with a daily milk increase of 0.35
64 kg/cow/day (Sanchez et al., 2004a). However, such an approach does not take into account the
65 effect of different helminths and exposure levels on production losses. In addition, evidence
66 suggests that anthelmintic drugs could directly stimulate cow milk production (Purvis and
67 Whittier, 1996). In other studies, high levels of bulk tank milk antibody against *O. ostertagi*
68 were associated with an annual drop of cow milk production (Sanchez and Dohoo, 2002;
69 Charlier et al., 2005). However, the use of pooled samples also makes the interpretation of
70 these results difficult (Sekiya et al., 2013). In addition to these effects on milk production, cattle
71 helminths could also reduce calving interval and number of breeding at conception and increase
72 the mortality rate in a dairy herd (Walsh et al., 1995; Stromberg et al., 1997; Sanchez et al.,
73 2002a; Delafosse, 2013). Interestingly, although heifers represent a capital investment for dairy
74 farmers and are among the most vulnerable to this type of infections and production losses,
75 little has been done to explore impacts of helminth infections in first-lactation heifers, with
76 very few, inconclusive studies available (Blanco-Penedo et al., 2012; Liedtke et al., 2013).
77 Moreover, it is not clear whether losses in milk yield due to helminth infections can be
78 compensated during the subsequent lactations of the cow. Finally, although there is clear
79 evidence that *O. ostertagi* actively suppresses cattle immune responses (Gasbarre, 1997) , there
80 is no evidence from studies conducted on farms of the effects of this parasite on cattle
81 susceptibility to other diseases.

82 Climatic conditions and herd management vary greatly between countries, which
83 ultimately influences measures of impact (Williams, 1999; Sanchez et al., 2002a). Moreover,
84 infections such as helminth infections affect cattle systems at different levels (e.g. animal, farm,
85 livestock sector and national) and dimensions (e.g. milk production, reproduction, health and

welfare), for which the individual level represent a start (Rushton and Bruce, 2016). In this study, we examine the relationship between individual exposure to helminths on pasture and the production performances of first-lactation heifers in England, taking the gastrointestinal nematode (GIN) *O. ostertagi* as a case study. Besides overcoming methodological limitations in the current literature, we also discuss the value of epidemiological approaches in assessing the effects of cattle helminth infections on production performances under real farm conditions.

2. Materials and methods

2.1. Study heifers

Since individual milk (IM) antibody levels against *O. ostertagi* highly vary within-farm (Charlier et al., 2007a), the sampling aimed to sample more heifers per farm across the seasons than farms. Heifers came from a convenience and purposive sample of dairy farms, all members of the Quality Milk Management Services (QMMS) recording scheme, Somerset, England. Farms were selected to allow the representation of different levels of heifer exposure to helminths on pasture and heifer management. Farm inclusion 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 with data recording, agreeing with the study protocol and sharing farm records. There were no restrictions on the type of cattle housing (i.e. housed all-year-round, in the winter only, and varied) or the practices of anthelmintic treatments. In total, 43 English dairy farms were included in the study. The average size of herds sampled was 150 cows, of which 46 were first lactation heifers. Heifer IM samples were obtained from samples routinely collected and stored by QMMS. 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 significant association between outcomes (i.e. heifer production, reproduction and health) and predictors (i.e. *O.*

ostertagi milk antibodies) (Dohoo et al., 2009). Heifer sample size calculation was based on available estimates of association between anti-*O. ostertagi* milk antibody levels and milk production in adult cows (Sanchez et al., 2004a). Considering the origin of the farms, no estimate of likely dropouts and withdrawals was taken into consideration in the heifer sample size determination. A total of 1,500 heifers were included in the study from March 2014 to March 2015 - with 35 heifers (i.e. 1,500/43) regularly sampled throughout the seasons on each farm and tested for *O. ostertagi* antibodies. A more detailed description of the heifer samples selection criteria and the sampling process is available in Bellet et al. (2018).

