

Association between *Coxiella burnetii* seropositivity and abortion in dairy cattle of Northern Italy

Clotilde S. Cabassi, Simone Taddei, Gaetano Donofrio, Francesca Ghidini, Chiara Piancastelli, Cesidio F. Flammini, Sandro Cvirani

Università di Parma, Dipartimento di Salute Animale, Parma, Italy

SUMMARY

Coxiella burnetii, the agent of Q fever in humans, has been associated with abortion in cattle. In this study 650 sera from cattle with abortion and 600 randomly-selected control sera were examined for antibodies to *C. burnetii* by ELISA. Two hundred and ninety-two (44.9%) out of 650 animals which experienced abortion were seropositive versus 132 (22%) out of 600 of the control group. A statistically significant difference resulted from the comparison of the seroprevalence of aborted cattle with that of controls ($p < 0.001$). Moreover, a significant higher prevalence was disclosed in cattle which aborted during late gestation ($p < 0.002$) and in the autumn ($p < 0.001$).

KEY WORDS: *Coxiella burnetii*, abortion, bovine, serology, zoonosis, raw milk

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Coxiella burnetii, classified in the order *Legionellae*, family *Coxiellaceae*, is a strictly intracellular, gram-negative coccobacillus responsible for infection in man and animals (Babudieri, 1959; Maurin and Raoult, 1999). It has been isolated from a wide variety of tick species that play an important role in the transmission of infection among animals (Babudieri, 1959).

Humans are usually infected by contaminated aerosols from domestic animals, particularly after contact with parturient females and their birth products. Since mammals also shed *C. burnetii* in milk, consumption of raw milk could be a source of infection (Fishbein and Raoult, 1992). In cattle, *C. burnetii* is implicated in abortion, placentitis, infertility, and other reproductive dis-

orders (Bildfell *et al.*, 2000; Hassig and Lusben, 1998). In most cases, abortion occurs at the end of gestation without specific clinical signs (Tainturier, 1987). Stillbirth, lower birth-weight, and the delivery of weak offspring are the most common clinical manifestations in neonatal calves (Babudieri, 1959; To *et al.*, 1998).

Since specimens potentially infected with *C. burnetii* must be handled at least in biosafety level 3 laboratories (Fournier *et al.*, 1998), serological procedures are of primary importance for *C. burnetii* diagnosis. ELISA shows good sensitivity either in human or cattle sera (Peter *et al.*, 1987; Jasper *et al.*, 1994) and is considered a useful tool for seroepidemiological survey, also due to its low cost.

The present study aimed to evaluate the association between the prevalence of *C. burnetii* antibodies and bovine abortion in dairy cattle in three provinces of Northern Italy, namely Parma, Reggio Emilia and Modena. For this purpose 1250 serum samples were collected. Six hundred-fifty sera were from cows with abortion, and 600

Corresponding author

Prof. Sandro Cvirani

Università di Parma

Dipartimento di Salute Animale

Parma, Italy

E-mail: sandro.cvirani@unipr.it

sera from a control group of randomly-selected cows. The number of control sera ($n = 600$) exceeded the estimated minimum sample size ($n = 323$) as calculated from the formula $n = 4Z^2p(1-p)/L^2$, where $Z = 2.56$ (90% confidence interval), $L = 10\%$ (meaning that the length of the prevalence interval cannot be $>10\%$) and $p = 14.4\%$ (value of estimated seroprevalence in the area of concern) (Jenicek and Cl eroux, 1987). An overall value of seroprevalence recently reported for *C. burnetii* infection in cattle in Italy was considered for the p value (Capuano *et al.*, 2001). However, the number of control sera can be considered adequate for a seroprevalence value as higher as 35.5%, considering the seroprevalence values reported worldwide, which are higher than those reported for Italy (Houwens and Richardus, 1987; Htwe *et al.*, 1992; Rehacek *et al.*, 1993; Hatchette *et al.*, 2002).

A commercial indirect ELISA test (CHEKIT-Q-Fever, Bommeli Diagnostics, Bern, Switzerland) was used to determine the antibodies level to *C. burnetii* in serum samples.

Statistical analysis was carried out by chi-square test with Yates correction if appropriate. Differences were considered statistically significant for values of $p < 0.05$. Table 1 summarizes the results obtained by ELISA on serum samples from cattle which aborted and from a control group of randomly-selected cows. Two hundred and ninety-two (44.9%) out of 650 animals which experienced abortion were seropositive versus 132 (22%) out of 600 of the control group. The statistical analysis showed a significant difference ($\chi^2_{\text{Yates}} = 72.28$; $p < 0.001$) between the seropositivity of aborted cattle and the control animals.

