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Ostertagia ostertagi antibodies in milk samples: Relationships with herd management and milk production parameters in two Mediterranean production systems of Spain

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ABSTRACT

The present study analyzed *Ostertagia ostertagi* antibodies by indirect ELISA in milk samples in two cattle systems in Mediterranean Spain to indirectly monitor gastrointestinal nematode (GI) parasitism effects on production. Individual samples from 10 animals and the corresponding milk herd samples were collected from 133 herds in Girona (intensive management) and 123 herds in Minorca (extensive management). Both locations showed high and significant positive relationships between average optical density ratios (ODR) of individual animals and ODR in their milk tank. Although antibodies levels were low, there were significantly higher in Minorca. Negative correlations between ODR values and milk production were found in both systems. Importantly, in Minorca, average herd milk production was higher in the herds that treated their animals against GI nematodes compared to those that did not treat. The ELISA technique was valuable to indirectly assess differences in the level of GI nematode infection even in cattle production systems with low levels of infection.

1. Introduction

Gastrointestinal (GI) nematodes, especially *Ostertagia ostertagi*, constitute an important constraint on the productivity of cattle in temperate regions. Gastrointestinal parasitism reduces heifer growth rates and may adversely affect milk production in dairy herds (Ploeger et al., 1990; Maclean et al., 1992; Gross et al., 1999). Therefore, anthelmintic treatment of lactating cows can result in greater milk yields (Gross et al., 1999; Charlier et al., 2007a).

Classically, diagnosis of GI infections has been based on faecal egg counts (FEC). However, FEC are highly variable, both within and between animals, and they have been shown to be effective only during the first grazing season in young animals (Eysker et al., 2000; Eysker and Ploeger, 2000). Two of the most promising diagnostic methods to evaluate GI infections are pepsinogen levels and the assessment of antibody titres using the enzyme-linked immunosorbent assay (ELISA) (Eysker and Ploeger, 2000). Pepsinogen assays have been found to be useful in diagnosing gastric infections. However, these assays may overestimate the adult parasite burden in adult animals due to a hypersensitivity type reaction (Berghen et al., 1993). An ELISA technique using a crude adult *O. ostertagi* antigen was developed by Keus et al. (1981) and evaluated by Kloosterman et al. (1993) in The Netherlands. More recently the ELISA technique has been used to analyze antibodies against *O. ostertagi* in milk samples and it has been shown to be a useful technique to measure exposure in adult dairy cows (Sanchez and Dohoo, 2002) and a promising parameter to identify dairy cows or herds with production losses due to GI nematodes (Charlier et al., 2006, 2007b). A relationship between levels of antibodies against *O. ostertagi* in bulk tank and milk production has been shown in several studies mainly in Canada (Guitián et al., 2000; Sanchez and Dohoo, 2002; Sanchez et al., 2002a) and Belgium (Charlier et al., 2005a). An increase in antibody levels from the observed 25th percentile to the 75th percentile was associated with a drop in the annual milk production yield of 1.2 kg/cow/day in Prince Edward Island (Canada) and 1.1 kg/cow/day in Belgium, respectively (Sanchez and Dohoo, 2002; Charlier et al., 2005a).

In Mediterranean areas of Spain GI nematodes also cause concern due to decreased productions in dairy cattle, but due to drier weather conditions low parasitism generally is expected and usually only subclinical infections are observed (Almería et al., 1996; Nicolàs et al., 2006). In a recent European study, *O. ostertagi* antibody levels in bulk milk samples from Spain showed high optical

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density ratios (ODR) comparable to those observed in Portugal and UK/Ireland (mean ODR 0.53 for Spain, 0.61 for Portugal and 0.60 for UK/Ireland, respectively) and higher than those observed in Denmark, Netherlands and Germany (0.45–0.48) (Forbes et al., 2008). The farms analyzed in Spain included farms from North of Zaragoza and South of Navarra (Mediterranean weather), but were mainly from farms in the humid areas of Spain (North of Navarra and Basque Country) (Atlantic weather) (J. Charlier and P. Rodriguez, personal communication), and this fact could explain the relatively high *O. ostertagi* antibody levels observed in the study. The main objective of the present study was to examine the associations between *O. ostertagi* indirect-ELISA measures in milk samples in two production systems associated to different levels of exposure to GI parasitism in drier Mediterranean areas of Spain to assess the potential of the ELISA technique as a monitoring tool for parasite burden in dairy cattle in those farming conditions.