2.2. Data collection

Detailed retrospective and prospective information on demographic and management was obtained for each heifer, from birth to the end of the study (i.e. one year after the last heifer sampling). These included information on housing, food (including grazing), vaccination and anthelmintic treatments, before and after individual sampling. The collection of data relied on the use of different tools and approaches, including questionnaires, face-to-face and telephone interviews and QMMS' information management system. Individual parameters of heifers' milk production, reproduction and health were extracted from QMMS laboratory's information management system and processed using the dairy herd data analysis program TotalVet (QMMS Ltd/SUM-IT Computer Systems). In order to collect one-year of prospective production data for each heifer, data covered the period between March 2014 (i.e. start of the milk samples collection) and April 2016 (i.e. the end of the study). At the time of milk sampling (t_S), heifers' individual records included season, age, breed, milk yield, fat, protein, somatic cell counts (SCC), calving date and status of offspring (i.e. alive or dead). Cumulative milk, protein and fat yields of heifers at day 305 of heifer lactation (t_{305}) were obtained if heifers had reached this stage at the end of the study (t_E). These were calculated beforehand by QMMS, using the 'test-interval' method (ICAR, 2016). The interval between the first and second

136 calving of heifers was computed from the corresponding calving dates, if present. Since
137 farmers' assiduousness to record varied by farm and variables, only accurate health variables
138 with a sufficient number of observations were extracted from TotalVet and considered for the
139 analysis. These health variables included individual levels of milk antibody against
140 *Mycobacterium paratuberculosis (M. paratuberculosis)* during the first lactation of heifers and
141 heifer's health status at t_E (i.e. present, dead and absent (culled or dead)).

142 *2.3. ELISA milk testing*

143 Considering the fact that heifer samples would be stored for a period of several months
144 before testing, a pilot study was conducted to evaluate the effect of milk sample storage for
145 over a one-year period on ELISA results using IM samples from adult cows. Cow samples that
146 had been tested for *O. ostertagi* antibodies in 2012 and then stored at QMMS at -20 °C, were
147 tested again under similar laboratory conditions in March 2014. No significant differences were
148 obtained between the results of the two years (Bellet et al., 2018). After collection on farm,
149 heifer IM samples were preserved using bronopol/natamycin and kept at ambient temperature
150 until arrival at the laboratory. In the laboratory, the samples were processed, tested for SCC,
151 fat and protein, before being frozen at -20 °C (±2 °C) until further testing; this was achieved
152 within the first 48 h after samples collection on farms. In order to account for possible cross-
153 reactivity between the *O. ostertagi* test and *Fasciola hepatica* (*F. hepatica*) (Bennema et al.,
154 2009), levels of farm exposure to *F. hepatica* were determined by antibody-detection ELISA
155 applied on bulk tank milk (BTM) at the end of the grazing season 2014 (i.e. from October to
156 December 2014). BTM samples were also tested for *O. ostertagi* antibodies. IM and BTM
157 samples were defrosted, defatted by centrifugation (2000 x g, 2 min) and their supernatant
158 collected. Samples were tested undiluted according to the kit manufacturer's instructions. All
159 tests were conducted by the same technician, who was blinded to the identity of the animal.
160 The *F. hepatica* test was performed using 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* tests were performed using the Svanovir® kit sourced from Svanova Ltd. (Uppsala, Sweden), which is an indirect ELISA based on crude saline-extracts of *O. ostertagi* adult worm antigens (Keus et al., 1981; Sanchez et al., 2002b). Results were expressed as an Optical Density Ratio (ODR) of the sample to guarantee test repeatability (Sanchez et al., 2002b), after the measure of OD from both sample and positive and negative controls at 405 nm.

