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IFAT and ELISA phase I/phase II as tools for the identification of Q fever chronic milk shedders in cattle

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ABSTRACT

Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*. In cattle the bacterial shedding can persist without symptoms for several months and the shedders identification is a critical issue in the control of the infection at herd level. Following the example of the human protocols for the assessment of Q fever infection status, the aim of this study was the evaluation of the antibody response dynamics to phase I and phase II antigens in *C. burnetii* shedder dairy cows by means of a phase-specific serology, to verify the suitability of the investigated tools in recognising milk shedders. A total of 99 cows were monitored during time and classified on the basis of serological and PCR results in five groups identifying different shedding patterns. The 297 sera collected in three sampling times were tested by means of ELISA IgG for differential phase I and phase II antibodies detection, while a selection of 107 sera were tested by means of phase specific IgM and IgG IFAT. Both ELISA IgG and IFAT IgG highlighted a low reactivity in non-shedder seropositive animals compared to chronic milk shedder animals. ELISA IgG seemed to perform better than IFAT IgG–IgM, showing significant serological differences among groups that allowed recognising specific serological group patterns, in particular for chronic and occasional milk shedders. These results supported the hypothesis that an animal classification based on phase patterns is reasonable, although it needs to be further investigated.

1. Introduction

Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacterium with a wide range of hosts. In domestic ruminants, which represent the major source of human infection, the disease is frequently subclinical, but late abortions, stillbirths and reproductive disorders can occur (Arricau-Bouvery and Rodolakis, 2005). Shedding of bacterium into the environment mainly occurs during parturition or abortion, but infected animals can also shed bacteria in milk, urine, faeces and vaginal mucus (Rodolakis, 2009). The shedding can last for variable time depending on species and excretion route. Infected cows can persistently shed bacteria in milk for several months without symptoms, while sporadic or intermittent shedding can occur in faeces

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or vaginal mucus (Guatteo et al., 2007); the faecal and vaginal excretion has been debated especially in goats as possibly due to environmental contamination (Roest et al., 2012).

Phase variation, similar to the smooth to rough transition of Enterobacteriaceae, is a significant characteristic of *C. burnetii*. Phase I is the virulent form that can be isolated from acutely infected animals, while phase II is the avirulent one that appears after several passages in cell culture (Raoult and Parola, 2007). The phase transition induces a detectable immunological response that in human medicine allows the differentiation between acute and chronic statuses: high titres of IgG to phase II antigens and lower titres of IgG to phase I antigens are associated to an acute stage of infection, while in chronic Q fever, the IgG titres to phase I and phase II antigens may both be high (Angelakis and Raoult, 2010). Phase II IgM increases before phase II IgG and allows the identification of the early stage of infection.

In veterinary medicine the commercially available immunological assays do not allow individual identification of animals that shed *C. burnetii*, although the identification of the stage of infection associated to the excretion patterns is a critical issue for the control of Q fever at herd level, considering that the presence of chronic shedders is reported in ruminants with or without clinical signs (Guatteo et al., 2007). The EFSA Opinion in 2010 (Sidi-Boumedine et al., 2010) underlined the need of a better awareness concerning the pathogenesis of Q fever in domestic animals, the infection kinetics and shedding patterns and emphasised the need for an improved diagnosis based on phase I and phase II antibodies detection.

The aim of this study was to evaluate in dairy cattle the antibodies response dynamics to phase I and phase II antigens in chronic and occasional milk shedders of *C. burnetii*, using a commercial experimental kit ELISA IgG for the differential detection of IgG anti-*Coxiella* phase I and phase II and an IFAT IgG–IgM kit targeted to the human diagnosis, experimentally modified for the detection of anti-*Coxiella* phase I and phase II antibodies in cattle.

2. Materials and methods

2.1. Background

The samples used in the study originated from animals farmed in four herds with confirmed Q fever cases. The herds were selected among those participating in the Veneto Regional Programme for surveillance and diagnosis of abortions, for which the laboratory analyses are routinely performed at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy.

The Regional Programme includes direct diagnoses on placenta and/or aborted foetus (BVDV, SBV, *Chlamydia* spp., *C. burnetii*, *Neospora caninum*, broad-spectrum microbiological tests) and serological tests on the aborting cow (IBRV, BVDV, *Neospora caninum, Brucella abortus/melitensis*, *Chlamydia* sp., *C. burnetii*). The Veneto region is free from Bluetongue and Brucellosis.

2.2. Herds selection and description

The four herds were selected according to the following criteria:

- At least one *C. burnetii* positive PCR result on an aborted foetus during the last 12 months;
- Herd size with an average of at least 100 cows milked;
- No other confirmed causes of abortion;
- No vaccination against *C. burnetii*.

These criteria were established because in the herds with PCR-positive abortions there is a high probability to detect shedder cows in milk, and milk excretion of *C. burnetii* could last up to 32 months (Angelakis and Raoult, 2010). Furthermore a herd size of at least 100 lactating cows allows obtaining an acceptable number of milk shedder cows, considering that the percentage of milk shedders is estimated around 14% (Guatteo et al., 2007).

- Herd 1: 215 cattle; breed Brown Swiss, an average of 107 milking cows, freestalls housing and a tandem milking parlour. During the last year, three cases of abortion with *C. burnetii* positive PCR were found in the farm. Poor fertility performances were reported.
- Herd 2: 200 cattle; breed Holstein, an average of 100 milking cows, freestalls housing and a herringbone milking parlour. During the last year, two cases of abortion with *C. burnetii* positive PCR were found in the farm. No specific problems on fertility were reported.
- Herd 3: 230 cattle; breed Holstein, an average of 100 milking cows, freestalls housing and a herringbone milking parlour. During the last year, one case of abortion with *C. burnetii* positive PCR was found in the farm. Poor fertility performances were reported.
- Herd 4: 606 cattle; breed Holstein, an average of 294 milking cows, freestalls housing and a herringbone milking parlour. During the last year, two cases of abortion with *C. burnetii* positive PCR were found in the farm. Poor fertility performances were reported.

2.3. Herds monitoring during time

At the first sampling (S0) all the lactating cows were screened on individual milk by means of real time PCR and on sera for total IgG anti-*Coxiella* by means of a commercial ELISA in order to assess their initial status concerning *C. burnetii* infection. The animals were then classified into four groups:

- Positive to PCR in milk, seropositive (ELISA+_PCR+);
- Positive to PCR in milk, seronegative (ELISA−_PCR+);
- Negative to PCR in milk, seropositive (ELISA+_PCR−);
- Negative to PCR in milk, seronegative (ELISA−_PCR−).

To follow the evolution of the shedding patterns in the groups during time, an average of 10 animals for each group were initially selected in each herd and monitored by collecting individual milk and blood samples every 2 months for three further times (S1, S2, S3). Some groups, such as ELISA−_PCR+ and ELISA+_PCR+, were scarcely represented, so a number <10 has been accepted.