Veterinary Parasitology 197 (2013) 212–220

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

The association between *Ostertagia ostertagi* antibodies in bulk tank milk samples and parameters linked to cattle reproduction and mortality

Arnaud Delafosse*

Groupement de Défense Sanitaire de l'Orne, 61004 Alençon, France

ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form 10 May 2013
Accepted 18 May 2013

Keywords:
Ostertagia
ELISA
Bulk tank milk
Production parameters

ABSTRACT

In Western Europe, gastrointestinal nematodes are widespread in dairy cattle. This study was carried out to evaluate the relationship between optical density ratio (ODR) measured on bulk tank milk with an indirect *Ostertagia ostertagi* ELISA and reproduction/mortality parameters. Data were collected between 2008 and 2010 from monitoring carried out on 1643 dairy herds (Normandy, Western France). ODR values of 3 samples from each farm taken from November 2008 to 2010 were averaged and then transformed into a categorical variable. Reproductive and mortality data were obtained from 1444 herds using cow records from government databases. Statistical analysis was carried out using ordinary logistic regression (OLR). The outcome variables were the case–control status of a herd for reproductive factors, age at first calving and inter-calving intervals, and mortality ratios of various age classes. The effect of the categorical ODR variable was studied and several potential confounder herd factors were used to improve the model fit. A significant relationship was found between high *Ostertagia* ODR levels and a late age at the first calving (>34.5 months) (odds ratio (OR) = 1.94, $p < 0.001$). No significant relationship was observed with OLR for inter-calving intervals although bivariate analysis showed that herds with high ODR levels had longer inter-calving intervals than herds with low ODR level (first inter-calving interval in herds with low vs. high ODR levels = 412 days vs. 422 days, $p < 0.001$; other inter-calving intervals = 408 days vs. 413 days, $p < 0.01$).

A high ODR level was also associated with high mortality of calves between 0 and 30 days of life (mortality ratio > 6%) (OR = 1.43, $p < 0.05$) and between 91 and 365 days (ratio > 3%) (OR = 1.72, $p < 0.01$). No significant relationship was observed with multivariate approach for mortalities in other classes by age, but bivariate analysis showed that herds with high ODR level had higher mortalities than herds with low ODR levels (mortality between 31 and 90 days in herds with low vs. high ODR levels = 1.89% vs. 2.91%, $p < 0.001$; mortality after 365 days = 1.67% vs. 2.93%, $p < 0.001$).

In conclusion, our results confirm the usefulness of ELISA as an indicator for production losses in dairy herds. This inexpensive tool could be advantageous, used to aid farmers and veterinarians to carry out appropriate control measures.

1. Introduction

In Western Europe, gastrointestinal (GI) parasitic nematodes are widespread, particularly in dairy cattle, and the most important species are *Ostertagia ostertagi* and *Cooperia oncophora* (Kenyon and Jackson, 2011). Infections with

---

* Tel.: +33 02 33 80 38 38; fax: +33 02 33 31 94 40.
E-mail address: arnaud.delafosse@gdsco.fr

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.05.023

GI nematodes were considered to be mainly important in first-season grazing calves but studies have demonstrated that subclinical GI-nematode infections can impair milk yield in adults (Gross et al., 1999; Sanchez et al., 2004b). The major problem however is to identify the herds where the infection level is high enough to justify an anthelmintic treatment (Vercruysse and Claerebout, 2001).

Diagnostic techniques such as faecal egg count and serum pepsinogen assays have been shown to be of limited use in adult dairy cattle (Ploeger et al., 1989, 1990; Berghen et al., 1993). Enzyme-linked immunosorbent assays (ELISAs) have been used as a diagnostic tool to quantify the impact of gastrointestinal nematodes in dairy cattle by measuring *O. ostertagi* antibodies in milk. Higher levels of *O. ostertagi* antibodies measured by ELISA methods, referred to as optical density ratios (ODRs), are associated with decreased milk production in dairy cattle (Sanchez and Dohoo, 2002a; Guiot et al., 2007).

The main impact of gastrointestinal parasites for farmers is linked to loss of milk production and weight gain. However, some studies suggest that *O. ostertagi* in cattle can provoke a non-specific immune suppression, reducing the ability of the animal to respond to heterologous antigens and increasing general susceptibility to disease (Wiggin and Gibbs, 1989; Hawkins, 1993). As a result, a high herd exposure to GI nematodes could increase the mortality of post-weaning calves, heifers and adult cows, and reduce fertility. Pre-weaned calves may also be affected by the absorption of antibody-poor colostrum. Another consequence of reduced weight gain in calves and heifers is an extended time in reaching breeding weight, thus involving reproductive costs.

The major problem remains of how to monitor GI nematode infections in an adult dairy cow to indicate if the infection is causing an impact on productivity. At the herd level, *Ostertagia*-specific antibody level determination in bulk tank milk is the most promising method for this purpose (Charlier et al., 2009).

A limitation of using bulk tank milk is that information is only obtained from the lactating cows, ignoring calves, heifers and cows in the dry period. However, significant relationship was found between an increased exposure to pasture of the heifers and higher ODRs, suggesting that bulk tank milk ELISA at the end of the grazing season is a tool to evaluate the herd's exposure to GI nematodes by the end of the grazing period (Charlier et al., 2009).

