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Change in milk production after treatment against gastrointestinal nematodes according to grazing history, parasitological and production-based indicators in adult dairy cows

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ABSTRACT

To investigate future tools for targeted selective treatment against gastrointestinal nematodes (GIN) in adult dairy cows, we evaluated herd and individual cow factors associated with the post-treatment milk production (MP) response over time. A field trial involving 20 pasturing dairy herds in Western France was conducted in autumn 2010 and autumn 2011. In each herd, lactating cows were randomly allocated to a treatment group (fenbendazole) (623 cows), or a control group (631 cows). Daily cow MP was recorded from 2 weeks before until 10 to 14 weeks after treatment. Individual serum anti-*Ostertagia* antibody levels (expressed as ODR), pepsinogen levels, faecal egg count (FEC), and bulk tank milk ODR were measured at the time of treatment. Moreover, in each herd, information regarding heifers' grazing and treatment history was collected to assess the Time of Effective Contact (TEC, expressed in months) with GIN infective larvae before the first calving. TEC was expected to reflect the development of immunity against GIN, and TEC = 8 months was a cautious threshold over which the resistance to re-infection was expected to be established. Daily MP averaged by week was analyzed using linear mixed models with three nested random effects (cow within herd and herd within year). The overall treatment effect was significant but slight (maximum = +0.85 kg/d on week 6 after treatment), and the evolution of treated cows' MP differed significantly according to several factors. At the herd level, cows from low-TEC herds responded better than cows from high-TEC (≥8 months) herds; cows from herds in which the percentage of positive FEC was >22.6% (median value) responded better than those from herds where it was lower. At the individual cow level, primiparous cows, cows with days in milk (DIM) < or = 100 at the time of treatment, and cows with low individual ODR (< or = 0.38) responded better than multiparous cows, cows with DIM > 100, and cows with higher ODR, respectively.

These results highlight the variability of the treatment response, suggesting that whole herd anthelmintic treatment are not always appropriate, and propose promising key criteria for targeted selective treatment for GIN in dairy cows. Particularly, the TEC is an original criterion which lends support for a simultaneous on-farm qualitative analysis of grazing management factors.

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1. Introduction

The prevalence of gastrointestinal nematodes (GIN) infection can be high in pasturing adult dairy cows. In abattoir surveys carried out in Belgium, in The Netherlands and in France, worms were found in 91%, 96% and 84% of the abomasa examined, respectively, *Ostertagia ostertagi* being the most frequently recovered species (Agneessens et al., 2000; Borgsteede et al., 2000; Chartier et al., 2013). Although this infection is considered to be subclinical in most adult cattle, it can induce a decrease in milk production (MP) and could be responsible for chronic and insidious economic losses in adult dairy cows (Gross et al., 1999; Sanchez et al., 2004a; Charlier et al., 2009). Whole-herd anthelminthic treatments have often been proposed as control measures, due to their relatively low cost, ease of use and lack of effective alternative options. However, this blanket application of chemical treatments shows serious potential drawbacks. (i) It could exercise a heavy selection pressure leading to possible emergence and diffusion of anthelmintic resistance, particularly when persistent activity pour-on products are used (Sutherland and Leathwick, 2011; Demeler et al., 2009). (ii) It can leave unwanted residues harmful for the environment (Lumaret et al., 2012). (iii) It can also negatively impact the image of vets and farmers, especially with the increasing societal demand for circumspect use of drugs. A reduction in the use of anthelmintics is therefore needed.

The distribution of parasites in adult dairy cows is overdispersed: a majority of cows has a low parasitic burden due to their resistant status to new infection, whereas some cows have a parasitic burden supposed to be high enough to negatively impact MP (Agneessens et al., 2000; Borgsteede et al., 2000). Thus, at the individual cow level, we can hypothesize that the impairment of MP is variable. Moreover, at herd level, a between-herd variability of this negative impact on MP has been also reported (O'Farrell et al., 1986; Ploeger et al., 1989, 1990; Mason et al., 2012). Consequently, we need indicators to discriminate herds and cows within herds that would benefit from a targeted selective treatment.

Several studies have focused on the relationships between parasitological indicators and MP response to anthelmintic treatment. The value of the anti *O. ostertagi* antibody level (in serum or milk) to predict MP response has been widely examined, with inconclusive results. At the individual cow level, several studies have suggested that a beneficial treatment response can be expected for cows with high milk *O. ostertagi* antibody levels (Sanchez et al., 2002, 2005; Vanderstichel et al., 2013). However, Charlier et al. (2010) highlighted that the value of this parasitological indicator remains equivocal to predict individual MP response. At the herd level, the mean herd serum *O. ostertagi* antibody titre and the bulk tank milk (BTM) *O. ostertagi* antibody level were found to be potentially good predictors of the MP response (Ploeger et al., 1989; Kloosterman et al., 1996; Sithole et al., 2005; Charlier et al., 2007); but these results, depending on studies, were either not confirmed (Ploeger et al., 1990), or lacked statistical significance (Kloosterman et al., 1996; Sithole et al., 2005), or were not fully consistent (Charlier et al., 2007). The values of faecal egg counts (Michel et al., 1982; O'Farrell et al., 1986; Sithole et al., 2005) and serum pepsinogen concentrations (O'Farrell et al., 1986; Ploeger et al., 1989, 1990) were only investigated at the herd level and were not related to the treatment response. Among herd level indicators, the duration of contact with GIN larvae before the first calving (grazing history), reflecting at least in part the resistance to reinfection (Vercruysse and Claerebout, 1997), has never been studied regarding its relation with treatment response. However, we can assume that it could contribute to explain the variability of the effect of anthelmintic treatment on MP.

Production-based indicators have also been investigated for their impact on MP response to anthelminthic treatment. Parity was reported to be related to the treatment response by Charlier et al. (2010) and McPherson et al. (2001), with a better response for multiparous cows, whereas in other studies parity did not influence the MP response (Mason et al., 2012; Ploeger et al., 1990; Michel et al., 1982; O'Farrell et al., 1986). Similarly, production level was found to be positively and significantly linked to the treatment response in one study (Ploeger et al., 1989), but did not interact with treatment in other studies (Ploeger et al., 1990; Mason et al., 2012). Finally, it was suggested that a positive MP response only occurred when the treatment was performed in the first half of lactation (Charlier et al., 2010), or that cattle responded maximally to treatment during mid lactation (Mason et al., 2012).

When studying relationships between indicators and MP response to anthelmintic treatment, different approaches have been used (Ploeger et al., 1989, 1990; Charlier et al., 2007; Vanderstichel et al., 2013). However, there is no study in which grazing history, individual cow production-based indicators as well as individual and herd-level parasitological indicators have been examined all together in the same sample.

The objectives of this study were, in adult pasturing dairy cows, (1) to assess the effect of an anthelmintic treatment on MP over time, (2) to identify factors associated with the treatment response at both the herd and individual cow level, by investigating, on the same sample, the relationships between treatment response and grazing history, production-based as well as parasitological indicators. The factors identified could then be candidate indicators for targeted selective treatment against GIN.

2. Materials and methods

2.1. Farms and animals

The study sample was a convenience sample, constructed thanks to a network of contacts between the Nantes-Atlantic College of Veterinary Medicine and Food Sciences and Engineering (Oniris), veterinarians and farmer organizations. The major herd recruitment criteria were the breed (Holstein), an access to pasture during a large grazing season (at least 4 months on pasture with a use of grass in the diet), the absence of anthelmintic treatment on adult dairy cows, and a daily recording of milk production (automatic milking system, or milking parlor with milk meters). In each farm, the majority of the lactating cows were included in the study provided that they were planned to be milked for at least 4 weeks after treatment and in apparent good health at the time of treatment.

Ten dairy herds in the North-West of France were visited from November 2010 until January 2011. The following autumn, from October 2011 until December 2011, 5 of these herds plus 10 new herds were visited. This field trial was thus conducted twice, including 25 visits in 20 different herds in total.

