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Original article

Ostertagia ostertagi antibodies in bulk tank milk from dairy cattle in Italy: A nation-wide survey

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ABSTRACT

The objective of this study was to investigate the exposure to *Ostertagia ostertagi* in dairy cattle herds across Italy through measurement of antibody concentration in the bulk tank milk (BTM) and to evaluate the possible effect of regions, seasons and pasture management practices on the level of exposure to the parasite. The *O. ostertagi* antibody levels in milk were determined using a commercial ELISA kit (SVANOVIR® *O. ostertagi*-Ab) and expressed as optical density ratio (ODR). From March 2015 to September 2017, BTM samples were collected in 1209 dairy cattle farms located in 15 regions of Northern, Central and Southern Italy. As reported in previous studies, BTM samples were classified in three different categories according to the level of exposure to *O. ostertagi* as follows: (i) positive with ODR values > 0.60; (ii) negative with ODR values < 0.30 and (iii) grey zone with ODR values between 0.30 and 0.60. Finally, differences in ODR values between regions, seasons and herd management practices were studied using the one-way analysis of variance. The overall mean ODR revealed a value of 0.49 in Italy; in particular, dairy cattle farms located in Southern Italy showed the highest *O. ostertagi* BTM ODR values than the other regions ($p < 0.05$). The ODR values ranged from 0.45 in autumn to 0.53 in spring and the seasonal difference was significant ($p < 0.05$). Furthermore, a positive association was found between the ODR values and the access to pasture; herds with access to pasture showed significantly higher titres ($p < 0.0001$) of *O. ostertagi* antibodies in milk (mean ODR = 0.63) than in those in total-confinement housing (mean ODR = 0.42). This study, the first of its kind in Italy, will provide a quantitative assessment exposure to *O. ostertagi* of Italian dairy herds and represents a significant step forward in evidence-based medicine for dairy veterinarians, advisors and farmers.

1. Introduction

Parasitic gastroenteritis in European cattle result principally from infections with *Ostertagia ostertagi*, a common gastrointestinal nematode (GIN). These abomasal infections, single or together with other GIN (e.g. *Haemonchus*, *Cooperia*, and *Oesophagostomum*), are usually chronic and can be associated with hidden subclinical losses such as reduced weight gain, milk yields and reproductive performance (Bennema et al., 2010; Charlier et al., 2018).

Neither faecal egg counts (FEC) nor plasma pepsinogen values have been shown to provide quantitative measures of worm burdens, or their impact, in adult cattle (Eysker and Ploeger, 2000; Vercruysse and Claerebout, 2001). Furthermore, FEC must be followed by faecal culture to identify GIN at genus/species level by larval morphology/morphometric analysis (Van Wyk and Mayhew, 2013). In order to perform a rapid diagnosis at the dairy farm level, an enzyme-linked immunosorbent assay (ELISA) has been developed to detect antibodies against *O. ostertagi* in bulk tank milk (BTM) samples (Charlier et al., 2005b). This ELISA can be used to assess and monitor the level of exposure to *O. ostertagi* of lactating herd and it enables, combined with other information, to better identify herds subjected to production losses and where an anthelmintic treatment could be justified (Charlier et al., 2005b; Fanke et al., 2017). Higher levels of *O. ostertagi* antibodies measured by BTM ELISA methods, referred to as optical density ratios (ODRs), are associated with decreased milk production in dairy cattle (Sanchez et al., 2005; Charlier et al., 2007, 2010). Furthermore, Charlier et al. (2012) developed a web-based application tool (Paracalc®) to estimate the economic costs of *O. ostertagi* (and *Fasciola hepatica*) infection in dairy cattle herds; results show that GIN infections cost a farmer on average €721.38 per year. Some studies investigated

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the relationship between farm management practices (e.g. pasturing techniques) and ODR values against *O. ostertagi* (Charlier et al., 2005a; Almería et al., 2009; Vanderstichel et al., 2012). These studies found positive associations between pasture exposure and ODRs. In Spain, Pablos-Tanarro et al. (2013) investigated possible differences related to seasonal variations and no significant association was found between BTM *O. ostertagi* ODR values and season. Otherwise, Sekiya et al. (2013) reported that herds managed by summer grazing and winter housing demonstrated a seasonal pattern of high ODR in late summer and early autumn and low ODR in winter. For the multiple reasons mentioned above, it is crucial to develop and implement a systematic monitoring based on comprehensive knowledge of basic epidemiologic information, including the wide range of factors that influence the level of exposure to *O. ostertagi* in a target geographical area. Currently, data on the level of exposure to *O. ostertagi* assessed by BTM ELISA in dairy cattle are limited in Italy. The only data are reported in Forbes et al. (2008) in a limited number of farms, showing low BTM ODRs in Italy (0.31) compared to ODR values in other European countries. Therefore, the objective of this study was to investigate in Italy the exposure to *O. ostertagi* in dairy cattle herds through measurement of antibody concentration in the BTM samples and evaluate the effect of pasture management practices and seasons on BTM ODR, but also the regions, in order to adapt regionally monitoring and to rationalize the anthelmintic treatments based on the local conditions.