The effect of GI nematodes on reproductive performance was studied in dairy cattle but remains equivocal. Some studies of anthelmintic treated cows, compared with untreated controls, showed an increase in cow conception rate, calving rate, reductions in calf mortality and reductions in the calving to breeding interval (Hawkins, 1993; Gross et al., 1999). At the herd level, little work has been published on the relationships between ODRs and reproductive performance (Guiot et al., 2007). No studies have explored the relationship between GI nematode infections in cow at the herd level and mortality.

The present study was carried out to evaluate the relationship between ODRs measured on bulk tank milk and reproduction/mortality parameters. Data were collected between 2008 and 2010 by monitoring carried out on all dairy herds in the Orne department (Normandy, Western France). The aim was to clarify the significance of ODRs in bulk tank milk as an indicator for production losses in dairy herds.

2. Materials and methods

2.1. Sample collection and laboratory methods

All dairy farms in the Orne department (Normandy, Western France) were included in the study. A bulk tank milk sample was taken during a routine milk collection from the dairy cooperatives on 3 occasions: once in November 2008, once in November 2009 and once in November 2010. During the collection in 2008, 2091 herds were analysed against 2005 in 2009 and only 1927 in 2010. All samples arrived at the laboratory between 24 and 72 h after collection from the farms. During all handling procedures the samples were constantly stored at 4 °C. Bulk tank milk samples from each farm were tested with an indirect ELISA using a crude *O. ostertagi* antigen (SVANOVIR® *O. ostertagi*-Ab ELISA Kit). The test results were expressed as an optical density ratio (ODR). Analysis was conducted by two professional dairy laboratories (LILANO, ANALIS) following the manufacturer's recommendations.

2.2. Collection of farm and production data

For each herd, the optical density ratio (ODR) values from 3 years – 2008, 2009, 2010 – were averaged and then transformed in a categorical variable using 2 cut-off points producing 3 levels: low $\leq$0.70; medium >0.70 and $\leq$0.90; high >0.90. Only 1643 herds had results over the 3 years studied.

Information on reproductive and mortality parameters were obtained from computerized cow records from the governmental database (BDNI). Selected herds in the study had at least 25 breeding dairy females (>24 months) on 1 January 2008/2009/2010. Only 1461 herds met this criterion and 1444 had ODR values. Reproductive performance of dairy cows as measured by: 1 – age at the first calving; 2 – first inter-calving interval (interval between first and second calving); 3 – other inter-calving intervals (all inter-calving intervals other than the first). Data from each herd were calculated by year (2008/2009/2010) and then averaged for 3 years.

Mortality was estimated by several ratios depending on the age group. Cattle births (including stillbirths) were taken into account between 1 January 2008 and December 31, 2010. The output (including mortalities) was recorded until July 10, 2011 (the oldest animals were 1287 days = 3.5 years). Cattle that died or were sold during this period were included in the denominator for half the time.

- Mortality ratio to 0 day = number stillborn calves/number of calves born (including stillbirths)
- Mortality ratio from 0 to 30 days = number of calves died between 0 and 30 days/[(number of calves born − number dead 0J) − (0.5 × number of calves dead or sold between 0 and 30 days)]

- Mortality ratio between 31 and 90 days = (number of calves died between 31 and 90 days)/[number of calves present at 30 days – (0.5 × number of calves dead or sold between 31 and 90 days)]
- Mortality ratio between 91 and 365 days = (number of dead cattle between 91 and 365 days)/[number of cattle present at 90 days – (0.5 × number of cattle dead or sold between 91 and 365 days)]
- Mortality ratio after 365 days = (number of cattle died after 365 days)/[number of cattle present at 365 days – (0.5 × number of cattle dead or sold after 365 days)]

Several potential confounder herd factors were used to improve the model fit (Table 1). Factors were divided into health status (fasciolosis and BVD), general management (number of reproductive dairy cows, proportion of Holstein breed, proportion calving in winter, proportion of first-calving cows) and somatic cell counts in bulk tank milk (SCC) which was used as indicator of poor hygiene practices. Moreover, a study had demonstrated a relationship between SCC and *O. ostertagi* ODR values. Serum total IgG concentrations are approximately 35-fold higher than total IgG concentrations in mature milk (Butler, 1986) and mastitis can cause a flow of specific and non-specific antibodies from the serum to the milk (Caffin et al., 1983; Charlier et al., 2006).

2.3. Statistical analysis

The outcome variables in the study were the case–control status of a herd for several factors: 1 – age at first calving (*case*: >34.5 months vs. *control*: ≤34.5 months); 2 – first inter-calving interval (*case*: >415 days vs. *control*: ≤415 days); 3 – others inter-calving intervals (*case*: >410 days vs. *control*: ≤410 days); 4 – mortality to 0 day (*case*: >5% vs. *control*: ≤5%); 5 – mortality 0–30 days (*case*: >6% vs. *control*: ≤6%); 6 – mortality 31–90 days (*case*: >2% vs. *control*: ≤2%); 7 – mortality 91–365 days (*case*: >3% vs. *control*: ≤3%); 8 – mortality after 365 days (*case*: >1.5% vs. *control*: ≤1.5%).

Optimal case–control cut-off values were chosen after consideration of variable distributions by looking for inflection points and ensuring balance the numbers in each group.

The bivariate association between ODR status and each studied parameter was obtained from Wilcoxon test. The effect of categorical ODR variable on the outcome variables was then assessed by a multivariable logistic regression. The covariates listed in Table 1 were included in the eight multivariable models. Multivariate analysis uses a backward stepwise logistic regression with a procedure minimizing the Akaike Information Criterion (AIC) (Akaike, 1974). The stepwise method based on AIC that we use is based on the stepAIC function of Venables and Ripley (1999) (STEP procedure for glm models, R statistical software). The R function calculates the AIC according to the formula of Sakamoto et al. (1986). The starting candidate model is based on using all of the retained predictors. Subsequent models are based on omitting a variable from the current candidate model or adding a variable that is not in the model, with the choice based on minimizing AIC. The final model is found when adding or omitting a variable does not reduce AIC further.