The visits took place during the housing period. When limited access to pasture was still possible, we ensured that grass represented less than 5% of cows total dry matter intake.

2.2. Anthelmintic treatment

In each herd, lactating cows were randomly allocated to a treatment group and a control group. In order to have comparable groups for milk production, cows were previously matched on three criteria: they were stratified first by parity (first, second, third and more), then by days in milk (DIM) classes (less than 35 DIM, 35 to 100 DIM, 100 to 200 DIM, and more than 200 DIM), and then ranked and paired by ascending expected production level (last test-day milk yield). Then, cows of each pair were assigned to either the treatment group or the control group using a random number table.

Fenbendazole (Panacur® 10%) was chosen as the anthelmintic treatment because of its narrow spectrum against nematodes, its oral administration and zero-withdrawal time for milk in France. In each herd, cows belonging to the treatment group were all treated on the day of visit with a single dose: 60 mL, which is the dose for 800 kg body weight (7.5 mg/kg). The treatment was applied by one of the study operators or by the farmer. This administration was always under the control of another operator: if the dose was not well swallowed, the operator required that a new half-dose or a full dose be administered.

2.3. Samples and laboratory analysis

On the day of treatment, individual blood and faecal samples were taken once from all treated cows and control cows, and a BTM sample was collected. Samples were kept on ice during transport. Then, blood samples and milk samples were centrifuged, fat was skimmed off for milk samples, and milk and sera were frozen and stored at −20 °C until analysis.

For ELISA testing, milk samples were tested undiluted whereas sera were diluted at 1/160 (Charlier, personal communication). Individual anti-*Ostertagia* serum antibody levels, and bulk tank milk anti-*Ostertagia* antibody levels were determined, following the kit procedure, with an ELISA technique using the commercially available SVANOVIR® *O. ostertagi*-Ab ELISA kit (Svanova Biotech, Uppsala, Sweden), which is based on a crude adult worm capture antigen. Results were expressed as an optical density ratio (ODR) using the following formula: $ODR = (OD_{test\ sample} - OD_{negative\ control})/OD_{positive\ control} - OD_{negative\ control})$, where OD is the optical density.

Individual serum pepsinogen levels were determined following the simplified method described by Kerboeuf et al. (2002). Results were expressed in milli-Units of tyrosine (mUTyr).

Individual faecal egg counts (FEC) per 5 g of feces were determined using the modified Wisconsin Sugar Centrifugal flotation method (Bliss and Kvasnicka, 1997).

2.4. Indicators characterizing cows and herds

Each cow was characterized by 3 production-based indicators: parity, DIM at the time of treatment (DIMt), and production level. To assess the latter, for each multiparous cow included in this study, all test-day milk yields of the previous lactation collected as part of the milk-recording scheme were obtained, and the 305-day milk production was estimated by the Test Interval method (TIM) (ICAR, 2012) and adjusted for parity. For primiparous cows, the production level was estimated by the maximal test-day milk yield over the first 80 days of the current lactation.

Each cow was also characterized by three parasitological indicators: FEC, serum *O. ostertagi* ODR, and serum pepsinogen level.

Each herd was characterized by two parasitological indicators: *O. ostertagi* BTM ODR and percentage of positive FEC. Each herd was also characterized by the duration of contact with GIN infective larvae before the first calving. This last indicator was assessed by collecting, in each herd, information regarding heifers' grazing management with a standardized questionnaire including: the number of grazing seasons before the first calving (1 or 2), dates of turn out and dates of housing (duration of grazing seasons), dates and type of anthelmintic treatment(s) (persistent or not), duration of drought periods, duration of high supplementation period (herbage too scarce to cover entirely heifers' nutritional requirements). The Time of Effective Contact (TEC, expressed in months) with GIN larvae before the first calving was then calculated, for each herd, as follows: TEC = duration of grazing season – [duration of persistency of anthelmintic treatments + duration of drought and high supplementation periods]. When heifers grazed two seasons before the first calving, one TEC was calculated for each grazing season and both were added to give the final value. As heifers were born all over the year, date of turn-out was not necessarily the same for all heifers within a herd. As a result the duration of the first grazing season could be variable within a herd. The duration of the second grazing, before entering the adult herd, could also vary from one heifer to another according to its date of first calving. Therefore, TEC at the first calving could be variable within a herd. Several TECs were calculated in each herd according to different scenarios related to the pattern of dates of birth and age at calving. In each herd a minimal TEC (TEC_{min}) and a maximal TEC (TEC_{max}) were drawn from these different scenarios.

2.5. Daily milk production data

Daily individual cow milk production data were recorded from 14 days before treatment until 60 days after treatment in 2010, and until 100 days after treatment in 2011. This daily milk production was highly variable in herds using an automatic milking system, due to an irregular frequentation of the automatic milking system. Therefore daily milk productions were averaged by week. In order to have only one reference point before treatment, we calculated the average daily MP over the period of 14 days before treatment.

2.6. Statistical analysis

2.6.1. Coding and classification of variables (Table 1)

Raw data were entered into an Access database (Microsoft Corp., Redmond, WA). They were then transferred into SAS 9.2 (SAS Institute Inc., Cary, NC) to build the variables of interest and carry out statistical analyses.

The daily MPs averaged by week were considered as repeated measures through time (week after week). The treatment was coded differently depending on the week, and on whether the cow was treated or untreated, thanks to the creation of a categorical variable "week-trt" divided into 17 categories: "week-trt" took the values −1 (before treatment period), and 0, 1, 2, ..., 14 (after treatment period, week 0 being the week of treatment) for a treated cow, and only took the value 99 whatever the week for an untreated cow.

The 3 production-based indicators were categorized as follows: parity in 3 classes (1, 2, 3 or greater), DIM at the time of treatment (DIMt) in 3 classes (DIMt ≤ 100, 100 < DIMt ≤ 200, DIMt > 200 days), and production level in 3 classes (low, moderate and high) according to the terciles of each within-herd distribution of the previous 305-days milk production for multiparous cows, and of maximal test-day milk yield over the first 80 days of the current lactation for primiparous cows. Thus, each cow was considered as a high, moderate or low producing animal in its herd.

The 3 individual parasitological indicators were categorized as follows: FEC in 2 classes (positive FEC and negative FEC, i.e. < 0.2 epg), serum *O. ostertagi* ODR in 3 classes according to the terciles of the between-herd distribution (ODR ≤ 0.38, 0.38 < ODR ≤ 0.62, ODR > 0.62), and serum pepsinogen level (pep) in 3 classes according to the terciles of the between-herd distribution (pep ≤ 952 mUTyr, 952 mUTyr <pep ≤ 1402 mUTyr, pep > 1402 mUTyr).

The 3 herd-level parasitological indicators were categorized as follows: the *O. ostertagi* BTM ODR and the percentage of positive FEC were both classified in 2 classes according to the medians of the distributions: BTM ODR < 0.74, or ≥0.74, and % positive FEC ≤ 22.6%, or > 22.6%, respectively. Two classes were defined for the TEC with GIN larvae at the first calving: high-TEC herds when $TEC_{min} \geq 8$ months, and low-TEC herds otherwise. $TEC_{min} \geq 8$ months was a cautious threshold over which the resistance to re-infection was expected to be established.

For each indicator, the proportion of treated cows and control cows in each category was compared using Chi-square tests (level of significance set at $p \leq 0.05$).

2.6.2. Assessment of the overall treatment effect over time

In a first step, the overall effect of treatment on daily MP averaged by week over time was studied using a first linear mixed model (model 0), with three nested random effects (cow within herd and herd within year). Daily MP averaged by week expressed in kg per day was the outcome variable. It was normally distributed (14,023 observations, mean = 29.53 kg, sd = 8.87 kg). This model 0 included the following independent variables: week-trt (variable of interest, reference = 99), parity (1, 2, 3 or greater), days in milk (DIM) (expressed as week in milk: 1 to 52, and 52 or greater), production level (low, moderate and high), month of milk production (October to March), and a two way interaction between parity and DIM.

