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Serological screening for *Coxiella burnetii* infection and related reproductive performance in high producing dairy cows

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ABSTRACT

The possible relationship between *Coxiella*-seropositivity and the reproductive performance of cows during the previous year to the serological screening was examined in three high producing dairy herds. The herds had a history of subfertility (<25% of pregnancies for the total number of AI), abortion (>18% abortions) and a positive polymerase chain reaction test for *Coxiella burnetii* in the bulk tank milk with an excretion higher than 10^4 *Coxiella* /ml for all three herds. Antibodies against *C. burnetii* were detected in 50.2% of the 781 parous cows analyzed. *Coxiella* seropositivity was linked to placenta retention, to changes in the interval from parturition to conception (with the lowest interval parturition-conception for cows with low level of seropositivity), early pregnancy (cows becoming pregnant before Day 90 postpartum) and maintenance of gestation during the early fetal period, while it failed to affect rates of abortion after Day 90 of gestation or stillbirth.

1. Introduction

Coxiella burnetii is an obligate intracellular Gram-negative bacterium that causes Q fever, a ubiquitous zoonosis that can produce acute or chronic disease in humans (Maurin and Raoult, 1999; Woldehiwet, 2004; Arricau-Bouvery and Rodolakis, 2005; Carcopino et al., 2009). While acute disease is mainly a flu-like syndrome, chronic disease has been linked to chronic fatigue syndrome and endocarditis. Q fever is also associated with premature delivery or abortion in pregnant woman (Carcopino et al., 2009). Humans become infected mainly by inhalation of contaminated aerosols or dusts containing the *C. burnetii* spore-like form shed by infected animals. Although many wild and domestic mammals, birds and arthropods can act as reservoirs for the bacterium, the main sources for human infection are cattle, goat and sheep (Maurin and Raoult, 1999; Woldehiwet, 2004; Arricau-Bouvery and Rodolakis, 2005; Carcopino et al., 2009).

In dairy cattle, the bacteria are shed via birth fluids, placenta, vaginal mucus, milk, feces, urine and semen, although the extent of infection is probably underestimated since infected animals often show no symptoms of infection (Guatteo et al., 2007; Rodolakis, 2009). Relationships between infection and reproductive disorders such as abortion, stillbirth, weak offspring, postpartum metritis and infertility have been suggested (To et al., 1998; Hassig and Lubsen, 1998; Bildfell et al., 2000; Barlow et al., 2008). In contrast, other study series were not able to link *C. burnetii* seroprevalence with any reproductive disorder (Rodolakis, 2009; Literák and Kroupa, 1998; Masala et al., 2004). There is worldwide evidence suggesting a decline in the fertility of dairy cattle along with increased milk production over the past decades (Lucy, 2001; Royal et al., 2000; Butler, 2003; López-Gatius, 2003). Therefore, the objective of the present study was to examine the possible relation between factors such as age, stillbirth and a retained placenta on *Coxiella*-seroprevalence in subfertile and aborting high producing dairy herds. In addition, since the intracellular coccidian parasite *Neospora caninum* causes abortion worldwide in dairy cattle (Anderson et al., 2000; Dubey, 2003) and thus affects the success of gestation, we also explored a possible interaction between the two diseases.

2. Materials and methods

2.1. Cattle and herd management

The data examined were generated during a reproductive control program conducted at the University of Lleida on three well-managed, high producing, Holstein–Friesian dairy herds in

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northeastern Spain. A retrospective study based on serological screening for *C. burnetii* infection was conducted. Reproductive performance was evaluated for the period of June 2008 to May 2009, from 0 to 365 days before serological analysis (June 2009). The three herds comprised 811 parous cows (615, 100 and 96 respectively) and were selected based on a recent history (last 12 months) of low fertility and high incidence of abortions. During the study period, the mean pregnancy rate was (percentage of pregnancies for the total number of AI) 22.7% (25%, 23% and 20% for each herd, respectively) and the rate of abortions was 20.8% following a positive pregnancy diagnosis. Two further herds (herd 4 and 5) comprising 1101 and 1270 cows, respectively, were used as controls. During the study period, the control herds had a mean rate of pregnancy of 38% and abortion of 11%. In previous studies on factors affecting the fertility including all five herds (three under examination and two controls), the herds had a similar pregnancy rate (García-Ispierto et al., 2007) and abortion rate (López-Gatius et al., 2004a).