Multicollinearity among predictors in logistic regression creates problems for the validity of the model for the investigation. In particular, it affects the validity of the statistical tests of the regression coefficients by inflating their standard errors (Hair et al., 2006). Lack of multicollinearity among the independent continuous variables was supported by the obtained variance inflation factor (VIF) values that were calculated using bivariate correlations (R statistical software). The maximum bivariate correlation ($R = 0.30$, VIF = 1.1) was observed between ODR and SCC variables. All VIF were well below the cut-off value of 10 (Field, 2009).

3. Results

3.1. Descriptive data

Descriptive statistics on continuous variables showed an average of 0.82 for the mean 2008/2009/2010 *Ostertagia* ODR, 34.3 months for age to first calving, 416 days for the first inter-calving interval and 410 days for others inter-calving intervals. Mortality ratios were 5.35%, 7.79%, 2.45%, 3.86% and 2.10% for neonates calves (0 day), young calves (0–30 days), preweaned calves (31–90 days), postweaned calves (91–365 days) and adults (after 365 days), respectively (Table 2).

3.2. Bivariate analysis

Overall, parameters studied were better in herds with low average ODR (≤0.70). Thus, age to first calving was 1.5 months later in the medium ODR class and 2.5 months later in the high class relative to low class (Wilcoxon test, $p < 0.001$). Similarly, the first inter-calving interval was 8 and 10 days longer in the high class comparatively to medium and low classes (Wilcoxon test, $p < 0.001$). The same trend was found with the other inter-calving intervals but the differences were less marked (significant differences between high class and low/medium classes, Wilcoxon test, $p < 0.01$). Mortality at birth was similar in the three classes. Mortality was then higher in the medium and high classes, particularly between 91 and 365 days where 92% increase was observed in high class vs. low class (Table 3).

3.3. Multivariate analysis

3.3.1. Reproductive parameters

Ostertagia herd score was significantly and increasingly associated with a high age at first calving. Thus, adjusted odds ratio were 1.40 for the medium ODR class and 1.94 to the high ODR class relative to the reference class. In contrast, no significant association was observed between the ODR and the two inter-calving intervals studied. Others factors retained in the models and strongly associated with reproductive parameters were the proportion of Holstein cows and SCC in bulk tank milk (Table 4).

Table 1
Categorical variables offered for the multivariate logistic-regression models.

Variable	Definition	Levels
Fasciolosis	*E*/*P* values obtained in bulk-tank milk with ELISA Pourquier in November 2008/2009/2010 Interpretation: Negative ≤5; low positive >5 and ≤30; strong positive >30 (Delafosse, 2011)	Low = also *E*/*P* ≤ 5 Medium = at least once a *E*/*P* > 5 and never *E*/*P* > 30 High = at least once a *E*/*P* > 30
BVD	% inhibition values obtained in bulk-tank milk with ELISA competition LSI in November 2008/2009/2010 Interpretation: Negative <35% (0); low positive ≥35% and <60% (1+); strong positive ≥60% (2+)	Low = score in November 2008/2009/2010 = 0/0/0 or 0/1/0 or 1/0/0 or 0/0/1 Medium = 1/1/1; 1/1/0; 1/0/1; 0/1/1; 2/1/0; 0/1/2; 2/0/1; 1/0/2; 1/2/0; 0/2/1 High = 2/2/2; 2/2/1; 2/2/0; 2/0/2; 2/1/2; 0/2/2; 1/2/2
Number of dairy cows 24 months old or more	Average of numbers at 1 January 2008/2009/2010	≤50 >50 and ≤75 >75 and ≤100 >100
Proportion of dairy cows of Holstein breed	Calculated using the numbers at 1 January 2008/2009/2010	≤30% >30% and ≤60% >60% and ≤90% >90%
Proportion of calving in winter	Proportion of calving between October 1 and February 28 – period considered 2008/2009/2010	≤35% >35%
Proportion of first calving cows	Proportion of cows at first calving – period considered 2008/2009/2010	≤35% >35%
Somatic cell counts (SCC) in bulk tank milk	Average of monthly values – period considered August 2008 and June 2011	≤200,000 cells/ml >200,000 and ≤300,000 cells/ml >300,000 cells/ml

3.3.2. Mortality parameters

Ostertagia herd score was significantly and increasingly associated with mortality for 2 periods: 0–30 days and then 91–365 days (Tables 5 and 6). Thus, adjusted odds ratio of death between 0 and 30 days were 1.22 for the medium ODR class and 1.43 for the high ODR class relative to the reference class. These values were 1.46 and 1.72 for mortality between 91 and 365 days.

Others factors retained in the models and strongly associated with early mortality (≤30 days) were the number of breeding females in the herd, the proportion of calving in winter and SCC in bulk tank milk (Table 5). Others factors strongly associated with late mortality (>30 days) were the number of breeding females in the herd, fasciolosis and BVD status and SCC in bulk tank milk (Tables 5 and 6).

4. Discussion

The anthelmintic treatment history of the herds would have been interesting to include in the confounding factors because several products, such as endectocides, are also effective against external parasites that could have an impact on the parameters studied.