This model 0 was of the following form:

$$(\text{Daily MP averaged by week})_{wijk} = \mu + \Sigma\beta_{wijk}X_{wijk} + \Sigma\beta_{ijk}X_{ijk} + \upsilon_k + \nu_{jk} + \omega_{ijk} + \varepsilon_{wijk}$$

with

$$\upsilon_k \sim N\left(0, \sigma^2_{\upsilon}\right), \quad \nu_{jk} \sim N\left(0, \sigma^2_{\nu}\right), \quad \omega_{ijk} \sim N\left(0, \sigma^2_{\omega}\right)$$

$$\varepsilon_{wijk} = \left[\varepsilon_{1ijk}, \varepsilon_{2ijk}, \ldots, \varepsilon_{14ijk}\right] \sim N\left(0, \sigma^2_{\varepsilon}\right)$$

where $(\text{Daily MP averaged by week})_{wijk}$ = MP for cow i in farm j, on year k, on week w, μ = average MP after adjusting for covariates, β_{wijk} = coefficients for X_{wijk}, X_{wijk} = variables varying between daily MP averaged by week (DIM, week-trt, month of milk production, and DIM × parity), β_{ijk} = coefficients for X_{ijk}, X_{ijk} = variables varying between cows (parity, production level), υ_k = year random effect, ν_{jk} = farm random effect nested into year, ω_{ijk} = cow random effect nested into herd, ε_{wijk} = residual at week w. The random effects υ_k, ν_{jk}, ω_{ijk} and the residual ε_{wijk} were assumed to be normally distributed with mean 0 and variance $\sigma^2{}_{\upsilon}$, $\sigma^2{}_{\nu}$, $\sigma^2{}_{\omega}$ and $\sigma^2{}_{\varepsilon}$, respectively.

Residuals and predicted values were plotted to evaluate their heteroscedasticity and their normality.

2.6.3. Individual or herd-level indicators and milk production response after anthelminthic treatment

In a second step, the relationships between the treatment response and the nine categorical indicators characterizing the herds and the cows were evaluated. Following the stepwise approach described just below, different multilevel linear mixed models were constructed, including the same independent variables as the model 0, and in addition all the indicators of interest in interaction with week-trt.

Firstly, we assessed the variability of the treatment response according to easy-to-use herd or individual-level indicators: operational indicators that are either directly accessible to the farmer (parity, DIMt, production level), or with low additional cost for sample and laboratory analysis (*O. ostertagi* BTM ODR and TEC with GIN larvae), or related to the possibility of sampling a part of the herd (percentage of positive FEC). These easy-to-use individual and herd-level indicators were put all together in a same model in interaction with week-trt, after examination of all biologically plausible two-by-two associations (Chi-square tests, level of significance set at $p \leq 0.05$).

Second, we aimed to assess the additive value of the three individual parasitological indicators which are used

Table 1
Description of grazing management practices of the adult cows in the 20 herds included in the field trial.

	Minimum	Q1	Median	Mean (*std*)	Q3	Maximum
Months on pasture	2.2	7.3	8.2	7.8 (*1.4*)	8.6	10
Average number of lactating cows per herd	37	47	55	57 (*17*)	67	120
Number of grazing plots per herd	1	3	5	5.5 (*3*)	7	14
Size of grazing plots (ha)	0.4	1	1.5	2.4 (*1.95*)	3	10
Stocking rate per plots (number of cows/ha) *(number of grazing days at this stocking rate)*	7.2 *(10 grazing days)*	22.5 *(5 grazing days)*	31.3 *(3 grazing days)*	36 (*20.5*) *(4.7 grazing days)*	50 *(1 grazing day)*	100 *(0.5 grazing day)*
% grass in the diet[a]	10%	50%	55%	56% (*18.6%*)	70%	80%
Hours per day on pasture[a,b]	3	9	17	15.8 (*8.2*)	24	24

[a] Two data not available.
[b] In herds where the barn is always open (access to the automatic milking system, or access to feed in the trough), cows freely move from the barn to the pasture. The information given here is thus the duration of free access to pasture per day.

for the diagnosis of GIN infections in ruminants (FEC, serum pepsinogen level and serum *O. ostertagi* ODR). We could suspect here potential associations between (i) individual parasitological indicators and herd level parasitological indicators, (ii) individual parasitological indicators and individual production-based indicators, (iii) individual parasitological indicators (one related to another). Thus, two-by-two associations were examined using Chi-square tests or mean comparison tests (level of significance set at $p \leq 0.05$). According to the significant associations found, as many models as necessary were constructed.

The fixed effect structure of each model used was determined by backward elimination procedure, starting with interaction terms.

For each model constructed, residuals and predicted values were plotted to evaluate their heteroscedasticity and their normality.

2.6.4. *Calculation on the estimates for the assessment of the milk production gain*

After this two-step modeling approach, we calculated the treated cows' MP gain. The statistical models estimated the mean weekly MP of treated and control cows, for the whole population (model 0), and within each category of the investigated indicators (models with two way interactions terms between week-trt and indicators). Each week, the treated cows' MP gain resulted from the following calculation: if D_{-1} is the difference of the estimated daily MP averaged by week between future treated cows and control cows before treatment in week$_{-1}$, and D_i is this difference after treatment in week$_i$, then G_i is the estimated treated cows' MP gain in week$_i$ with $G_i = D_i - D_{-1}$. Thus, the initial difference of MP between treated cows and control cows is taken into account each week to calculate the gain of MP (G_i). Indeed, despite the randomization of treatment, the average MP of the treated and control groups before treatment could be different. Consequently, to assess thoroughly the treated cows' MP gain, we had to take into account this initial gap between treated cows and control cows.

The average treated cows' MP gain for the whole period of follow up was the arithmetic mean of the G_is (calculated only for the overall treatment effect).

To test each week if G_is were significantly different from zero, we used student tests with adjusted *p*-values for multiple testing. Similarly, to investigate, each week, the statistical significance of the differences between G_is in each category of indicators put in interaction with week-trt, we used contrasts with adjusted *p*-values for multiple testing.

3. Results

3.1. *Description of the study sample*

1254 cows from 20 different herds were initially included in the study (511 cows in 2010 and 743 cows in 2011). There were 26 to 109 cows included per herd (average: 50 cows). Compared to the average number of lactating cows in each herd, the percentage of cows included in the study ranged from 63% to 98%. Fifteen herds were equipped with an automatic milking system, 5 with a milking parlor with milk meters. Nineteen herds had an access to pasture lasting from 6.5 months until 10 months. Only one herd had a shorter access to pasture of 2.2 months. Among the 9 herds with the largest % of grass in the diet in spring (≥60%), 7 herds were equipped with an automatic milking system. General information regarding grazing system of dairy cows of our sample is provided in Table 1. Moreover, details related to heifers' grazing practices are provided in Table 2. Anthelmintic treatments of first season grazing calves were applied as follows: in 10 herds, they were treated at housing, in 7 herds they were treated in spring or summer with an endectocide or a benzimidazole, in 2 herds they were not treated, and in 1 herd heifers did not graze before the first calving (and thus were not treated). Second season grazing heifers were treated at housing in 4 herds, in spring or in summer with an endectocide or a benzimidazole in 6 herds, or not treated in 10 herds.

623 cows were treated while 631 cows remained untreated. The time between housing and anthelmintic treatment was on average 17 days.

In two herds, a few cows could sometimes be treated by the farmer. Then, these cows could be included only if after the "farmer treatment" they had been exposed to

Table 2
Description of grazing management practices of the heifers in the 20 herds included in the field trial.