2.4. Data collation and statistical analyses

Computer data entry was conducted using Microsoft Excel and Access (Microsoft, 2013). Data were collated and initially analysed using STATA 12.1 (STATA Inc., Texas, USA). Due to the nature and the complexity of individual information on grazing management, a systematic process of data entry was performed for each heifer included in the study (Bellet et al., 2018). Data were collated and initially analyzed using Stata 12.1 (Stata Inc., College Station, TX). As farmers did not report significant changes in their farming in the last four years, a general profile of demographic and management practices (except grazing) was established for each farm. Descriptive and graphical analyses were carried out to explore data on farms and heifers. Three sets of statistical modelling analyses were conducted in MLwiN 2.30 (Rasbash et al., 2012), according to the nature of the production outcome (i.e. milk production, reproductive performances and health). Since, for all models, several heifers originated from the same farm, the independence of the observations could not be assumed and the models had heifers' IM ODR nested within farms. Therefore, all statistical models incorporated two hierarchical levels: level 1 (i), a heifer level, level 2 (j), a farm level. In each analysis, all collected variables were first tested in a univariable multilevel model. Association

between outcomes and collected variables was 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. We explored interactions among predictors that were found to be significant in main effects model (Dohoo et al., 2009). The scale of the coefficient of the ELISA predictors were converted to be interpreted as the effect of a 0.1 unit increase of the ELISA predictor on the outcome.

2.4.1. Association between individual levels of milk antibody against Ostertagia ostertagi and first-lactation heifers' milk production

Six multilevel linear regression models were used to estimate the association between IM ODR and the following outcomes: (1) milk yield at t_S, (2) protein yield at t_S, (3) fat yield at t_S, (4) milk yield at t_{305}, (5) protein yield at t_{305}, and (6) fat yield at t_{305}. The models were developed using a reweighted generalised iterative least squares algorithm (Rasbash et al., 2012) and took the form (1):

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

Where: y_{ij} = outcome, i.e. the milk production parameter of the *i*th heifer from the *j*th farm; 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} = farm random effect and e_{0ij} = heifer level residual, both assumed to be normally distributed. Information on known confounding variables, as identified from previous literature (Klesius, 1993; Kloosterman et al., 1993; Sanchez et al., 2004b), was collected and these variables were retained in the final models. These included herd size, BTM ODR, BTM PP, breed, record season, DIM, log(SCC) and age. The effect of DIM on milk yield was included using the Wilmink's function (Wilmink, 1987). Model goodness-of-fit was assessed

209 by examination of QQ plots and kurtosis of residual distributions (Dohoo et al., 2009; Rasbash
210 et al., 2012).

211 *2.4.2. Association between individual levels of milk antibody against Ostertagia ostertagi and*
212 *first-lactation heifers' reproductive performances*

213 A multilevel binomial regression model was first built to investigate the association
214 between IM ODR and the probability of heifers to have a dead calf at first calving (i.e. to abort
215 or have a stillborn calf). The model used a logit link function (Rasbash et al., 2012) and took
216 the form (2):

217 $$logit\left(\pi_{ij}\right) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + \mathrm{u}_{0j} \quad (2)$$

218 Where: π_{ij} = the outcome, i.e. the probability of the *i*th heifer of the *j*th farm to have a dead
219 offspring at first calving; β_0 = intercept value; β_1 = vector of coefficients for x_{ij}; x_{ij} = vector
220 of covariates associated with each heifer; β_2 = vector of coefficients for x_j; x_j = vector of
221 covariates associated with each farm; u_{0j} = the random effect to account for residual variation
222 between farms, assumed to be normally distributed.

223 A multilevel discrete time survival model was also built to express the hazard of a heifer
224 to calve for the second time in an interval *t*, given that the heifer had not calved before the start
225 of this interval. The time follow-up of the survival analysis was set at 681 days, i.e. one year
226 plus the time of a subsequent gestation. The heifers that had not conceived a second time by
227 that time were considered as censored. The continuous time interval between first and second
228 calving was divided into four discrete categories of time at 120 days intervals. The time interval
229 was nested within heifers; therefore a third hierarchical level was incorporated in the model.
230 The model used a complementary log-log function to express the outcome probability, given
231 this function is based on the assumption of the proportional hazards (Dohoo et al., 2009;
232 Rasbash et al., 2012) and took the form (3):

$$cloglog\left(h_{tij}\right) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_{0j} + e_{0ij} \quad (3)$$

