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Anti-*Neospora caninum* antibodies in milk in relation to production losses in dairy cattle

Marta González-Warleta*, José Antonio Castro-Hermida, Carmen Carro-Corral, Mercedes Mezo

Laboratorio de Parasitología, Centro de Investigaciones Agrarias de Mabegondo, Instituto Galego de Calidade Alimentaria–Xunta de Galicia, Carretera AC-542 de Betanzos a Mesón do Vento, Km 7,5, CP 15318, Abegondo (A Coruña), Spain

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ABSTRACT

A comprehensive field study was carried out with the following objectives: (a) to assess the usefulness of individual and bulk tank milk analysis for determining *Neospora caninum* serostatus in individual cows and herds, and (b) to study the associations between *N. caninum* infection status (based on milk testing), and several productive and reproductive parameters in the animals. Antibodies were detected with a commercially available ELISA test (Bio K 192/5). Analysis of paired serum and milk samples from 1134 lactating cows on 38 farms revealed that 97.6% of the ELISA results were coincident, irrespective of whether serum or milk samples were used. Moreover, multiple linear regression analysis revealed that 86.0% of the variations in ELISA values in milk were due to variations in the serum. The measurement of antibodies in bulk tank milk was a good estimator of the herd level status of *N. caninum* infection, and enabled detection of infection in 94.7% herds with ≥10.0% seropositive cows and/or in all herds with >4% highly seropositive cows. The odds ratio for abortion in seropositive animals was 9.1 times higher than in seronegative animals. The infection serostatus was also a significant risk factor, as the odds ratio for abortion was even higher (12.0 times) in cows categorized as highly seropositive. ELISA values for the bulk milk from 387 randomly selected herds were negatively associated with average milk production. Moreover, milk production losses mainly occurred on farms categorized as highly positive (i.e. herds with ≥20.0% seropositive cows).

1. Introduction

Neospora caninum is a protozoan parasite considered as one of the most frequent causes of abortion in dairy cattle worldwide (Dubey, 2003). However, abortion does not always occur, and cattle may harbour chronic *Neospora* infections without showing any clinical symptoms (Björkman et al., 1996). The efficiency of vertical transmission of neosporosis is very high (around 93%; Schares et al., 1998) and foetal death and abortion may occur from 3 months of pregnancy onwards. However, in most cases, the foetuses do not die and calves are born alive and clinically normal, although carrying congenital infections that are likely to persist throughout life (Williams et al., 2009) and that may have adverse effects on the animals and their productivity.

Information about the effects of chronic neosporosis on cattle productivity is scarce and inconclusive, probably because of differences among countries, regions and herds, especially in relation to the prevalence of infection, the strains of *N. caninum* involved and the diagnostic tests used. Further research involving the effects of subclinical neosporosis on production and cattle health is therefore warranted.

No treatments or vaccines have yet been shown to be safe and effective against bovine neosporosis, and the most common strategies for controlling transplacental

* Corresponding author. Fax: +34 981673656.
E-mail address: marta.gonzalez.warleta@xunta.es (M. González-Warleta).

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transmission include different combinations of the following: (1) test and cull seropositive dams or seropositive aborting dams; (2) test and inseminate the progeny of seropositive dams with beef bull semen only; and (3) test and exclude the progeny of seropositive dams from breeding programs (Hall et al., 2005; López-Gatius et al., 2005a,b). At present, diagnosis of bovine neosporosis relies on the detection of specific circulating antibodies against the parasite, and several ELISA kits based on different antigen preparations are now commercially available, some of which have been adapted for use with milk samples (Schares et al., 2004; Bartels et al., 2005). However, most of these tests have not been evaluated for their capacity to estimate potential production losses associated with infection of cows and/or herds, which is essential for supporting control measures based on objective criteria.

Previous epidemiological studies carried out in Galicia (NW Spain). (Bartels et al., 2006; González-Warleta et al., 2008) have demonstrated that the prevalence of *N. caninum* in dairy herds in the region (63–79%) is one of the highest in Europe, although the percentage seropositivity within the herds varied widely (between 1.2% and 69%). Although the impact of this high prevalence of infection on dairy herd productivity has not been quantified before, it may be considerable, since Galicia is the largest milk-producer region in Spain.