	Minimum	Q1	Median	Mean (*std*)	Q3	Maximum
Duration of the FGS[a] (months)	0	4.5	6	5.6 (*2.4*)	7.5	8.5
Duration of drought and high supplementation periods during the FGS (months)	0	0.75	2	1.9 (*1.4*)	2.75	5.5
Duration of the SGS[b] (months)	0	2	6.5	5.2 (*3.2*)	8	10
Duration of drought and high supplementation periods during the SGS (months)	0	0	1	1.3 (*1.3*)	2	5.5
TECmin[c] (months)	0	3.75	6.75	5.9 (*3.4*)	7.25	12
TECmax[c] (months)	0	6.5	10	9.2 (*4*)	12.25	15

[a] FGS = first grazing season, the duration of the FGS can be variable from one herd to another and within a herd according to date of birth and age at the first turn-out.
[b] SGS = second grazing season, the duration of the SGS can be variable from one herd to another and within a herd according to the date of first calving.
[c] TEC = time of effective contact with GIN larvae before the first calving. A minimal TEC (TEC_{min}) and a maximal TEC (TEC_{max}) were calculated in each herd according to the pattern of date of birth and the age at first calving.

re-infection for at least three months before our experimental treatment.

166 cows were excluded because of: absence of recorded daily milk production data (for 109 cows, daily MP data had not been correctly recorded on farm), illness or death during the study period (9 cows), administration of an anthelmintic treatment by the farmer (26 cows exposed to re-infection for less than three months before the experimental treatment), impossibility of estimating the production level (necessary data not recovered from the milk-recording scheme) (20 cows), mistake in the identification of the blood samples (2 cows).

A number of 1088 cows were finally included for the statistical analysis regarding (i) the assessment of the global treatment response and (ii) the variations of the treatment response according to individual production-based indicators, parasitological herd-level indicators and TEC: 541 treated cows and 547 control cows, 421 cows for 2010, 667 cows for 2011, and 109 present in 2010 and 2011. Few individual parasitological results were lacking and led to the exclusion of 11 cows for the statistical analysis regarding the variations of the treatment response according to the individual parasitological indicators, which was thus carried out on 1077 cows.

After treatment, during the follow up, the number of cows decreased each week because cows could be dried off or culled. Moreover, as cows included in 2010 were followed for 9 weeks after treatment (0 to 8) versus 15 weeks (0 to 14) in 2011, from $week_9$ only cows included in 2011 remained in the data set. As a result, the decrease was from 1088 cows on $week_0$ until 980 cows on $week_8$, and from 623 cows on $week_9$ until 564 cows on $week_{14}$.

The final dataset is described in Table 3. For each indicator, there was no difference in the proportion of treated cows between categories (Chi-square test, $p > 0.05$).

3.2. Overall milk production response after anthelmintic treatment over time

In model 0, all the usual factors associated with the variations of MP were significant, and the variable of interest week-trt was significant ($p < 0.0001$). The overall evolution of the 541 treated cows' daily MP averaged by week was thus significantly different from the evolution of the 547 control cows' daily MP averaged by week. The evolution of the treated cows' MP gain (G_is) over time is displayed on the Fig. 1. This overall treatment effect over time remained slight: the maximal MP gain is +0.85 kg/cow/day in $week_6$ after treatment (Fig. 1), and the average MP gain is +0.27 kg/cow/day for a 15-week period of follow-up.

3.3. Variations of milk production response after anthelminthic treatment

Three models were finally constructed to assess the variability of the treatment response, accounting for the associations found between indicators (Table 4).

3.3.1. Variation of the treatment response according to easy-to-use indicators: Grazing history (TEC), other herd-level indicators and individual cow level production-based indicators

After examination of the two-by-two associations (Table 4a and b), model 1 included all together the TEC, the two other herd-level indicators (BTM ODR and % of positive FEC), and the three individual production-based indicators (parity, DIMt and production level) in interaction with week-trt. Figs. 2 and 3 display the evolution of the treated cows' MP gain (G_is) over time according to the three herd level indicators, and to the three individual production-based indicators, respectively.

The interaction between TEC and week-trt was highly significant ($p < 0.0001$), cows from low-TEC herds responding better to treatment than cows from high-TEC herds (Fig. 2a).

The interaction between % positive FEC and week-trt was also significant ($p = 0.002$), cows from herds with % positive FEC $\leq$ 22.6% showing a better treatment response than cows from herds with % positive FEC > 22.6% (Fig. 2b).

The interaction between BTM ODR and week-trt was not significant ($p = 0.12$), but cows from high-BTM ODR herds (BTM ODR $\geq$ 0.74) tended to respond better than cows from low-BTM ODR herds (BTM ODR < 0.74) (Fig. 2c).

The evolution of treated cows' MP differed significantly according to parity (interaction between parity and week-trt: $p < 0.0001$). The evolution of the treated cows' MP

Table 3
Description of the dataset (1088 cows), distribution of the variables, classification and number of treated cows and control cows per classes of each indicator:.

Indicators	Variables	Minimum	Maximum	Mean *(std)*	Median	Classes' thresholds and number of cows per classes			
							Treated cows	Control cows	Total
Individual production- based indicators	Parity	1	8	2.1 (*1.2*)	2.0	1	204	206	410
						2	165	173	338
						3 and greater	172	168	340
	Days in milk at the time of treatment (DIMt) (days)	1	513	182 (*136*)	154	DIMt ≤ 100	180	182	362
						100 < DIMt ≤ 200	166	154	320
						DIMt > 200	195	211	406
	Production level (kg) (multiparous cows)[a]	5893	15,507	10,710 (*1693*)	10,682	Low[c]	107	122	229
						Moderate[c]	121	106	227
						High[c]	109	113	222
	Production level (kg) (primiparous cows)[b]	15.9	48.5	31.6 (*5.5*)	31.6	Low[c]	79	68	147
						Moderate[c]	70	65	135
						High[c]	55	73	128
Individual parasitological indicators	Serum pepsinogen level (mUTyr)	185	4313	1291 (*570*)	1214	Pepsi ≤ 952[d]	161	166	327
						952 < pepsi ≤ 1402	179	175	354
						Pepsi > 1402[d]	193	203	396
	Serum *O. ostertagi* ODR	−0.16	1.35	0.51 (*0.27*)	0.51	ODR ≤ 0.38[d]	180	182	362
						0.38 < ODR ≤ 0.62	172	184	356
						ODR > 0.62[d]	181	178	359
	FEC (eggs per 5 g)	0	67	0.95 (*4.2*)	0	Positive FEC	122	133	255
						Negative FEC	411	411	822
Herd-level parasitological indicators	TEC[e] with GIN larvae before first calving (months)	0	15	7.9 (*3.95*)	8	High-TEC (TEC_{min} ≥ 8)	159	164	323 (7 herds)
						Low-TEC(otherwise)	382	383	765 (18 herds)
	O. ostertagi Bulk Tank Milk ODR	0.36	1.07	0.77 (*0.19*)	0.74	BTM ODR < 0.74[f]	222	223	445 (11 herds)
						BTM ODR ≥ 0.74	319	324	643 (14 herds)
	% positive FEC in the herd	4.5	77.8	24.9 (*16.8*)	22.6%	%FEC+ ≤ 22.6[f]	299	311	610 (13 herds)
						%FEC+ > 22.6	242	236	478 (12 herds)
Milk production	Daily MP averaged by week	3.70	61.36	29.53 (*8.87*)	28.74				

[a] 305-days milk production of the previous lactation estimated by the Test Interval method and adjusted for parity.
[b] Maximal test-day milk yield over the first 80 days of the current lactation.
[c] According to the terciles of the within-herd distribution (a cow is considered a high, moderate or low producing cow in its herd).
[d] Terciles of the distribution (all cows).
[e] TEC = time of effective contact with GIN infective larvae before the first calving.
[f] Median of the between-herd distribution.