Similarly, a large number of other confounder factors, such as etiological agents involved in mortality of calves (*Escherichia coli*, rotavirus, coronavirus, *Cryptosporidium parvum*) or fertility (herpesvirus, etc.), should ideally have been included in our statistical analysis, however accurate data sets are difficult to collect.

Another problem is multicollinearity which occurs when predictor (independent) variables are not statistically independent and result in unstable estimates of

Table 2
Descriptive statistics of continuous variables: *Ostertagia* ODR (mean 2008/2009/2010), reproductive parameters and mortality parameters.

Variable	Nb	Average	SD	Min	Max
Ostertagia – ODR	1643	0.82	0.18	0.09	1.27
Reproductive parameters					
Age to first calving (months)	1444	34.3	3.9	24.6	58.1
First inter-calving interval (days)	1444	416	28	357	633
Others inter-calving intervals (days)	1444	410	24	352	574
Mortality parameters					
Ratio – 0 day (%)	1443	5.35	3.1	0	20.1
Ratio – 0–30 days (%)	1443	7.79	5.3	0	36.6
Ratio – 31–90 days (%)	1435	2.45	2.7	0	24.7
Ratio – 91–365 days (%)	1435	3.86	4.8	0	80.0
Ratio – >365 days (%)	1435	2.10	3.5	0	53.5

Table 3
Reproduction and mortality parameters by class of ODR in bulk milk tank.

	Ostertagia – levels ODR								
	≤0.70			>0.70–≤0.90			>0.90		
	Nb	Average	SD	Nb	Average	SD	Nb	Average	SD
Reproductive parameters									
Age to first calving (months)	334	32.9[a]	3.51	659	34.4[b]	3.82	451	35.4[c]	3.83
First inter-calving interval (days)	334	412[d]	25	659	414[e]	27	451	422[f]	30
Others inter-calving intervals (days)	334	408[g]	23	659	409[h]	24	451	413[i]	25
Mortality parameters									
Ratio – 0 day (%)	334	5.31[j]	2.7	659	5.37[k]	3.1	450	5.34[l]	3.4
Ratio – 0–30 days (%)	334	6.92[m]	4.5	659	7.74[n]	5.3	450	8.50[o]	5.8
Ratio – 31–90 days (%)	334	1.89[p]	2.1	659	2.42[q]	2.7	450	2.91[r]	3.2
Ratio – 91–365 days (%)	330	2.54[s]	2.9	659	3.84[t]	4.3	446	4.87[u]	6.2
Ratio – >365 days (%)	330	1.67[v]	2.0	659	1.75[w]	2.1	446	2.93[x]	5.4

$p \leq 0.001$ = ab, ac, bc, df, ef, mo, pr, st, su, vx, wx.
$p \leq 0.01$ = gi, hi, pq, tu.
$p \leq 0.05$ = no, qr.
$p > 0.05$ = de, gh, jk, jl, kl, mn, vw.

Table 4
Factors retained in three OLR models after examination of the statistical liaison with the outcomes of interest (reproductive parameters).

Variable	Levels	Regression coefficient	p	Adjusted OR [CI]
Model 1 (age to first calving)				
Ostertagia (ODR)	≤0.70	Ref.	–	–
	>0.70 and ≤0.90	0.34	0.02[*]	1.40 [1.05–1.88]
	>0.90	0.66	<0.0001[***]	1.94 [1.40–2.68]
Number of dairy cows 24 months old or more	≤50	Ref.	–	–
	>50 and ≤75	0.27	0.0[NS]	1.32 [0.97–1.77]
	>75 and ≤100	−0.09	0.6[NS]	0.91 [0.65–1.28]
	>100	−0.18	0.3[NS]	0.84 [0.55–1.26]
Proportion of dairy cows of Holstein breed	≤30%	Ref.	–	–
	>30% and ≤60%	−0.31	0.1[NS]	0.74 [0.50–1.08]
	>60% and ≤90%	−0.57	0.0007[***]	0.57 [0.41–0.79]
	>90%	−1.38	<0.0001[***]	0.25 [0.19–0.33]
SCC in bulk tank milk	≤200,000	Ref.	–	–
	>200,000 and ≤300,000	0.45	0.0008[***]	1.57 [1.21–2.05]
	>300,000	1.29	<0.0001[***]	3.62 [2.60–5.05]
Model 2 (first inter-calving interval)				
Proportion of dairy cows of Holstein breed	≤30%	Ref.	–	–
	>30% and ≤60%	1.04	<0.0001[***]	2.82 [1.93–4.14]
	>60% and ≤90%	1.64	<0.0001[***]	5.13 [3.68–7.16]
	>90%	1.81	<0.0001[***]	6.13 [4.62–8.12]
Proportion of first calving cows	≤35%	Ref.	–	–
	>35%	−0.17	0.1[NS]	0.84 [0.67–1.07]
SCC in bulk tank milk	≤200,000	Ref.	–	–
	>200,000 and ≤300,000	0.50	0.00031[***]	1.64 [1.26–2.15]
	>300,000	1.13	<0.0001[***]	3.09 [2.24–4.26]
Model 3 (others inter-calving intervals)				
Proportion of dairy cows of Holstein breed	≤30%	Ref.	–	–
	>30% and ≤60%	1.41	<0.0001[***]	4.10 [2.79–6.02]
	>60% and ≤90%	1.87	<0.0001[***]	6.48 [4.62–9.07]
	>90%	2.14	<0.0001[***]	8.53 [6.41–11.36]
SCC in bulk tank milk	≤200,000	Ref.	–	–
	>200,000 and ≤300,000	0.34	0.01[*]	1.41 [1.07–1.84]
	>300,000	0.61	<0.0001[***]	1.85 [1.34–2.55]

Model 1: residual deviance with 1433 degrees of freedom: 1767.5, AIC: 1789.5.
Model 2: residual deviance with 1437 degrees of freedom: 1756.5, AIC: 1770.5.
Model 3: residual deviance with 1438 degrees of freedom: 1706.6, AIC: 1718.6.
[**] $p \leq 0.01$.
[*] $p \leq 0.05$.
[***] $p \leq 0.001$.
[NS] $p > 0.05$.

