

Diagnosis of bovine paratuberculosis: sensitivity of a commercial ELISA test on bovine bulk milk

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52 dairy herds, infected with paratuberculosis, were submitted to repeated bulk milk sampling. 183 bulk milk samples (an average of 3.5 samples/herd) were tested by a commercial ELISA test validated on milk (Institut Pourquier); 30.1% of samples and 40.4% of herds resulted reactive (positive or inconclusive) to paratuberculosis. The sensitivity of this ELISA test applied on bulk milk appears strongly correlated to the herd prevalence. The repeated sampling enhances the sensitivity of this diagnostic tool.

Introduction

Paratuberculosis, an infectious and contagious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), is widely spread in all the European (IDF 2001) and Italian territory, as shown by the results of the surveys carried out in Veneto (Robbi et Al. 2002), Lazio (Lillini et Al. 2005) and Lombardia Regions (Arrigoni et Al. 2006).

At present, ELISA test on bulk milk is widely used, as a low-cost diagnostic tool, in surveillance epidemiological and prophylactic plans against Brucellosis and Enzootic Bovine Leukosis, but the performances for Paratuberculosis are not well defined.

Serological ELISA test on individual milk has given promising results in paratuberculosis diagnosis, showing a sensitivity only slightly lower than the same test on blood samples (K=0.81-0.91) (Arrigoni et Al. 2004, Van Weering et Al. 2007).

The reported specificity of Pourquier ELISA test on milk varies between 99.8-100% on individual milk and 100% on bulk milk (Van Weering et Al. 2007, Arrigoni et Al. 2004).

The objective of this study was to investigate the relative sensitivity of a commercial ELISA test in detecting antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in bulk milk of infected herds, compared with the results of ELISA individual test on blood.

Materials and Methods

This study was carried out on 52 dairy herds, previously classified as positive for *Map* infection by an ELISA test (Institut Pourquier), performed on individual blood samples of cows over one year of age. When the number of seropositive animals was below a statistical threshold, the positivity was confirmed by faecal culture (Arrigoni et al. 2006).

On the basis of the prevalence of seropositive animals, the herds were classified as:

- “low prevalence” herds (<5%);
- “moderate prevalence” herds (5-15%);
- “high prevalence” herds (>15%).

The bulk milk of the infected herds was repeatedly sampled, each farm being submitted to an average of 3.5 samples, at a frequency interval of at least 30 days, with the aim of sampling all the lactating cows, resulting in a total of 183 bulk milk samples being collected.

The ELISA test on milk was performed by *Mycobacterium paratuberculosis* ELISA kit (Institut Pourquier), following the manufacturer's instructions.

Results

In the statistical analysis, all the positive and inconclusive samples were classified as “reactive”, following the manufacturer's instructions.

As showed in Table 1, 55 samples out of 183 controlled (30.1%) tested positive and were distributed as follows: 8 samples (11.1%) out of 72 coming from “low prevalence” herds, 32 samples (33.7%) out of 95 coming from “moderate prevalence” herds, and 15 samples (93.8%) out of 16 coming from “high prevalence” herds.

On the whole, taking into consideration the herds, 21 herds out of 52 controlled (40.4%) registered at least one positive sample, and were distributed as follows: 4/21 (19.0%) coming from “low prevalence” herds, 13/27 (48.1%) coming from “moderate prevalence” herds, and 4/4 (100.0%) coming from “high prevalence” herds.

Among the 21 infected herds with positive ELISA test on bulk milk: 7 herds, tested positive on a single sample; 6 herds, on more than one sample, but not on all the samples; and 8 herds, on all the samples.

Among the 8 herds that were positive on all the samples, 3 were “high prevalence” herds, 4 were “moderate prevalence” herds and one was a “low prevalence” herd.

Among the 4 “high prevalence” herds, the only herd that didn’t turn out positive in all the samples, had 3 positive and 1 negative result.

The overall relative sensitivity of ELISA test on bulk milk resulted 30.1% (C.I.95% : 27.5-37.3%). The repeated sampling enhances the sensitivity values of the test; in fact, testing 3.5 times the bulk milk of the same herd, it was possible to reveal 40.4% (C.I.95% : 27.0-54.9%) of the infected herds.

Prevalence	Tested		ELISA positive			
	Herds	Samples	Herds	% pos.	Samples	% pos.
Low (<5%)	21	72	4	19.0	8	11.1
Moderate (5-15%)	27	95	13	48.1	32	33.7
High (>15%)	4	16	4	100.0	15	93.8
Total	52	183	21	40.4	55	30.1

Table 1 – Results of bulk milk ELISA Test on Map infected bovine herds

Discussion and conclusions

The ELISA test is a rapid, economic and cost-effective diagnostic tool, with a high-throughput for the laboratory; furthermore, the ELISA paratuberculosis test can be applied on bulk milk samples collected for Brucellosis and Leukosis diagnosis, lowering the sampling costs.

The data shown in this paper agrees with the results of analogous studies carried out in Holland, where the sensitivity was 24-30% (Van Maanen et Al. 2005, Van Weering et Al. 2007).

The overall sensitivity of the ELISA test appeared strongly correlated with the herd seroprevalence; in particular, the test was very sensitive (93.8% on a single sample, 100% on repeated samples) in herds where the seroprevalence was over 15%; for this reason the use of this test could be recommended to detect high prevalence herds, more frequently producing Map contaminated milk.

On the other hand, because the low sensitivity (30.1% on a single sample, 40.4% on repeated samples) the ELISA test on bulk milk isn’t a suitable tool in Paratuberculosis certification programmes.

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