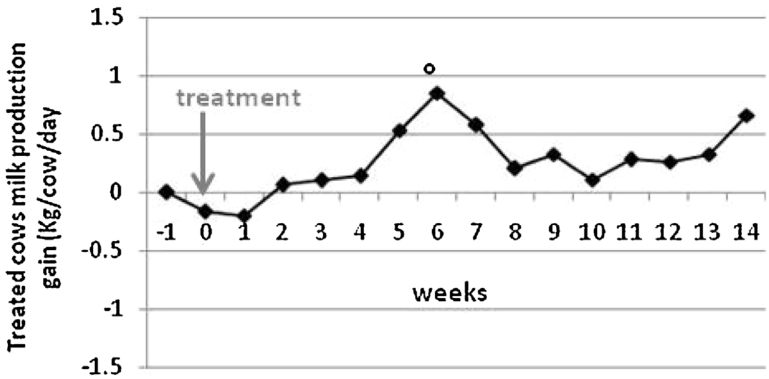

Fig. 1. Evolution of the treated cows' milk production gain (G_is) over time in comparison with control cows (model 0, n = 1088 cows, 541 treated cows, 547 control cows) (week$_0$ = week of treatment, the first day of week$_0$ is the day of treatment) (°G_i significantly different from zero, adjusted p-value <0.05).

gain over time according to parity suggested that primiparous cows responded slightly better than multiparous cows until week$_8$ (Fig. 3a).

The interaction between DIMt and week-trt was significant ($p < 0.0001$), cows with DIMt ≤ 100 days responding better to treatment than cows with higher DIMt (Fig. 3b).

Finally, although a significant interaction between production level and week-trt ($p = 0.002$), indicating a different pattern of treatment response between high, moderate and low producing cows, we notice in Fig. 3c that there was no obvious trend towards a better treatment response pattern for one or two of these 3 categories.

Table 4
Description of the two-by-two associations between: (a) the 6 categorized individual-level indicators, (b) the 3 categorized herd-level indicators, (c) the 3 individual-level parasitological indicators and the 3 herd-level parasitological indicators.

(a)

	Parity	DIM at the time of treatment	Production level	FEC	Serum *Ostertagia* ODR	serum pepsinogen level
Parity		NS[a]	NS[a]	NS[a]	NS[a]	NS[a]
DIM at the time of treatment			NS[a]	$p < 0.0001$[a,1]	NS[a]	NS[a]
Production level				NS[a]	NS[a]	NS[a]
FEC					NS[a]	NS[a]
Serum *Ostertagia* ODR						p = 0.008[a,2]

(b)

	Bulk tank milk *Ostertagia* ODR	TEC	% positive FEC
Bulk tank milk *Ostertagia* ODR		NS[a]	NS[a]
TEC			NS[a]

(c)

		Herd-level parasitological indicators: Bulk tank milk *Ostertagia* ODR	TEC	% positive FEC
Individual-level parasitological indicators	FEC	$p = 0.002$[a,3]	$p = 0.002$[a,4]	$p < 0.0001$[a,5]
	Serum Ostertagia ODR	$p < 0.0001$[b,6]	$p < 0.0001$[b,7]	NS[b]
	Serum pepsinogen level	$p < 0.0001$[b,8]	$p = 0.002$[b,9]	NS[b]

[1] Early lactation cows (DIM ≤ 100 days) have more often a positive FEC than cows with higher DIM (100 < DIM ≤ 200 days or DIM > 200 days).
[2] When the serum pepsinogen level is low, the serum Ostertagia ODR is most often low, and when the serum pepsinogen level is high, the serum Ostertagia ODR is most often high.
[3] Among cows from high-BTM ODR herds, 27% have a positive FEC, whereas among cows from low-BTM ODR herds, 19% have a positive FEC.
[4] Among cows from high-TEC herds, 30% have a positive FEC, whereas among cows from low-TEC herds, 21% have a positive FEC.
[5] Among cows from high-% positive FEC herds, 39% have a positive FEC, whereas among cows from low-% positive FEC herds, 12% have a positive FEC.
[6] Individual serum Ostertagia ODR is on average lower in low-BTM ODR herds than in high-BTM ODR herds (0.44 versus 0.56).
[7] Individual serum Ostertagia ODR is on average lower in low-TEC herds than in high-TEC herds (0.47 versus 0.61).
[8] Individual serum pepsinogen level is on average higher in low-BTM ODR herds than in high-BTM ODR herds (1374 versus 1233 mUTyr).
[9] Individual serum pepsinogen level is on average lower in low-TEC herds than in high-TEC herds (1266 versus 1348 mUTyr).
[a] Chi-square tests (level of significance set at $p \leq 0.05$).
[b] Mean comparison tests (level of significance set at $p \leq 0.05$). NS = not significant.

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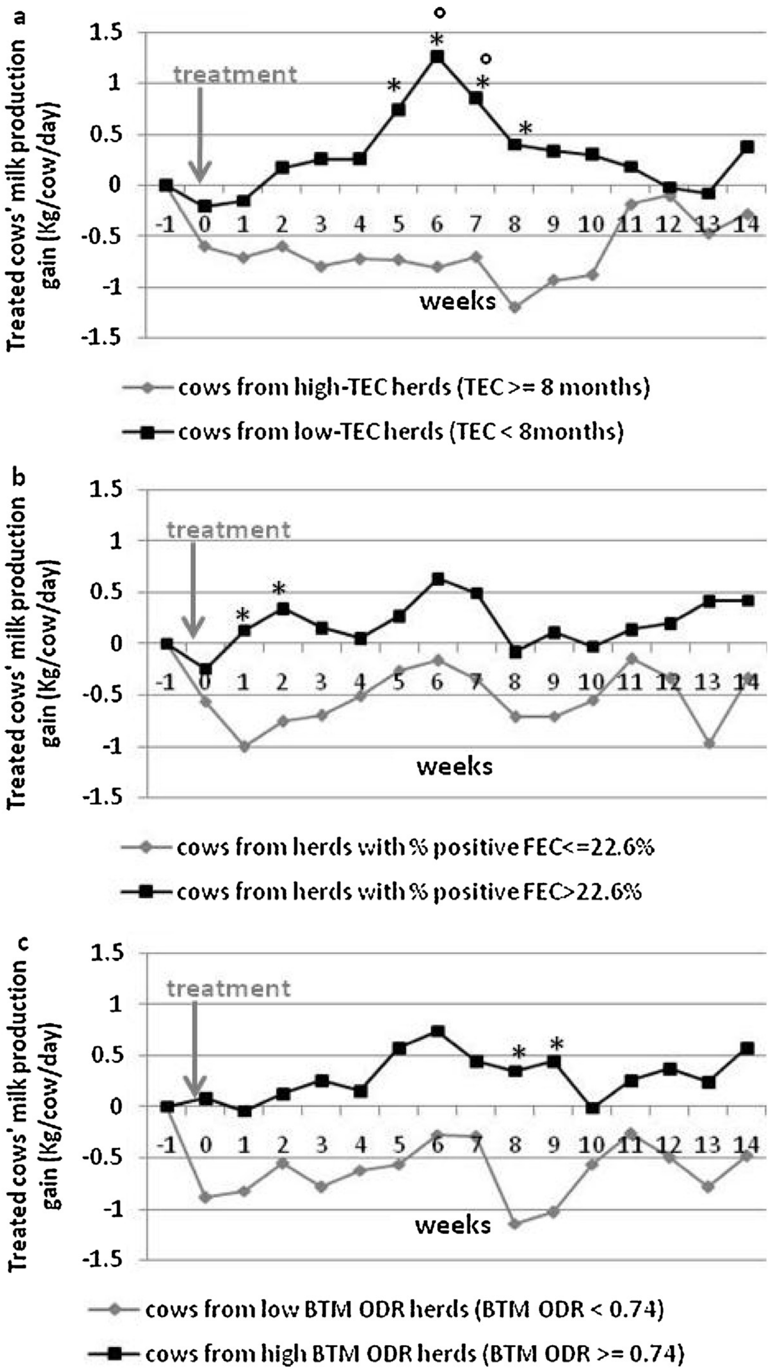

Fig. 2. Evolution of the treated cows' MP gain (G_is) over time in comparison with control cows according to the three herd level indicators (model 1, n = 1088 cows): (a) Time of Effective Contact (TEC) with GIN larvae before first calving, (b) % of positive FEC in the herd, (c) Bulk tank milk O. ostertagi ODR. (°G_is significantly different from zero, * weeks during which G_is are significantly different, adjusted p-value <0.05) (See Table 1 for the number of treated and control cows in each category).