Table 5
Factors retained in three OLR models after examination of the statistical liaison with the outcomes of interest (mortality parameters: 0 day, 0–30 days and 31–90 days).

Variable	Levels	Regression coefficient	p	Adjusted OR [CI]
Model 1 (mortality ratio to 0 day)				
Number of dairy cows 24 months old or more	≤50	Ref.	–	–
	>50 and ≤75	0.41	0.004^{**}	1.50 [1.14–1.99]
	>75 and ≤100	0.68	$<0.0001^{***}$	1.97 [1.43–2.71]
	>100	0.57	0.004^{**}	1.77 [1.20–2.61]
Proportion of dairy cows of Holstein breed	≤30%	Ref.	–	–
	>30% and ≤60%	0.006	0.9^{NS}	1.01 [0.70–1.45]
	>60% and ≤90%	0.61	0.0002^{***}	1.84 [1.34–2.53]
	>90%	0.32	0.01^{*}	1.38 [1.07–1.77]
Proportion of calving in winter	≤35%	Ref.	–	–
	>35%	0.38	0.0006^{***}	1.46 [1.18–1.81]
Model 2 (mortality ratio from 0 to 30 days)				
Ostertagia (ODR)	≤0.70	Ref.	–	–
	>0.70 and ≤0.90	0.20	0.1^{NS}	1.22 [0.96–1.61]
	>0.90	0.36	0.03^{*}	1.43 [1.04–1.96]
Fasciolosis (*E*/*P*)	Low	Ref.	–	–
	Medium	0.27	0.03^{*}	1.32 [1.02–1.70]
	High	−0.12	0.4^{NS}	0.89 [0.65–1.20]
Number of dairy cows 24 months old or more	≤50	Ref.	–	–
	>50 and ≤75	0.24	0.1^{NS}	1.27 [0.96–1.69]
	>75 and ≤100	0.51	0.002^{**}	1.67 [1.21–2.31]
	>100	0.86	$<0.0001^{***}$	2.36 [1.57–3.54]
Proportion of dairy cows of Holstein breed	≤30%	Ref.	–	–
	>30% and ≤60%	0.18	0.3^{NS}	1.20 [0.83–1.75]
	>60% and ≤90%	0.22	0.1^{NS}	1.24 [0.90–1.71]
	>90%	0.47	0.0004^{***}	1.60 [1.23–2.07]
Proportion of calving in winter	≤35%	Ref.	–	–
	>35%	0.29	0.009^{**}	1.34 [1.08–1.67]
SCC in bulk tank milk	≤200,000	Ref.	–	–
	>200,000 and ≤300,000	0.64	$<0.0001^{***}$	1.90 [1.47–2.45]
	>300,000	0.87	$<0.0001^{***}$	2.39 [1.74–2.28]
Model 3 (mortality ratio from 31 to 90 days)				
Fasciolosis (*E*/*P*)	Low	Ref.	–	–
	Medium	0.37	0.003^{**}	1.45 [1.13–1.85]
	High	0.37	0.01^{*}	1.45 [1.08–1.94]
Number of dairy cows 24 months old or more	≤50	Ref.	–	–
	>50 and ≤75	0.08	0.5^{NS}	1.08 [0.81 –1.45]
	>75 and ≤100	0.40	0.01^{*}	1.50 [1.08 –2.07]
	>100	0.49	0.02^{*}	1.63 [1.10 –2.41]
SCC in bulk tank milk	≤200,000	Ref.	–	–
	>200,000 and ≤300,000	0.49	0.0002^{***}	1.63 [1.26–2.11]
	>300,000	1.05	$<0.0001^{***}$	2.86 [2.12–3.88]

Model 1: residual deviance with 1435 degrees of freedom: 1938.4, AIC: 1954.4.
Model 2: residual deviance with 1429 degrees of freedom: 1886.8, AIC: 1914.8.
Model 3: residual deviance with 1435 degrees of freedom: 1900.8, AIC: 1916.8.

* $p \le 0.05$.
** $p \le 0.01$.
*** $p \le 0.001$.
^{NS} $p > 0.05$.

regression coefficients in logistic regression models (Dohoo et al., 1996). The results of the analysis showed that our data did not violate the multicollinearity assumption with all VIF values well below the cut-off value of 10.

Somatic cell counts (SCC) pose a problem of interpretation because they are used here as an indicator of poor breeding practices and are frequently retained in models. Nevertheless, parasitism can have a negative impact on immunity (Wiggin and Gibbs, 1989; Yang et al., 1993) which could favour the development of mastitis infections and increase SCC. A positive correlation was also found in other study between ODR and SCC (Sanchez et al., 2004a). The question remains whether there is a causal relationship between anti-*Ostertagia* antibody levels and SCC. Its use as a predictor probably led to the minimizing or the elimination of regression coefficients of *Ostertagia* ODR from several

Table 6
Factors retained in two OLR models after examination of the statistical liaison with the outcomes of interest (mortality parameters, 91–365 days and after 365 days).