Table 1 summarizes the seropositivity results in aborted cattle also with regard to quarter of gestation and season. Data indicate a statistically significant difference of seropositivity to *C. burnetii* comparing the different quarters of gestation ($\chi^2 = 36.93$; $p < 0.001$). In particular, no significant difference in seropositivity was demonstrated between cattle experiencing abortion during the first quarter of gestation and those that aborted during the second quarter ($\chi^2_{\text{Yates}} = 0.984$; $p > 0.1$). However, the comparison of combined results regarding the first two quarters of gestation with the third quarter showed a higher prevalence of antibodies to *C. burnetii* in cattle during the advanced period of gestation ($\chi^2_{\text{Yates}} = 35.6$; $p < 0.002$). Moreover, a significant difference appeared comparing seropositivity rate in cattle which aborted during autumn and during the other seasons ($\chi^2_{\text{Yates}} = 104.34$; $p < 0.001$).

Seropositivity for *Coxiella burnetii* in cattle has been described worldwide. Seroprevalence rates are reported to be up to 40% in Germany, 82% in the USA, 39% in the Netherlands, 46% in Japan, 24% in Canada (Houwens and Richardus, 1987; Htwe *et al.*, 1992; Rehacek *et al.*, 1993; Hatchette *et al.*, 2002). In Italy, seroepidemiological surveys indicate a seroprevalence of 8.5% in the province of Brescia (Massirio *et al.*, 1992) and 4.4% in an Apenninic area of the Emilia-Romagna Region (Martini *et al.*, 1994). A recent study reported an overall seroprevalence of 14.4% in cattle reared in Southern Italy (Capuano *et al.*, 2001).

An association between seropositivity to *C. burnetii* and reproductive disorders has been reported in France (Durand and Strohl, 1978), Netherland (Houwens and Richardus, 1987),

TABLE 1 - Seropositivity to *C. burnetii* in aborted cattle and in randomly-selected control cows, and distribution of abortion cases in relationship to time of gestation and season.

| | Control | | | Abortion | | | |
|-------------------------|---------------------------|----------------------------|-----------------------------|--------------------|-------------------|--------------------|-------------------|
| | Time of gestation | | | Season of abortion | | | |
| | I ^o Quarter | II ^o Quarter | III ^o Quarter | Spring | Summer | Autumn | Winter |
| Examined/Pos. (%) | 200/67 (33.5%) | 220/85 (38.6%) | 230/140 (60.8%) | 120/46 (38.3%) | 250/76 (30.4%) | 165/131 (79.4%) | 115/39 (33.9%) |
| Total Examined/Pos. (%) | 600/132 (22.0%) | | | 650/292 (44.9%) | | | |

Hungary (Rady *et al.*, 1985), Germany (Reusch *et al.*, 1984) and Japan (To *et al.*, 1998).

A previous survey in Italy indicated antibody response to *C. burnetii* in dairy cows with infertility (Caracappa *et al.*, 1988) or abortion (Morganti *et al.*, 1987), but no association between seropositivity and reproductive disorders has been demonstrated.

Conversely, the data obtained by this study pointed out a statistically significant association between seropositivity towards *C. burnetii* and abortion in dairy cattle reared in some provinces of Northern Italy. The distribution of seropositivity in cows with abortion, in the area of concern, shows a peak in the autumn season. This could be linked to the higher presence of ticks during the warm season in the considered area. Moreover, we found a significantly higher prevalence of seropositivity to *C. burnetii* in cattle which aborted in the late stage of gestation. This trend was shown by Tainturier (1987) but in that report no association between *C. burnetii* seropositivity and the time of abortion was demonstrated. Taken together, our data might indicate the autumn season and the late stage of gestation as factor risks for abortion by *C. burnetii* in the considered cattle population.

Due to the association between *C. burnetii* seropositivity and abortion in dairy cattle reared in Northern Italy, serological investigations for *C. burnetii* could be useful in the course of reproductive failures occurring in the considered area. In addition, seropositivity to *C. burnetii* in dairy cattle reared in Northern Italy has to be considered as a risk factor for Q fever in humans, particularly in farmers or people used to drinking raw non pasteurized milk. This is a real risk since in the Emilia Romagna Region farmers have been authorized to sell raw milk directly to the public (D.P.R. 54/97, Emilia Romagna Regional Circular no. 17, 5th October 2005).

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