3.3.2. Variation of the treatment response according to individual cow level parasitological indicators

It was not possible to assess the additive value of the three individual cow level parasitological indicators in Model 1 as each one was associated with at least two of the three herd-level indicators (Table 4c). Particularly, TEC and individual serum ODR were significantly associated: individual serum *Ostertagia* ODR was on average lower in low-TEC herds than in high-TEC herds (0.47 versus 0.61, p < 0.0001) (Table 4c), and 80% of low-ODR cows were cows from low-TEC herds (whereas only 58% of high-ODR cows came from low-TEC herds). Moreover, serum pepsinogen

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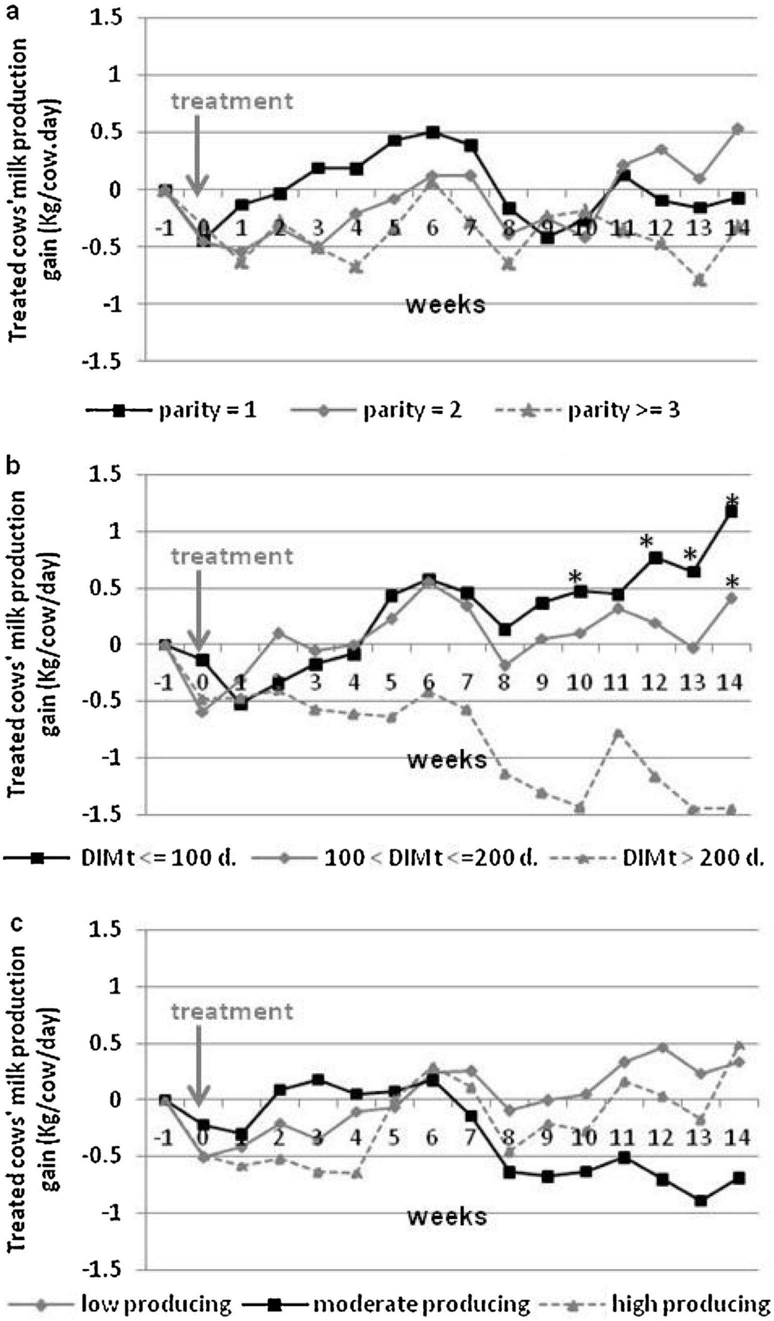

Fig. 3. Evolution of the treated cows' MP gain (G_is) over time in comparison with control cows according to the three individual production-based indicators (model 1, $n = 1088$ cows): (a) Parity, (b) Days in milk at the time of treatment (DIMt), (c) Production level. (* weeks during which G_is of cows with DIMt ≤ 100 days or 100 < DIMt ≤ 200 days are significantly different from G_is of cows with DIMt > 200 days, adjusted p-value < 0.05). (See Table 1 for the number of treated and control cows in each category.)

levels and individual *O. ostertagi* ODRs on the one hand, FEC and DIMt on the other hand, were associated (Table 4a). Therefore, they were not put in the same model and two other models had to be constructed: models 2 and 3. Model 2 initially included parity, production level, pepsinogen level and FEC in interaction with week-trt. Model 3 initially included parity, production level, individual ODR and FEC in interaction with week-trt. In models 2 and 3 the interaction between week-trt and FEC was not significant ($p = 0.3$ and $p = 0.4$, respectively), FEC was thus removed from these two models. FEC was not a factor associated with the treatment response at the individual cow level.

Fig. 4 displays the evolution of the treated cows' MP gain (G_is) over time according to the three individual cow level parasitological indicators.

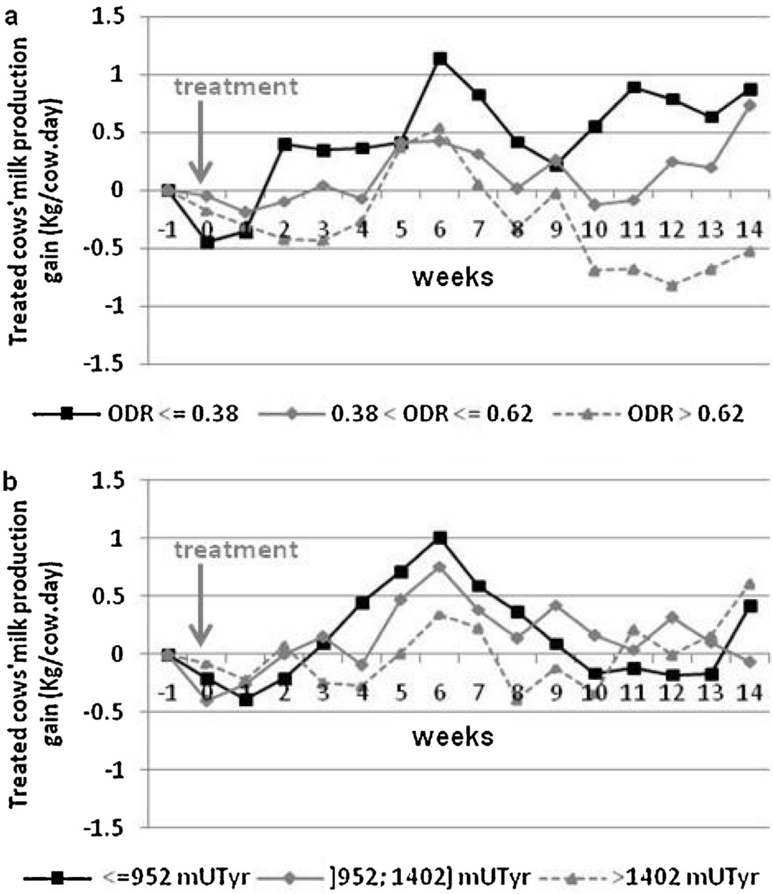

Fig. 4. Evolution of the treated cows' MP gain over time in comparison with control cows according to the individual parasitological indicators (models 2 and 3, $n = 1077$ cows): (a) Individual serum O. ostertagi ODR, (b) Individual serum pepsinogen level. (See Table 1 for the number of treated and control cows in each category.)