2. Materials and methods

2.1. Study population and sample collection

A total of 133 herds in Girona and 123 herds in Minorca were randomly selected to obtain bulk-tank herd samples and individual milk samples from 10 cows were randomly selected in each herd in 2003. Minorca is a small Balearic island (702 km^2) located in the Mediterranean Sea. The weather is of a maritime temperate Mediterranean type. For the period 1971–2000, the mean annual temperature was 17 °C, the mean annual rainfall 600 mm, and relative humidity 73% (www.aemet.es). Most of herds in Minorca are allowed to graze outdoors practically during the whole year. The main grazing season starts in September, coinciding with the main calving period in most of the herds, to March–April when pasture is still abundant. During the summer, although pasture is scarce, the animals are still kept outdoors in most of the herds. On the other hand, in Girona, located in North-East Spain (maritime temperate Mediterranean weather) there was a mean annual temperature of 14 °C, mean annual rainfall of 725 mm and relative humidity of 72% for the period 1971–2000) (www.aemet.es), most herds have limited outdoor exposure and they are fed a total mixed ration that meets the nutritional requirements of the cows.

Individual and bulk tank milk samples were collected once during a period of 3 months (January–March) in 2003. This time was selected because it coincided with the last three months of the main grazing season in Minorca. Samples were stored frozen with preservative Bronopol (San Ramon, CA). After thawing, the samples were centrifuged at 16,000*g* 4 min at 4 °C, fat was skimmed off, supernatant was collected, and centrifuged again under the same conditions to assure that all fat from milk was removed.

At the time of collection, a survey was conducted in each herd to gather information about the farm-management practices that could potentially be associated with GI parasitism. The questions referred to items such as herd characteristics, grazing information and parasite control.

The study was performed following the ethical considerations of the Animal Welfare Committee of the Autonomous University of Barcelona.

2.2. ELISA procedure

Samples were tested using an indirect *O. ostertagi* ELISA using crude-antigen of adult parasites as described by Sanchez et al. (2002b), with minor modifications. Briefly, flat-bottom 96 well microplates (Immulon 2HB, Vitaltech, Bayern, Germany) were coated with 100 μl/ml of antigen per well (1 μg/ml in a carbonate–bicarbonate buffer, pH 9.6). Plates were incubated overnight at 4 °C. After three washes in a saline solution with Tween (ST), the plates were blocked by adding 200 μl of ST/3% fetal calf serum (FCS) (Gibco BRL, Life Technologies, St. Paul, MN) for 1 h. Plates were then frozen at −20 °C until subsequent readings. After thawing, plates were washed as before. Milk samples were thawed and 100 μl of the supernatant were dispensed into each well. Samples were analyzed in duplicates. Plates were incubated for 1 h at 25° C.

Bulk tank milk samples with high and low antibody titres were run on each plate in duplicate as positive and negative controls and were used to asses the repeatability of the technique. Similarly, a negative control sample for background was added consisting of ST/1% FCS. Control samples were dispensed at 100 μl per well.

Rabbit anti-bovine IgG conjugated to horseradish peroxidase was used as conjugate at 1:1500 in ST/1% FCS. One-hundred microlitre of 1:1500 diluted conjugate were added to each well. The plate was incubated for 1 h at 25 °C. After being washed, 50 mg of ABTS substrate (2,2′-azinobis-3-ethilbenzothaizoline-sulfonic acid) (Roche, Basel, Switzerland) was diluted in 50 ml freshly-prepared working ABTS buffer solution. The working solution was dispensed at 100 μl per well and the plates were incubated for 30 min in the dark at 25 °C. Absorbance was read at 405 and 492 nm.

Optical density values were recorded for all samples and controls. The final OD values (raw data) were the result of the subtraction of OD reading at 492 nm from OD at 405 nm. Normalization of OD values as optical density ratios (ODR) was performed using the following method: $ODR = (OD - neg)/(pos - neg)$. Where pos: mean OD value of milk bulk-tank high antibody positive control; neg: mean OD value of milk bulk-tank low antibody negative control.

2.3. Surveys

A survey was conducted in each farm at the time of collection of milk samples. The questionnaire was designed to gather information about the management practices that would potentially influence the extent of parasite exposure and included herd description (total number of animals (size) and number of lactating cows, days in milk, lactation number, individual milk yield and average herd milk yield), herd grazing information (access or not to pasture, hectares of land available/herd and hectares of irrigated land/herd) and information about parasite control (which herds treated against parasites and against GI nematodes in particular, and, if parasite control was performed, the products used).

Individual milk production data at the time of sample collection were obtained from the local dairy herd improvement association (DHIA) (Semega and Ibab, Girona and Minorca, respectively, Spain). To obtain actual milk production at the time of milk sampling, the survey and milk sample collection of each farm coincided with the DHIA control date.

2.4. Statistical analyses

Continuous variables, such as hectares available for pasture, hectares of irrigated land, stocking density, days in milk and milk production were regressed against both, the bulk tank ODR and the average ODR obtained from 10 animals per farm using mixed-effects linear regression analysis with location as a random effect. The effect of the remaining discrete variables or factors, such as access or not to pasture, parasite control treatment, products used for parasite treatment and moment of application, were also evaluated using a mixed-effects analysis of variance with the following linear mixed model:

$$Y = \mu + \text{Location} + \text{Independent variable} + \varepsilon,$$

where Y represents the dependent variable (average individual ODR or bulk tank ODR), μ represents the overall mean, ε represents the