

# Performances of an ELISA applied to serum and milk for the detection of antibodies to *Coxiella burnetii* in dairy cattle

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## SUMMARY

The performances of an ELISA test based on an antigen recovered from a *Coxiella burnetii* ovine strain (CbO1) when applied to either milk and serum for the detection of antibodies against *Coxiella burnetii* in dairy cattle were compared using 448 matched milk/serum samples collected from cows of 6 dairy herds known to be infected. All tests were performed blind using ELISA LSI Fièvre Q ruminants Serum®, (Laboratoire Service International, Lissieu, France). A total of 264 (257) serum (milk) samples were found positive (with an antibody titre > 40). In a first step, the level of agreement between results (positive vs negative) for serum and milk sample was determined by calculating the kappa statistic. In a second step, ELISA values in paired milk and serum samples were compared. The level of agreement between results for serum and milk samples was very good (kappa=0.89). The ELISA test applied to milk seems to be a convenient tool for establishing the serological status of lactating dairy cows towards *Coxiella burnetii* infection.

**Keywords :** Dairy cow, *Coxiella burnetii*, ELISA, serum, milk

## RESUME

L'objectif de cette étude était de comparer les performances d'un test ELISA basé sur un antigène obtenu à partir d'une souche de *Coxiella burnetii* isolée d'une brebis ayant avorté (CbO1) lorsque appliqué à des échantillons de sérum et de lait. Pour ce faire, 448 prélèvements concomitants de lait et de sang ont été réalisés chez des vaches en lactation de 6 troupeaux bovins laitiers connus pour être infectés par *Coxiella burnetii*. Les échantillons ont été testés en aveugle à l'aide du test ELISA LSI Fièvre Q ruminants Serum®, (Laboratoire Service International, Lissieu, France). Dans un premier temps, le degré de concordance des résultats sérologiques (positif vs négatif) obtenus dans le lait et le sérum a été déterminé et d'éventuelles différences dans les proportions de résultats positifs obtenus ont été recherchées. Dans un deuxième temps, les niveaux en anticorps obtenus dans le lait et le sérum des échantillons positifs ont été comparés. Le degré de concordance entre les résultats obtenus sur les prélèvements de lait et de sérum était excellent (coefficient Kappa de 0,89). Les proportions de résultats positifs ne différaient pas significativement selon le type de prélèvement testé. Les niveaux estimés en anticorps estimés dans le lait et le sérum étaient de plus corrélés positivement. Ainsi la détection des anticorps anti-*Coxiella burnetii* dans le lait apparaît comme un outil alternatif fiable et peu coûteux pour identifier les vaches laitières exposées à l'infection par *Coxiella burnetii* dans les troupeaux.

**Mots-clés :** Vache laitière, *Coxiella burnetii*, ELISA, sérum, lait

## Introduction

Q fever is an endemic and worldwide zoonosis caused by an obligate intracellular bacteria, *Coxiella burnetii* (BACA and PARETSKY, 1983; BEHYMER and RIEMANN, 1989). The ruminants and particularly sheep, goats and cattle are recognised as the main sources of human infection (FONTAINE *et al.*, 1975; CHALMERS *et al.*, 1998). In ruminants, this infection is frequently asymptomatic but it may cause abortion, metritis and infertility and the disease results in economic losses and restriction in commercial milk trade. Nowadays, PCR technique is recognised as the most suitable tool for *Coxiella burnetii* detection. But this technique remains costly and fastidious. Serological methods seem to be more appropriate in routine diagnosis and large-scale epidemiological studies aimed at demonstrating an exposure to *Coxiella burnetii*. Among available

serological methods, ELISA is known to be as sensitive as Indirect immunofluorescence assays and more sensitive than complement fixation assays (PETER *et al.*, 1987; COWLEY *et al.*, 1992; MAURIN and RAOULT, 1999). Regarding technical considerations ELISA seems to be more appropriate for using in large scale studies. Classically, evidence of antibody carriers against *Coxiella burnetii* in dairy cattle is performed on serum. However, for practical and economical reasons, it would be useful to evaluate if milk can be used alone to evidence exposure to the pathogen, as already described for brucellosis (CHAND *et al.*, 2005) or neosporosis, for example (FRÖSSLING *et al.*, 2006).

Thus, the present study aimed at comparing milk-ELISA and ELISA-serum performances for the detection of antibodies anti-*Coxiella burnetii* in dairy cattle.

## Material and methods

### SERUM AND MILK SAMPLES

A total of 448 milk samplings were performed from cows located in 6 commercial dairy herds in which *Coxiella burnetii* had been identified using PCR on vaginal mucus of an aborted cow were sampled on two occasions. To minimize the risk of contamination during the collection process, teats were washed with clean water to remove dirt. Then, each teat end was scrubbed with teat wipes impregnated with ethanol and chlorhexidine digluconate. Lastly, milk was collected from the 4 teats after elimination of the first streams. The samples were collected in 40 mL sterile plastic tubes, containing a tablet of preservative (2 bromo-2-nitro-propane-1,3-diol). In each herd, all lactating cows entering the milking parlour on the day of the visit were eligible for sampling. Additionally blood samples were collected on the same occasion from each cow sampled by the same investigator. Blood was collected into sterile vacutainers® by caudal venipuncture. All the samples were stored at +4°C during transport and conveyed to the laboratory on the sampling day. All assays were performed blind, that is, laboratories had no connective information (identification number) between milk and blood samples.