The evolution of treated cows' MP differed significantly according to the three classes of individual ODR values (interaction term between individual ODR and week-trt in model 3: $p = 0.005$). The evolution of the treated cows' MP gain over time according to the three classes of individual ODRs suggested that low-ODR cows ($ODR \leq 0.38$) responded better than cows with higher ODR ($0.38 < ODR \leq 0.62$ and $ODR > 0.62$) (Fig. 4a).

The interaction between pepsinogen level and week-trt was significant in model 2 ($p < 0.0001$), indicating a different pattern of treatment response between cows with low ($pep \leq 952$ mUTyr), moderate ($952 < pep \leq 1402$ mUTyr) and high ($pep > 1402$ mUTyr) serum pepsinogen levels. The evolution of the treated cows' MP gain over time according to the 3 classes of individual serum pepsinogen levels suggested a trend towards a better treatment response for low and moderate serum pepsinogen cows (Fig. 4b).

4. Discussion

4.1. Overall milk production response after anthelmintic treatment overtime

This is the first study looking at the effect of anthelmintic treatment on milk production in the North-West of France, and reporting the pattern/kinetics of the global treatment response obtained from daily milk production data averaged by week.

The maximal MP gain was achieved at $week_6$ post-treatment. This 6-week post-treatment delay is consistent with the results of Reist et al. (2011), based on monthly response, where the increase in milk production after anthelmintic treatment was most visible between day 22 and 62 after treatment. As depression in voluntary feed intake is an important feature of GIN infection (Fox, 1997), this time-lag could be the time needed for tissue repair and increase in feed intake. Nevertheless, our study design did not enable to assess the duration of this overall treatment effect beyond 15 weeks after treatment.

The amplitude of the overall treatment effect was slight on the 15-week period of follow up (+0.27 kg/cow/day on average with a maximal of +0.85 kg/cow/day in $week_6$). This result is in global agreement with the combined estimate of +0.35 kg/day after controlling for publication bias and/or small study effect in the meta-analysis of Sanchez et al. (2004a). However, conflicting results have been published recently. According to Mason et al. (2012), anthelmintic treatment had no overall effect on daily energy corrected milk in 3 New Zealander herds (923 cows), whereas Reist et al., 2011 found a higher treatment effect of +1.90 and +2.63 kg on the second and third test-days after treatment in 7 alpine farms in South Tyrol. Such conflicting results confirm the great variability of milk production response between trials reported previously by Gross et al. (1999).

This slight overall treatment effect on milk production in our study could also be related to (i) the drug used in our study design, (ii) the overdispersed distribution of parasites.

No anthelmintic resistance to fenbendazole has been reported in cattle in France. However, the efficacy of fenbendazole against L4 is often less than complete (ranging from 55% to 97.5% according to the administered dose (Williams et al., 1981, 1984; Williams, 1991), and this has to be kept in mind as the proportion of fourth stage inhibited larvae (L4) can be very high in adult dairy cattle, particularly in autumn (Borgsteede et al. (2000); Agneessens et al., 2000). On the other hand, the use of a more effective drug against L4 such as eprinomectin pour-on, the only macrocyclic lactone permitted during lactation in France, would not have been an appropriate option because the actual disposition of macrocyclic lactones administered as pour-on formulations has been shown to be largely influenced by both self-and allo-licking, leading to a possible partial anthelmintic efficacy in untreated animals (Bousquet-Mélou et al., 2011). Fenbendazole per os formulation was considered as the unique option in our study design because it has a zero withdrawal time for milk, a narrow spectrum without effect on ectoparasites, and an absence of diffusion of the anthelmintic substance from treated animals to control animals.

The distribution of nematodes is overdispersed: only a small proportion of cows would have a parasitic burden supposed to be high enough to negatively affect the milk production. This proportion ranges from 2% to 20% according to Borgsteede et al. (2000), Agneessens et al. (2000), and Chartier et al. (2013). Consequently, cows with an improved MP after anthelmintic treatment are supposed to be uncommon, and their individual treatment response could therefore be "concealed" in this overall slight treatment response of 541 treated cows versus 547 control cows. The further step is thus to try to identify the herds and/or cows that are contributing the most to the overall treatment response.

4.2. Variations of the treatment response according to the grazing history

Development of immunity against GIN depending both on the magnitude and duration of exposure (Vercruysse and Claerebout, 1997), the time of effective contact (TEC) with GIN infective larvae before the first calving was expected to reflect, at herd-level, the level of resistance to re-infection at the first calving. To qualify the herds as low-TEC or high-TEC herds, we chose the threshold $TEC_{min} \geq 8$ months on the basis of results obtained with experimental infection. Claerebout et al. (1998) reported that, after 5 to 6 months of effective contact with *Ostertagia* and *Cooperia* (trickle infections for 24 weeks), the establishment rate after a challenge infection was reduced by 65% compared to a control group which had not been in contact with GIN before this challenge infection (helminth naïve control before the challenge infection). Consequently with experimental infections, a time of effective "experimental" contact of 5 to 6 months could enable a high reduction of the *Ostertagia* burden. As experimental infections are an imperfect mimic of naturally infections, and as this reduction by 65% of the establishment rate could be the result of an only incomplete resistance, in our field study, we chose a cautious threshold of 8 months to expect an acquired complete resistance to re-infection. In low-TEC herds, the immune status of dairy cows is supposed to be heterogeneous due to the less than complete development of resistance to re-infection in the youngest (primiparous) cows. In high-TEC herds, this immune status should be more homogeneous because both young and older cows are supposed to be resistant. In our study cows from low-TEC herds responded better to treatment than cows from high-TEC herds, suggesting that the treatment response depends on the resistance status of the herd. The immune status of herds has, to our knowledge, never been taken into account in studies dealing with the effect of anthelmintic treatment on milk production. Yet its assessment through grazing history is feasible, as the information needed to calculate the TEC is easy to collect in farms. This indicator could therefore be one of the operational indicators helping practitioners and advisors to identify herds that could benefit from treatment.

4.3. Variations of the treatment response according to other easy-to-use indicators

Cows from high-BTM ODR herds tended to respond better than cows from low-BTM ODR herds in our study, but this difference was not significant. This result has to be compared with two studies investigating the relationship between the *Ostertagia* BTM antibody levels and treatment response. Kloosterman et al. (1996) showed that the response to treatment was higher in high antibody level herds than in low antibody level herds, but this difference lacked statistical significance. Charlier et al. (2007) observed the greatest positive treatment effect on milk yield in herds belonging to the highest BTM ODR category (BTM ODR > 0.84); but intriguingly the next greatest effect was observed in herds belonging to the lowest BTM ODR category (BTM < 0.50). Thus, the informative value of the BTM ODR to predict a potential treatment response on its own remains equivocal and debatable.

The treatment response was better for cows from high-% of positive FEC herds (>22.6%) than for cows from low-% of positive FEC herds. Sithole et al. (2005) failed to show a significant relationship between the treatment response and the % FEC in the herd; but this latter study was carried out in totally or semi-confined herds and only 8 cows per herd were sampled (4 first-parity and 4 second-and-above-parity milking cows). Nevertheless, as we found a significant association between FEC and DIM at the individual level, one can hypothesize that we measured more the influence of DIM on the treatment response than a direct relationship with the % of positive FEC. This is supported by the fact that, at herd level, the association between the

% of positive FEC and the % of cows in early lactation was marginally significant ($p = 0.07$).