2. Materials and methods

2.1. Sampling and laboratory procedure

BTM samples were collected from March 2015 to September 2017 in 1209 dairy herds from 15 regions of northern (Piedmont, Lombardy, Veneto, Trentino Alto-Adige, Friuli Venezia- Giulia and Emilia Romagna), central (Marche, Umbria, Tuscany and Lazio), southern (Abruzzo, Campania, Basilicata and Apulia) and insular (Sardinia) Italy. No specific criteria were imposed for farm inclusion in the study. Sampling was carried out by veterinary practitioners and for each dairy herd one sample of bulk milk was collected in a closed container, dated and labelled. During all handling procedures, the samples were constantly stored at 4 °C. All samples reached the CREMOPAR laboratories (Campania Region, southern Italy) between 24 and 72 h after collection from farms. On arrival at the laboratory, samples were aliquoted into duplicate 1.5 ml microtubes, centrifuged at 20,000 x g for 1 min, defatted and the supernatant frozen at −80 °C. BTM samples were tested in duplicate and examined by an indirect ELISA that uses a crude *O. ostertagi* antigen (SVANOVIR® *O. ostertagi*-Ab ELISA kit), sourced from Svanova Ltd. (Uppsala, Sweden), following the manufacturer's recommendations.

The substrate was measured at 405 nm using a spectrophotometer. The results from ELISAs were reported as optical density ratio (ODR) calculated as follows:

$$\text{ODR} = (\text{mean OD test sample–OD negative control}) / (\text{OD positive control–OD negative control}).$$

According to Fanke et al. (2017), BTM samples with an ODR < 0.30 were classified as negative, because at this level the infections of *O. ostertagi* have not considerable negative impacts on herd economics; BTM samples with an ODR > 0.60 were classified as positive, because at this level the production losses are mainly due to decreases of milk yield in cow on farms, and finally BTM samples with ODR values between 0.30 and 0.60 were considered as a "grey zone" that includes particularly animals within the pre-patency or weeks after treatment, when antibody titres are decreased (Malama et al., 2014).

2.2. Management information

At the time of collection, a questionnaire was gave out to each farmer to gather information about the farm-management practices that could potentially be associated with GIN parasitism. In particular, the first set of questions was directed to general herd size information. The second part of questionnaire included: anthelmintic treatments, herd management practices (with or without access to pasture) and administration of supplementary feed such as silage and green fodder; furthermore, sampling dates were recorded to investigate possible differences related to seasonal variations. The data were subsequently transferred on to a spreadsheet and the responses digitized in order to facilitate statistical analysis.

2.3. Data analysis

In order to investigate the relationship between BTM ODR results from dairy herds in Italy and management practices, the prevalence of positive samples (level of exposure with ODR > 0.60) and the distribution of the ODR values (mean, maximum, minimum, standard deviation, and 25th – 75th percentiles) were calculated in each region (northern, central, southern and insular), for each season (date of collection of the BTM sample: spring, summer, autumn or winter), and for each management practice (with or without access to pasture). Then, the differences in ODR values between the four regions and between the four seasons were analyzed using a one-way ANOVA followed by Bonferroni multiple comparison test to make the two-by-two comparisons. Finally, the association between ODR values and management practices was assessed using the Mann Whitney *U* test (95% Confidence Interval). Other variables, e.g. farm size and anthelmintic treatment, were excluded from the statistical analysis since only a low number of farmers answered the respective questions in the questionnaire. Statistical analysis was performed by GraphPad Prism 5.

3. Results

3.1. Bulk tank milk ELISA

The results of *O. ostertagi* BTM ODR profiles in Italy are reported in Table 1 and Fig. 1. The majority of farms ranged in the grey zone (57.7%) whereas 26.1% were classified as positive. Regional differences were observed for *O. ostertagi* BTM ODR values with a higher number of positive farms in Southern Italy (176/498 = 35.3%) compared to other regions ($p < 0.05$).

3.2. Seasonality

The median and interquartile ranges (box plot) of *O. ostertagi* ODR

Table 1
Ostertagia ostertagi BTM ODR profiles in Italy.

Italy	Prevalence positive farms (ODR > 0.60)	Mean ODR	25th percentile	75th percentile	SD	Minimum	Maximum
Northern	126/625 (20.2%)	0.46	0.32	0.57	0.18	0.05	1.19
Central	7/41 (17.1%)	0.49	0.40	0.58	0.21	0.0	1.12
Southern	176/498 (35.3%)	0.53	0.40	0.66	0.19	0.0	1.16
Island	7/45 (15.6%)	0.43	0.32	0.49	0.18	0.18	0.99
Entire country	316/1209 (26.1%)	0.49	0.35	0.61	0.19	0.0	1.19