Where: h_{tij} = the outcome, i.e. the hazard of the *i*th heifer of the *j*th farm to have her second calving in the interval *t* given that this heifer was present at the start of this interval; β_0 = logit(hazard) in the baseline time interval for a baseline heifer; β_1 and β_2 represented the heifer level and the farm level vectors of coefficients; x_{ij} and x_j were the heifer level and the farm level vectors of predictor variables; u_{0j} = farm random effect and e_{0ij} = heifer level residual, both assumed to be normally distributed. A term for the interaction between predictors and time was also added in the model 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).

Both models were fitted using a second-order penalised quasi-likelihood methods (RIGLS) to produce starting values for the second models using the method of Markov Chain Monte Carlo (MCMC). The convergence of the models were assessed visually (Hamra et al., 2013; Browne, 2015). MCMC chains were run for 100,000 and 500,000 iterations, respectively, after a burn-in of 5,000 iterations.

2.4.3. Association between individual levels of milk antibody against Ostertagia ostertagi and first-lactation heifers' health

A multilevel linear regression model was used to estimate the association between individual levels of milk antibody against *O. ostertagi* and *M. paratuberculosis.* Heifer samples were excluded from this analysis if the *M. paratuberculosis* test had been performed before the *O. ostertagi* test. A confounding variable, accounting for the time interval between the two serological tests was retained in the final model.

A multinomial regression model was also built to investigate the association between IM ODR and the probability of heifers to die before the end of the study (t_E). The model used

a logit link function to express the ratio probability of a given status to the probability of the reference score (Rasbash et al., 2012) and took the form of equation (4):

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

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

3. Results

3.1. Study heifers

Of the 43 dairy farms included, two withdrew shortly after the start of the study, resulting in a study participation of 95 %. Most of the study farms (76 %) were clustered around south-west counties, including counties of Somerset (N=18), Wiltshire (N=8), Devon (N=3), Cornwall (N=1), and Gloucestershire (N=1). A total of 1,454 heifer IM samples were included in the study, 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 (IQR), 25–75 %] of heifers sampled per farm was 34 (25–44). Sampled heifers were predominantly Holstein Friesian (91 %) and mainly born in 2012 (n = 1,013; 70 %). Main characteristics of the farms are presented in Table 1, in particular those related to food management, health and production

280 performances. Most heifers (59 %) had two grazing seasons before sampling, while the others
281 had one (17 %) or more than two (24 %).

282 *3.2. Association between individual levels of milk antibody against Ostertagia ostertagi and*
283 *first-lactation heifers' milk production*

284 The final models of association between IM ODR and heifers' yields in milk, protein
285 and fat at t_S and t_{305} are presented in Tables 2 and 3. At t_S, heifers' milk yield was significantly
286 associated with levels of heifer and farm exposure to *O. ostertagi* on pasture: for each 0.1 unit
287 increase in IM and BTM ODR, individual milk yield declined by 0.26 kg [95 % Confidence
288 Interval (CI): -0.40;-0.13] and 0.92 kg (95 % CI: -1.37;-0.48), respectively. Moreover, heifers
289 that originated from farms with high exposure to *O. ostertagi* at the end of the grazing season
290 had significantly lower milk yield at t_{305} [Coefficient (β) (95 % CI): -121.09 kg (-226.74;-
291 15.45)]. After controlling for milk yield, there were no significant association between yields
292 in protein and fat and levels of milk antibodies against helminths at both individual and farm
293 levels. Visual examinations of final residuals at each hierarchical level suggested that the six
294 models fitted well the data. Moreover, there was no effect of any outliers and, therefore, they
295 were left in the models.