In our study, the effect of parity on the treatment response was significant with primiparous cows responding slightly better to treatment during the 9 weeks following treatment. Nødtvedt et al. (2002) observed a similar trend, i.e. a greater effect of the treatment in first and second lactation animals. However, several other studies have reported either an absence of effect of parity (Michel et al., 1982; O'Farrell et al., 1986; Ploeger et al., 1990; Mason et al., 2012; Sithole et al., 2005), or a better treatment response for multiparous cows (McPherson et al., 2001; Charlier et al., 2010). Results regarding the relationship between parity and treatment response are thus variable and contradictory.

The effect of DIM at the time of treatment on the treatment response was also significant in our study. Cows with DIM ≤ 100 days at the time of treatment responded better than cows with higher DIM. Charlier et al. (2010) have shown similar results: in their study the treatment effect decreased with increasing DIM at the time of treatment, suggesting that a positive milk response only occurred when the treatment was performed in the first half of lactation. Mason et al. (2012) found that cattle responded maximally to treatment a little later, during mid lactation (approximately 150 days post-partum), the difference between treated and control cattle becoming smaller after this time. Indeed, cows in the first half of lactation have high nutritional requirements. The removal of GIN at the beginning of lactation could enhance appetite and, as a result, nutritional requirements might be better covered, and milk production improved.

Despite a significant relationship between treatment response and production level, this individual indicator did not appear as a reliable and discriminating factor of variation of the treatment response, since we notice in Fig. 3c that there was no obvious trend towards a better treatment response pattern for low, moderate or high producing cows. In the literature, results regarding the relationship between the treatment response and the production level are conflicting: Ploeger et al. (1989) showed that high producing cows benefited more from treatment than low producing cows, and similar observations were done in dairy goats (Chartier and Hoste, 1994), whereas Mason et al. (2012) did not find any relationship between treatment response and milk yield potential and suggested that further work is needed before strategic treatment of high or low producing cattle can be recommended.

4.4. *Variations of the treatment response according to the three individual cow level parasitological indicators*

As these three indicators are costly and time-consuming (high additional cost for sampling and laboratory analysis), they could be integrated in a selective treatment strategy only if they were very good discriminators. In this study no treatment response pattern related to one of these three indicators was sufficiently better than others to justify this cost.

Individual FEC was not associated with the treatment response in our study. Egg shedding is generally very low in adult cows, and the FEC is usually regarded as a poor indicator of the presence or the level of infection in adult cows (Charlier et al., 2009). However, Perri et al. (2011) and Mejía et al. (2011) showed a negative relationship between FEC performed around parturition and milk production level, positive-FEC cows producing less than negative-FEC cows, and concluded that FEC could be a useful tool to identify cows that would benefit from treatment. But these two studies have been conducted in an epidemiological context different from ours, making results difficult to compare. Indeed, they followed a single all year round fully grazing dairy farm, located in the humid Pampa (Argentina), with a grazing system probably enabling a higher and longer exposure to GIN than ours.

Previously, the relationship between serum pepsinogen values and treatment response had been investigated only at the herd level: mean herd milk yield response was not related to the mean serum pepsinogen levels, and this serological parameter could not be relied upon to identify herds that would benefit from treatment (O'Farrell et al., 1986; Ploeger et al., 1989, 1990). In our study, this relationship was investigated at the individual cow level. The individual pepsinogen level was significantly associated with the treatment response but appeared as a relatively poor discriminator. Moreover, a trend towards a better treatment response for low and moderate serum pepsinogen level cows was intriguingly found as this result did not fit with what is known about the value of this indicator for monitoring GIN infections in first and second grazing season calves (Kerboeuf et al., 2002; Charlier et al., 2011). In adult cows, the informative value of serum pepsinogen concentrations is poorly understood. Indeed, results regarding the relation between serum pepsinogen level and parasitic burden are conflicting: Agneessens et al. (2000) and Jacquiet et al. (2010) found a significant correlation whereas Vercruysse et al. (1986) and Chartier et al. (2013) did not. Moreover, high serum pepsinogen values can be observed in adult cows with low parasitic burden, and this has been attributed to a hypersensitivity resulting from infections in previous years (Charlier et al., 2009). It could also be attributed to other injuries of the abomasal mucosa because of the lack of specificity of this dosage.

Investigating the relationship between individual serum pepsinogen level and milk production response after anthelmintic treatment could have enabled to better understand this parameter in adult cows. However, this parameter has been scarcely evaluated in adult cows (Charlier et al., 2009), and additional work needs to be done.

Individual ODRs are often measured in milk samples, whereas in our study individual ODRs were measured in blood samples. Even if the serum antibody level is the most influential factor in determining the milk antibody titre, the correlation is moderate (Kloosterman et al., 1993), and other "milk factors" such as milk yield, stage of lactation, parity and mastitis also influence the milk antibody level (Kloosterman et al., 1993; Sanchez et al., 2002, 2004b, 2005; Charlier et al., 2006, 2010). In order to prevent from these influencing "milk factors", we chose to measure serum individual ODRs.

Individual serum ODR was significantly associated with the treatment response: low-ODR cows (ODR ≤ 0.38)

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responded better than cows with higher ODR. We noticed that the individual ODR and the herd immune status (measured by the TEC) were significantly associated. 80% of these low-ODR cows (versus 58% of high-ODR cows) came from low-TEC herds. As a result, this finding regarding the low individual ODR is consistent with the better treatment response found for cows from low-TEC herds. Nevertheless, the fact that low-ODR cows responded better than cows with higher ODR remains a puzzling result compared with those found in the literature. Indeed, the studies of Sanchez et al. (2002, 2005) and Vanderstichel et al. (2013) suggested on the contrary that a beneficial treatment effect could be expected for cows with high ODR. However, in these three Canadian studies individual milk ODR were globally low (average ODR: 0.297, 0.28 and 0.307, respectively), and the exposure to GIN was limited as the studied herds had only some access to pasture (Sanchez et al., 2002; Vanderstichel et al., 2013), or limited to none access to pasture (Sanchez et al., 2005). Consequently, we can assume that in these Canadian herds the level of resistance to re-infection is low, and that their results can hardly be extrapolated to our European grazing systems enabling a higher exposure and a higher development of immunity. In Belgian context, probably more like ours, Charlier et al. (2010) observed an increasing treatment response when the individual pre-treatment milk ODR increased, but these authors reported that they were not able to determine whether the individual ODR had an actual ability to predict the treatment response because of the relation with parity which was a confounding factor.

These conflicting results suggest that individual ODR values must be interpreted with caution: its predictive value regarding the treatment response, when demonstrated, should be related to the epidemiological context in which it has been demonstrated, and particularly related to the grazing management practices.

5. Conclusion

The overall slight treatment response found is this study confirms that blanket whole-herd treatment for GIN is debatable. At herd level, the Time of Effective Contact (TEC) with GIN infective larvae before the first calving appeared as a new promising tool for targeted treatment, and lends support for the on-farm qualitative analysis of grazing management factors. To use more reliably bulk tank milk and individual ODRs, this analysis of grazing history should be taken into account, and perhaps the definition of thresholds should depend on the TEC values.

At the individual cow level, days in milk could be integrated in a selective treatment strategy, and the individual TEC should be investigated. Since conflicting results regarding parity have been reported, this indicator should be investigated more deeply, and particularly in relation to the TEC, before determining if it could be a reliable tool for selective treatment. Moreover, the imperfect character of available indicators to predict a potential individual milk production response after anthelmintic treatment lead us to suggest that it would be more efficient to identify cows that would benefit from treatment with a combination of several indicators.

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