Variable	Levels	Regression coefficient	p	Adjusted OR [CI]
Model 4 (mortality ratio from 91 to 365 days)				
Ostertagia (ODR)	$\le$0.70	Ref.	–	–
	>0.70 and $\le$0.90	0.38	0.01[*]	1.46 [1.09–1.95]
	>0.90	0.54	0.001[**]	1.72 [1.25–2.37]
BVD	Low	Ref.	–	–
	Medium	0.21	0.1[NS]	1.23 [0.94–1.61]
	High	0.62	<0.0001[***]	1.86 [1.41–2.45]
Number of dairy cows 24 months old or more	$\le$50	Ref.	–	–
	>50 and $\le$75	0.52	0.0007[***]	1.69 [1.25–2.29]
	>75 and $\le$100	0.85	<0.0001[***]	2.33 [1.66–3.28]
	>100	1.02	<0.0001[***]	2.78 [1.84–4.21]
Proportion of dairy cows of Holstein breed	$\le$30%	Ref.	–	–
	>30% and $\le$60%	−0.20	0.2[NS]	0.82 [0.56–1.19]
	>60% and $\le$90%	−0.45	0.006[**]	0.64 [0.46–0.88]
	>90%	−0.67	<0.0001[***]	0.51 [0.39–0.66]
Proportion of first calving cows	$\le$35%	Ref.	–	–
	>35%	−0.20	0.0[NS]	0.82 [0.65–1.03]
SCC in bulk tank milk	$\le$200,000	Ref.	–	–
	>200,000 and $\le$300,000	0.49	0.0003[***]	1.63 [1.25–2.13]
	>300,000	1.08	<0.0001[***]	2.94 [2.13–4.07]
Model 5 (Mortality after 365 days)				
BVD	Low	Ref.	–	–
	Medium	0.19	0.1[NS]	1.21 [0.93–1.56]
	High	0.35	0.009[**]	1.42 [1.09–1.85]
Number of dairy cows 24 months old or more	$\le$50	Ref.	–	–
	>50 and $\le$75	0.18	0.2[NS]	1.20 [0.90–1.59]
	>75 and $\le$100	0.34	0.04[*]	1.40 [1.02–1.93]
	>100	0.56	0.009[**]	1.74 [1.18–2.58]
Proportion of first calving cows	$\le$35%	Ref.	–	–
	>35%	−0.18	0.1[NS]	0.83 [0.69–1.04]
SCC in bulk tank milk	$\le$200,000	Ref.	–	–
	>200,000 and $\le$300,000	0.53	<0.0001[***]	1.69 [1.32–2.18]
	>300,000	0.97	<0.0001[***]	2.63 [1.95–3.54]

Model 4: residual deviance with 1421 degrees of freedom: 1807.7, AIC: 1835.7.
Model 5: residual deviance with 1426 degrees of freedom: 1920.6, AIC: 1938.6.

[*] $p \le 0.05$.

[**] $p \le 0.01$.

[***] $p \le 0.001$.

[NS] $p > 0.05$.

models (first inter-calving interval, mortality 31–90 days and >365 days).

Multivariate analysis was conducted with logistic regression. This approach has the advantage of evaluating the significance of association between each hypothesized factor and the likelihood of infection while simultaneously controlling for the confounding effect of other significant factors. Moreover, logistic regression does not need many of the restrictive assumptions that we had with linear regression (Hosmer and Lemeshow, 1989). Variable selection methods attempt to balance the goodness-of-fit of a model with considerations of parsimony. However this is done, in theory one could look at all possible logistic regression models to find the "best" one, but this becomes computationally prohibitive when p is large. For this reason, it is more typical to use a stepwise procedure, where candidate models are based on adding or removing a term from the current "best" model. A flaw in this approach is that it does not directly address the crucial balance of goodness-of-fit vs. parsimony. Akaike (1974) proposed a measure (AIC) based on information considerations that explicitly quantifies this balance (Sakamoto et al., 1986). Model selection using AIC does better, more often resulting in improved performance relative to using the full logistic regression model, particularly for smaller samples sizes (Perlich et al., 2003). Stepwise methods are used to find a model to fit data in situations where causality is not of interest. Forward selection is more likely than backward elimination to exclude predictors involved in suppressor effects. As such, a forward method runs a higher risk of making error (Field et al., 2012).

In our study, a relationship was found between the *Ostertagia* ODR herd score and age at the first calving suggesting that higher parasite infection levels in the herd had an adverse effect on reproductive performance. The negative impact of GI parasitism in body weight has been demonstrated in many studies and the effect of anthelmintic treatment on growth has been studied in dairy breeds (Shaw et al., 1998; Hawkins, 1993). It was observed that dewormed cows and heifers grazed 50–90 min longer per day than untreated controls (Forbes et al., 2004). The reduced appetite in the controls may be a result of the increased gastrin levels associated with the increased abomasal pH, which is in turn a result of damage to the parietal cells. Other reported effects of GI parasitism are alterations in the GI-tract motility, in GI secretions and in digestion and absorption (Fox, 1997). Ploeger et al. (1996) observed that the infection-induced differences in weight gain during the first grazing season appeared to be permanent, at least up to the end of the second grazing season. Moreover, first-lactation yield was positively correlated with body weight at calving. This suggests that nematode infections occurring in the first 2 years of life negatively influence milk production by reducing weight gains. In our study, the strong parasite challenge in the herds with high *Ostertagia* level must increase the time required to reach breeding weight in replacement heifers. These results suggested that *Ostertagia* ODR of adult cows could be used to formulate appropriate preventive measures in young stock, for example regular treatments on farms with high ODR levels and exposure of cows to calf-contaminated pasture.