### DETECTION OF ANTIBODIES ANTI-COXIELLA

Each sample was tested using the kit LSI Fièvre Q ruminants Serum®, (Laboratoire Service International, Lissieu, France). The ELISA antigen was recovered from a *Coxiella burnetii* strain isolated from an aborted sheep. A cocktail of both antigen phases (I and II) were used in this assay to detect total immunoglobulins G (Ig G) anti-*Coxiella burnetii*. Both types of sample were analysed according to the manufacturer's instructions. Briefly, the serum samples were diluted 1/400 and milk samples 1/20 for testing as indicated by the manufacturer. The negative and positive controls were always included while examining milk and serum samples. 5 uL of sample was necessary for both milk and serum sample. After a 60-min incubation period at 37°C for serum samples and overnight incubation period at 4°C for milk samples, the plates were washed with phosphate buffer saline before the

addition of horseradish peroxidase (HRP) conjugated antibody. The absorbance was read using an ELISA reader at a wavelength of 450 nm. The criterion for considering a sample as positive (milk and serum) was based on ELISA value calculated as follows:

$$\text{ELISA value} = 100 \times \frac{[\text{Absorbance of test sample} - \text{Absorbance value of negative control}]}{(\text{Absorbance value of positive control} - \text{Absorbance value of negative control})}$$

Samples with antibody titre equal or above 40 were considered positive.

### STRATEGY OF ANALYSIS

The level of agreement between results (positive vs negative) for serum and milk sample was determined by calculating the kappa statistic. The McNemar test was used to compare paired population proportions of positive results when applying the ELISA to milk or serum. ELISA values in paired milk and serum samples were compared and the Pearson correlation coefficient was calculated to test the existence of a linear relationship between them.

## Results

The ELISA values varied widely in both milk and serum samples (Table I). ELISA values appear to be higher in serum than in milk. A total of 264 and 257 samples were found positive in serum and milk respectively. Among the 264 positive samples in serum, 249 were positive and 15 negative in milk. Among the 184 negative samples in serum, 8 were positive and 176 were negative in milk (Table II). The level of agreement between results was very good (kappa=0.89). The p value of the McNemar test indicated that proportions of positive results (*i.e.* relative sensitivities) for milk and serum samples were not significantly different (p=0.21).

Overall, ELISA values in matched milk and serum were positively correlated ( $r^2=0.67$ ) (Table 3). Regarding discrepant responses, positive samples in milk (n=8) with matched negative serum had low ELISA values (<100); all positive samples in serum (n=15) with matched negative milk had antibody titres <200 (10 out of 15 with ELISA values <100) (Table III).

	Minimum	Quartile 1	Median	Quartile 3	Maximum
Milk	0	7.68	52.72	127.75	480.61
Serum	1	17.01	71.12	153.20	522.31

TABLE I. Distribution of ELISA values in milk and serum samples

Serum-ELISA	Milk-ELISA		Total
	Positive	Negative	
Positive	249	15	264
Negative	8	176	184
Total	257	181	448

TABLE II. Cross-classification of results of an ELISA applied to serum and milk for the detection of antibodies to *Coxiella burnetii*

Serum	Milk							Total
	<40	40-100	101-200	201-300	301-400	401-500	>500	
<40	176	8	0	0	0	0	0	184
40-100	10	46	20	2	0	0	0	78
101-200	5	48	46	15	8	3	0	125
201-300	0	9	18	10	1	2	0	40
301-400	0	1	7	3	2	1	0	14
401-500	0	0	5	1	0	0	0	6
>500	0	0	1	0	0	0	0	1
Total	191	112	97	31	11	6	0	448

TABLE III. Comparison of ELISA values in milk and serum samples using the ELISA test

## Discussion

The aim of this study was to compare ELISA performances applied to serum and milk for the detection of antibodies against *Coxiella burnetii* in dairy cattle. To our knowledge, such a study has never been reported before.

Sampling milk rather than blood has several merits. Milk collection is non-invasive (allowing farmer's participation), easy to perform, and inexpensive. However, blood samples are still needed for non-lactating cows (dry cows or treated cows with milk withdrawal), heifers, and males.

From this study, the ELISA tested had comparable performances when applied to milk and to serum in order to evidence the exposure to *Coxiella burnetii* in dairy cows. Such a finding was expected, immunoglobulins being selectively transferred from blood to milk in lactating cows. The estimated kappa value was very good and the McNemar tests suggest that the relative sensitivity of milk-ELISA was comparable to that of the serum-ELISA. Such an observation was already reported for *Brucella melitensis* (Chand et al., 2005).

Eight cows were considered positive only in milk, but they had low ELISA value (<100). In these cows, a local antibody synthesis against an antigenic stimulation of the mammary gland (e.g., reactivation of *Coxiella* milk shedding, penetration of *Coxiella* via the teat canals) cannot be excluded. In 15 cows having positive serum, ELISA value in milk was found below the positivity limit. This very low amount of antibodies in milk could be related to either (i) low levels of antibodies in sera (likely for 10 cows with ELISA value in serum < 100) or (ii) disturbance in antibodies transport from blood to milk (in relation to inflammatory processes in the mammary gland) (CHAND *et al.*, 2005). Existence of discrepancies might be also related to a non-optimal dilution rate of milk samples. Indeed, the immunoglobulin content in milk and serum samples was here not standardized. To circumvent this limit, a strategy could have consisted in using a quantitative ELISA or a radial immunodiffusion assay to determine the IgG content of samples and then adjust the milk dilution. However, testing different dilution rates of milk for the

initially discrepant samples did not improve the level of agreement (data not shown).

In conclusion, the present results clearly demonstrated milk-ELISA is comparable to serum-ELISA and could be a convenient alternative to evidence exposure to *Coxiella burnetii* in lactating dairy cows.

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