For all of the aforementioned reasons, we carried out a field study to investigate whether there was any association between *N. caninum* infection (based on individual and bulk tank milk antibody testing) and the risk of abortion, calving to conception interval and milk production in dairy herds in Galicia. Milk analyses were carried out with a commercially available ELISA test (Bio K 192/5 from Bio-X Diagnostics). In a preliminary stage of the study, we evaluated the capacity of the kit to detect seropositive cows (with individual milk samples) and the percentage seropositivity within the herds (with bulk tank milk).

2. Materials and methods

2.1. Samples and data collection

Two groups of samples (Groups A and B) were collected from commercial dairy farms in Galicia, as described below.

The Group A samples were obtained from herds selected from among 276 dairy farms on which the within-herd seroprevalence for neosporosis had previously been determined (González-Warleta et al., 2008). In order to cover the whole range of within-herd seroprevalences (0–69.0%) found in this study, the original population was divided into 5 classes (within-herd seroprevalences of 0%, 1–20%, 21–40%, 41–60% and 61–80%), and farms were then chosen at random for each class. Thirty-eight farms (6–8 per class) were finally visited for the simultaneous collection of blood and milk samples from all cows (1189) that were lactating at the time of the visit. In addition, a bulk tank milk sample was also taken from each of the 38 farms and the number of milking cows contributing to each bulk tank and the parity number of each cow at the time of sampling were recorded. The veterinary services responsible for monitoring reproduction of the herds provided us with electronic format data on artificial insemination, monitoring of gestation at 35, 120 and 210 days after insemination, calvings and birth of dead foetuses. Data obtained between 12 months before and 18 months after the sampling date were used in the study.

The Group B samples were obtained from the bulk tank milk of 387 dairy herds randomly selected from those enrolled in the Galician Dairy Herd Improvement program. The cows in these dairy herds were Friesians, breeding was mainly by artificial insemination, calving occurred throughout the year and milking cows were fed conserved forage (hay and silage) and concentrate (average of 330 g/l milk), although grazing was also common. No vaccines against neosporosis were used. Reproductive data recorded from these farms were the same as those obtained for Group A. The milk production data for the lactation concurrent with sampling were obtained from the database provided by the Galician Animal Production Service, which only records data on lactations of usual duration, i.e. more than 250 days, according to Spanish legislation. The tank milk somatic cell count carried out on the date closest to sample collection was also provided by this service.

Individual and bulk tank milk samples from Group A were used to evaluate the usefulness of the 'Bio K 192/5' ELISA test (Bio-X Diagnostics, Jemelle, Belgique) for determining the *N. caninum* infection status at cow- and herd-level. The relationships between the levels of anti-*N. caninum* antibodies in milk, and reproductive parameters in lactating cows (odds ratio for abortion and days from calving to conception) were also studied in these samples.

Samples from Group B were used to study the association between *N. caninum* infection status, determined in bulk tank milk samples, and the average milk yield of the herd.

2.2. Anti-N. caninum antibody detection in serum and milk

Sera were obtained by centrifuging blood samples at $1000 \times g$ for 10 min. Milk samples were centrifuged at $2000 \times g$ for 10 min and the solid fat layer was then removed. All samples were stored at $-20\,^{\circ}C$ until testing.

For detection of immunoglobulin G1 antibodies against *N. caninum* in serum and milk samples, the commercial 'Bio K 192/5' ELISA test was used according to the manufacturer's recommendations. This test has previously been shown to be highly sensitive (100%) and specific (93.3%) for detection of anti-*N. caninum* antibodies in bovine serum (Ghalmi et al., 2009). Test results were expressed as percentage positivity of the sample in relation to an internal positive control, and the cow serostatus was classified, according to the cut-off recommended by the manufacturer, as negative (<10%) or positive ($\geq$10%).

2.3. Statistical analysis

The ELISA test cut-off for individual milk samples was determined by non parametric receiver operating curve (ROC) analysis, with serum samples used as the standard for comparison. The agreement between the results for serum and milk samples was assessed by calculating the