No significant relationship was observed here between inter-calving intervals and *Ostertagia* status with multivariate approach although the results show a significant effect with bivariate analysis, particularly on the first inter-calving interval. In contrast, some studies showed a reduction of the calving-to-conception interval was observed in anthelmintic treated cattle vs. placebo. Thus, in a Canadian study (549 cows in 20 herds) eprinomectin treated cows were significant lower calving-to-conception interval (117 days vs. 126 days)(Sanchez et al., 2002b). In the same study, ODR in milk was measured (109 cows) and the probability of conception was significantly lower for high-ODR cows compared to low-ODR cows. In a study using ivermectin in Australia, it was found that cows treated during the dry period had a 4.8 days shorter calving-to-conception interval than the untreated controls (430 cows in 5 herds) (Walsh et al., 1995). The same observation was made in France at the herd level in a study on 940 farms with an increased calving-to-conception interval of 1.5 days when the *Ostertagia* ODR measured on bulk tank milk increased from 0.482 to 0.720 (Guiot et al., 2007). In a study in Argentina all cows from a grass based dairy farm were followed monthly for egg parasite output in faeces and periodical bleedings for hormone determination were performed. Only a marginal effect on partum to first service interval were encountered but endocrine studies revealed a decrease in serum growth hormone (GH), type I insulin-like growth factor (IGF-I) and prolactin during lactation in cows with positive EPG in the first postpartum sample with respect to null EPG cows at that time (Perri et al., 2011). In contrast, a study in Canada (2381 cows in 35 herds) could not find a beneficial effect of eprinomectin treatment at calving on reproduction but the herds had a limited outdoor exposure (Sithole et al., 2006).

The relationship between GI nematodes infections in cow and calf mortality was only explored in studies of anthelmintic-treated beef cows, compared with untreated controls, with reductions in the treated groups (Hawkins, 1993; Gross et al., 1999). No work has been published on the effect of *Ostertagia* status measured in bulk milk tank on mortality parameters, especially during the first year of life. In our study, a relationship was observed with multivariate approach in two classes: first 0–30 days and then 90–365 days. The mortality in young calves could be explained by the poor quality of colostrum when dams were subjected to high parasite challenge. It seems that *O. ostertagi* can modulate the cattle's immune response, reducing the ability of the animal to respond to heterologous antigens (Wiggin and Gibbs, 1989). In addition, a study suggests a non-specific suppression of cellular immunity in *O. ostertagi* infected calves that were inoculated with *Brucella abortus* and IBR vaccines (Yang et al., 1993). However, a study of 103 cows in Belgium, Werbrouck et al. (2010) found no relationship between *O. ostertagi* ODR level in milk and concentration of immunoglobulin G in colostrum.

The impact of *Ostertagia* on post-weaning mortality (90–365 days) could be explained by the great sensitivity of first-season grazing cattle of GI nematodes (Shaw et al., 1998). Moreover, this excess mortality could also be a late consequence of poor passive transfer of immunity (colostrum) after birth. The results showed a significant effect of ODR for 31–90 days and >365 days mortalities only with bivariate analysis.

In conclusion, our results confirm the interest of *Ostertagia* specific antibody level in bulk tank milk at the end of the grazing season as indicator for production losses in adult dairy cows and also in heifers. This inexpensive tool could be advantageously used to inform farmers and veterinarians about exposure level to GI nematodes. Thereby, *Ostertagia* ODR could be incorporated in the decision-making process for adjusting appropriate control measures likely to reduce the mortality and to increase the fertility on farms with high ODR.

References

Akaike, H., 1974. A new look at statistical model identification. In: IEEE Transactions on Automatic Control, vol. AU-19, pp. 716–722.

Berghen, P., Hilderson, H., Vercruysse, J., Dorny, P., 1993. Evaluation of pepsinogen, gastrin and antibody response in diagnosing ostertagiasis. Vet. Parasitol. 46, 175–195.

Butler, J.E., 1986. Biochemistry and biology of ruminant immunoglobulins. Prog. Vet. Microbiol. Immun. 2, 1–53.

Caffin, J.P., Poutrel, B., Rainard, P., 1983. Physiological and pathological factors influencing bovine immunoglobulin G1 concentration in milk. J. Dairy Sci. 66, 2161–2166.

Charlier, J., Duchateau, L., Vangroenweghe, F., Claerebout, E., Burvenich, C., Vercruysse, J., 2006. The effect of an experimentally induced acute mastitis on the test results of an *Ostertagia ostertagi* milk ELISA. Vet. Parasitol. 136, 161–165.

Charlier, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2009. Gastrointestinal nematode infections in adult dairy cattle: impact on production, diagnosis and control. Vet. Parasitol. 164, 70–79.

Delafosse, A., 2011. Routine diagnosis of bovine fasciolosis in cattle. Point Vet. 316, 52–56.

Dohoo, I.R., Ducrot, C., Fourichon, C., Donald, A., Hurnik, D., 1996. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29, 221–239.

Field, A., 2009. Discovering Statistics Using SPSS. Sage, London.

Field, A., Miles, J., Field, Z., 2012. Discovering Statistics Using R. Sage, London.