296 *3.3. Association between individual levels of milk antibody against Ostertagia ostertagi and*
297 *first-lactation heifers' reproductive performances*

298 The final multilevel binomial regression model of association between IM ODR and
299 the probability of heifers to have a dead calf at first calving is presented in Table 4. After
300 controlling for other variables, the odds for a heifer to abort or have a stillbirth calf at first
301 calving significantly increased by 1.11 (95 % CI: 1.03;1.19) for each 0.1 unit increase in IM
302 ODR. A total of 1,423 heifers were included in the discrete time survival analysis, of which
303 225 (18 %) were censored. The final multilevel discrete time survival model of association

between IM ODR and hazard to have a second calving in an interval *t* is presented in Table 5. The hazard for a baseline heifer to calve for a second time after a first calving was 0.84 (95 % CI: 0.21;3.46). Heifers' hazard to calve for the second time significantly increased over time. After controlling for other confounders, the hazard for a heifer to calve for a second time at a time *t* decreased by 0.95 (95 % CI: 0.90;0.99) unit for a 0.1 unit in IM ODR. The visual examination of the MCMC diagnostic plots for each parameter included in both models suggested that models converged well.

3.4. Association between individual levels of milk antibody against Ostertagia ostertagi and first-lactation heifers' health

The final multilevel linear regression model of association between *M. paratuberculosis* and *O. ostertagi* ELISA results is presented in Table 6. A 0.1 unit increase in IM ODR was associated with a significant 0.48 unit (95 % CI: 0.16;0.61) increase in heifer's titre for *M. paratuberculosis* antibodies during the first lactation. Moreover, after controlling for other variables, a 0.1 unit increase in IM ODR increased the odds for a heifer to be dead by the end of the study by 1.12 (95 % CI: 1.01;1.25) (Table 7). When BTM predictors were included in this last model, neither individual nor BTM predictors were significantly associated with the outcome (data not shown). The visual examination of the models indicated a good overall fit at both levels.

4. Discussion

Epidemiology is one area of scientific enquiry that enables scientists to explore impacts of cattle diseases under real farm conditions (as opposed to laboratory conditions). However, as impact causality is increasingly understood to arise from an entanglement of host, pathogen and environmental variables, epidemiologists must use innovative approaches in their impact studies to incorporate and address this complexity.

329 In the current study, we used individual serological markers of response to helminths from
330 young animals (as opposed to bulk-tank-milk markers and adult dairy cows). This way, we
331 limited the bias and confounding often seen in previous research, which result from the age of
332 the animals (and their physiological state), the duration of exposure, the mixing of the samples
333 and the memory of farmers in relation to their own management practice (Sanchez et al., 2004b;
334 Dohoo, 2009; Sekiya et al., 2013). We also used a stratified random sampling approach for the
335 selection of heifers, took into account different seasons and farming systems from several
336 English counties, and collected an extensive range of individual data from different data
337 sources. All this allowed us to better control for bias and confounding effects in the different
338 models (Dohoo et al., 2009). Importantly, the participation of farmers remained particularly
339 high during the 2 years of the study (95 %); something that often hinders this type of research
340 (Goldstein et al., 2015). The choice of an ELISA diagnostic tool was also critical and depended
341 on the specificity and sensitivity of this approach, besides requirements in terms of time and
342 financial resources (Keus et al., 1981; Roeber et al., 2013; Charlier et al., 2014). Considering
343 that most of the sampled heifers had grazed for at least two years, *a priori* no limitation was
344 included in terms of immaturity of immune responses (Gasbarre, 1997). Given that the ELISA
345 test can cross-react with other GIN, for which no control was made in this study (Keus et al.,
346 1981), the test allowed for the assessment of exposure to GIN infections rather than simple *O.*
347 *ostertagi* infections. However, because ELISA techniques do not permit to differentiate
348 between past and present infections (Roeber et al., 2013), antibody levels were used as a marker
349 of heifer response to GIN infections rather than a tool for measuring GIN infection levels
350 (Charlier et al,, 2014). This represents a common and important limitation of epidemiological
351 surveys to measure impact (Knight-Jones et al., 2016).
352 Several key parameters of heifers' production were negatively associated with heifer
353 exposure to GIN on pasture. Heifers that had been highly exposed to GIN were more likely to