Herd management included housing in free stalls with cubicles and with concrete slatted floors, fans and water sprinklers used in the warm season, rigorous postpartum checks and the same reproductive health program for the three herds. The cows calved all year round and were milked three times (Herd 1) or twice (Herds 2 and 3) daily. Mean annual milk production for the study period was 11,825 kg (Herd 1), 10,204 kg (Herd 2) and 10371 kg (Herd 3), with an overall culling rate of 27%, 31% and 29%, respectively. Feeds consisted of cottonseed hulls, barley, corn, soybean, and bran, and roughage, primarily corn, barley or alfalfa silage and alfalfa hay. Rations were in line with NRC recommendations (National Research Council, 2001). Dry cows were kept in a separate group and transferred, depending on their body condition score and age, 7–25 days before parturition to a "parturition group". An early postpartum, or "fresh cow" group was established for postpartum nutrition and controls, and 7–20 days postpartum primiparous and multiparous lactating cows were transferred to separate groups. All cows were artificially inseminated using semen from bulls of proven fertility. The voluntary waiting period for the herds was 45 days for multiparous cows and 60 days for primiparous cows.

All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2009. Also determined in these checks, the mean *Neospora* seroprevalence in the herds was 10% for the study period. Sera were tested for antibodies against *N. caninum* using a commercial enzyme-linked immunosorbent assay (ELISA) kit (CIVTEST® anti-*Neospora*, HIPRA, Girona, Spain), based on the whole tachyzoite lysate of *Neospora* NC-1. This test, previously evaluated by the present authors (López-Gatius et al., 2004a) was performed according to the manufacturer's instructions and an antibody titre of $\geqslant 6.0$ units taken to denote seropositivity.

Vaccination programs were undertaken for the prevention of bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR). Modified live vaccines were used for animals 6–8 months old. Pregnant animals were given killed vaccines during the 7th month of each gestation period. Parous cows that were not pregnant on Day 150 post partum received a further killed vaccine. Concerning BVD control measurements beside vaccination, immunotolerant persistently infected animals for BVD (BVD-IPIs) could not be detected during the study period. The BVD control measurements included testing for the presence of antibodies to BVD in non-vaccinated calves from 6 to 8 months old (before vaccination) and yearly tests by polymerase chain reaction (PCR) in the bulk tank milk to screen for evidence of carriers with persistent BVD infection.

Only 781 parous cows reaching 90 days in milk during the study period were included in the study. The 30 remaining cows either had more than 90 days in milk at starting the study period or did not reach 90 days at the end of the period and were excluded of the study. Of the 30 excluded cows, 9 were *Coxiella*-seropositive.

2.2. Reproductive health management

Postpartum checks (daily) involved the treatment of the following puerperal diseases until resolved or until culling: signs of injury to the genital area (i.e., vaginal or recto-vulvar lacerations), metabolic diseases such as hypocalcemia and ketosis (the latter, diagnosed during the first or second week post partum), retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis (acute puerperal metritis diagnosed during the first or second week post partum in cows not suffering placenta retention).

The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by ultrasound from 30 to 36 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as pyometra or ovarian cysts were treated until resolved. Detectable intrauterine purulent fluid in the presence of a corpus luteum was interpreted as pyometra. An ovarian cyst was diagnosed when a follicular structure larger than 20 mm in diameter was detected in either one or both ovaries in the absence of a corpus luteum. Luteal cysts were not registered.

Cows with a retained placenta or primary metritis were always treated using oxytetracycline boluses introduced in the uterus. Prostaglandin F2α (Enzaprost, CEVA Salud Animal, Barcelona, Spain) was administered at the end of treatment for a retained placenta and primary metritis, and was also the luteolytic agent used to treat pyometra and ovarian cysts. In the latter case, treatment was subsequent to manual rupture of the cystic structure per rectum (Hanzen et al., 2008; López-Gatius et al., 2008). All postpartum reproductive disorders were resolved before 60 days in milk.

Cows 60 days in milk and not detected to be in estrus in the preceding 21 days (approximately 40% of cows during the study period) were examined weekly by ultrasound until estrus following specific treatment or until AI was performed during a natural estrus (López-Gatius et al., 2008).

2.3. Insemination and pregnancy diagnosis

Estrus was detected using a pedometer system (Herd 1) or by visually detecting estrous signs (Herds 2 and 3). Estrus was confirmed in cows detected in estrus by palpation per rectum, and the cows were inseminated at this time (López-Gatius and Camón-Urgel, 1991; López-Gatius, 2000). Only cows showing estrous signs with strong uterine contractility (determined by uterine tone) and copious, transparent vaginal fluid were inseminated. More than 90% of inseminations were performed by veterinarians. If cows returned to estrus, their status was confirmed by examination per rectum, and the animals were recorded as non-pregnant. In the remaining cows, pregnancy diagnosis was performed by ultrasound 28–34 days post-insemination. Pregnancy was confirmed by palpation per rectum 90–96, and 180–186 days post-insemination. Cows diagnosed as not pregnant were either returned to the reproductive program or registered for culling.

2.4. Types of pregnancy loss

Since management- and cow related factors of a non-infectious nature have been described to affect late embryonic and early fetal loss in our geographical area (López-Gatius et al., 2009; López-Gatius and Garcia-Ispierto, 2009), early fetal loss was recorded when the 90–96 day-diagnosis proved negative. Late fetal loss was re-