Forbes, A.B., Huckle, C.A., Gibb, M.J., 2004. Impact of eprinomectin on grazing behaviour and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. Vet. Parasitol. 125, 353–364.

Fox, M.T., 1997. Pathophysiology in infection with gastrointestinal nematodes in domestic ruminants: recent developments. Vet. Parasitol. 72, 285–297.

Gross, S.J., Ryan, W.G., Ploeger, H.W., 1999. Anthelmintic treatment of dairy cows and its effect on milk production. Vet. Rec. 144, 581–587.

Guiot, A.L., Charlier, J., Pravieux, J.J., Courtay, B., Vercruysse, J., 2007. Relation entre la mesure d'anticorps anti-Ostertagia sur lait de mélange et les paramètres de production laitière en France. Bull. G.T.V. 38, 75–79.

Hair, J.F., Anderson, R., Tatham, R.L., Black, W.C., 2006. Multivariate Data Analysis. Prentice Hall, Upper Saddle River, NJ.

Hawkins, J.A., 1993. Economic-benefits of parasite control in cattle. Vet. Parasitol. 46, 159–173.

Hosmer, D.W., Lemeshow, S.W., 1989. Applied Logistic Regression. Wiley, New York.

Kenyon, F., Jackson, F., 2011. Targeted flock/herd and individual ruminant treatment approaches. Vet. Parasitol. 186, 10–17.

Perlich, C., Provost, F., Simonoff, J.S., 2003. Tree induction vs. logistic regression: a learning-curve analysis. J. Mach. Learn. Res. 4, 211–255.

Perri, A.F., Mejía, M.E., Licoff, N., Lazaro, L., Miglierina, M., Ornstein, A., Becu-Villalobos, D., Lacau-Mengido, I.M., 2011. Gastrointestinal parasites presence during the peripartum decreases total milk production in grazing dairy Holstein cows. Vet. Parasitol. 178, 311–318.

Ploeger, H.W., Schoenmaker, G.J., Kloosterman, A., Borgsteede, F.H.M., 1989. Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematode infection. Vet. Parasitol. 34, 239–253.

Ploeger, H.W., Kloosterman, A., Bargeman, G., von Wijckhuise, L., van den Brink, R., 1990. Milk yield increase after anthelmintic treatment of dairy cattle related to some parameters estimating worm infections. Vet. Parasitol. 35, 103–106.

Ploeger, H.W., Kloosterman, A., Rietveld, F.W., Hilderson, H., Berghen, P., Pieke, E.J., 1996. Production of dairy replacement stock in relation to level of exposure to gastrointestinal nematode infection in the first grazing season: second-year calves and heifers. Vet. Parasitol. 65, 99–115.

Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike Information Criterion Statistics. D. Reidel Publishing Company, Dordrecht, Tokyo, Japan.

Sanchez, J., Dohoo, I., 2002a. A bulk tank milk survey of *Ostertagia ostertagi* antibodies in dairy herds in Prince Edward Island and their relationship with herd management factors and milk yield. Can. Vet. J. 43, 454–459.

Sanchez, J., Nødtvedt, A., Dohoo, I., DesCoteaux, L., 2002b. The effect of eprinomectin treatment at calving on reproduction parameters in adult dairy cows in Canada. Prev. Vet. Med. 56, 165–177.

Sanchez, J., Markham, F., Dohoo, I., Sheppard, J., Keefe, G., Leslie, K., 2004a. Milk antibodies against *Ostertagia ostertagi*: relationships with milk IgG and production parameters in lactating dairy cattle. Vet. Parasitol. 120, 319–330.

Sanchez, J., Dohoo, I., Carrier, J., DesCôteaux, L., 2004b. A metaanalysis of the milk-production response after anthelmintic treatment in naturally infected adult dairy cows. Prev. Vet. Med. 63, 237–256.

Shaw, D.J., Vercruysse, J., Claerebout, E., Dorny, P., 1998. Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. Vet. Parasitol. 75, 115–131.

Sithole, F., Dohoo, I., Leslie, K., DesCoteaux, L., Godden, S., Campbell, J., Keefe, G., Sanchez, J., 2006. Effect of eprinomectin pour-on treatment around calving on reproduction parameters in adult dairy cows with limited outdoor exposure. Prev. Vet. Med. 75, 267–279.

Venables, W.N., Ripley, B.D., 1999. Modern Applied Statistics with S-Plus, 3rd ed. Springer-Verlag, New York.

Vercruysse, J., Claerebout, E., 2001. Treatment vs. non-treatment of helminth infections in cattle: defining the threshold. Vet. Parasitol. 98, 195–214.

Walsh, T.A., Younis, P.J., Morton, J.M., 1995. The effect of ivermectin treatment of late pregnant dairy cows in south-west Victoria on subsequent milk production and reproductive performance. Aus. Vet. J. 72, 201–207.

Werbrouck, B., Van Aert Ugen, M., Charlier, J., 2010. Colostrum quality in Belgian beef cattle and its association with helminth infection. Vlaams Diergen.skund. Tijds. 79, 199–206.

Wiggin, C.J., Gibbs, H.C., 1989. Studies of the immunomodulatory effects of low-level infection with *Ostertagia ostertagi* in calves. Am. J. Vet. Res. 50, 1764–1770.

Yang, C., Gibbs, H.C., Xiao, L., 1993. Immunological changes in *Ostertagia ostertagi* infected calves treated strategically with an anthelmintic. Am. J. Vet. Res. 54, 1074–1083.