354 die before the end of the study. In fact, the effect of GIN on heifers' mortality disappeared
355 while accounting for *F. hepatica* BTM PP (data not shown), suggesting that this association
356 was related to cattle helminth infections rather than GIN infections. This agrees with previous
357 observations reported at farm level (Delafosse, 2013) and could be related to the poor digestion,
358 protein absorptions and, overall, poor cattle condition due to helminth infections (Hawkins,
359 1993), as well as other confounder factors that were not captured in the current study, such as
360 other etiological agents and disease control practices involved in heifer's mortality. This may
361 also explain why heifers with high levels of antibodies against GIN were more likely to lose
362 their calf at first calving and to present a delay in their second calving (Mejia et al., 1999;
363 Loyacano et al., 2002; Greer, 2008). Such persistence of the effect of GIN infections on
364 performances over time has been reported in young calves (Ploeger et al., 1990).
365 Many laboratory experiments suggest that *O. ostertagi* induces an important
366 immunosuppression in cattle, which can have an impact on cattle susceptibility to other
367 diseases and, ultimately, on cattle health (Gasbarre, 1997). However, there is very little field
368 evidence on this subject (Kloosterman et al., 1989; Gasbarre, 1997). On the other hand, the
369 capacity to exert a bystander effect on concurrent bacterial infections in the host has been
370 reported in the field for other cattle helminths (Aitken et al., 1978; Claridge et al., 2012; Gorsich
371 et al., 2014), especially in the case of infections due to *M. avium* subsp. *paratuberculosis*
372 (Lucena et al., 2017). In this study, we observed a significant association between individual
373 levels of milk antibody against GIN and *M. paratuberculosis*. Since there is no temporality
374 associated with this observation, the conclusions remain difficult. However, taking into account
375 the increasing number of Johne's cases in England (SAC, 2003), such an observation should
376 not be ignored. Other experiments should in fact be conducted to see whether GIN infections
377 increase the susceptibility of cattle to *M. paratuberculosis* infections or whether our

observation is due to different immunocompetence and immunoresponsiveness of the host (Greer, 2008).

It is not clear whether the negative association between IM ODR and heifers' milk yield was due to a negative effect of GIN infections on milk production or a dilution effect, as the one we observed for fat and protein yields (Kloosterman et al., 1993; Sanchez et al., 2004b). Moreover, one of the main objectives of the current study was to explore if GIN effects on heifers' milk yield persisted over time, i.e. at least until day 305 of heifer lactation. Our results suggest that it did not. However, it is worth noting that 31 % of the heifers (N=449) withdrew from this analysis. In addition, considering that heifers' milk yield at day 305 was significantly associated with BTM ODR, i.e. a pool of milk samples from all lactating animals including heifers at day 305 of the lactation period, it is questionable whether the different points made can be related to the choice of our indicator (i.e. the serological marker). In any case, it is important to note that there are a limited number of accurate and feasible methods that exist for the diagnostic of GIN infections in cattle (Roeber et al., 2013).

It is widely accepted that helminths have a negative impact on production and productivity in cattle systems (Charlier et al., 2014). However, there is insufficient evidence to allow for robust assessments of the impacts of helminths on the cattle industry. In this study, we observed how difficult it is to decipher the complexity of infectious processes based on the mere observation of association between predictors and outcomes and how the production of scientific knowledge can be therefore limited by the use of a single scientific approach, regardless of its quality. Therefore, frameworks that look at both direct losses attributable to the parasites and our responses to the presence or threat of these parasites are required (Rushton and Bruce, 2016). Of particular value are interdisciplinary and integrative approaches that consider the human, animal and environmental dimensions together. Without more comprehensive and integrated assessments of cattle helminth infections, prioritization

exercises in disease management will continue to rely on judgement calls by the various stakeholders involved in the dairy sector.

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