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Bovine abortion caused by *Coxiella burnetii*: report of a cluster of cases in Uruguay and review of the literature

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Abstract. A cluster of 4 bovine abortions caused by *Coxiella burnetii* occurred in a dairy herd in Uruguay during a 2-mo period. Case 1 consisted of a placenta from an aborted cow; cases 2–4 were fetuses and their placentas. Grossly, the placenta from one aborted cow had moderate, diffuse reddening of the cotyledons and loss of translucency of the intercotyledonary areas. No gross lesions were observed in the other 3 placentas. Microscopically, 2 of 4 placentas had fibrinonecrotizing placentitis with abundant intratrophoblastic gram-negative coccobacilli. *C. burnetii* was identified intralesionally by immunohistochemistry (IHC) in all 4 placentas, and by PCR and DNA sequencing in 3 placentas analyzed by these techniques. One fetus had mild neutrophilic alveolitis with multinucleate syncytial cells; no gross or microscopic lesions were observed in the other 2 fetuses examined. The lungs of the 3 fetuses were negative for *C. burnetii* by IHC. Tests performed to investigate other possible causes of abortions in the 4 cases were negative. *C. burnetii* causes Q fever in humans and coxiellosis in animals. Clusters of abortions in cattle by *C. burnetii* have not been reported previously, to our knowledge; this bacterium has been considered an opportunistic pathogen associated only with sporadic abortion in cattle. We present herein a cluster of 4 bovine abortions caused by *C. burnetii* in a dairy farm during a period of 2 mo and a review of the literature on *C. burnetii* infection in cattle.

Key words: bovine abortion; *Coxiella burnetii*; coxiellosis; Q fever; zoonosis.

Coxiella burnetii is a gram-negative, intracellular proteobacterium of worldwide distribution that causes query (Q) fever in humans and coxiellosis in animals.[1,2,25] Infection by *C. burnetii* has been reported in a variety of mammals, including but not limited to, cattle, sheep, goats, buffaloes, pigs, horses, pacific harbor seals, and rodents.[28,41] Several avian and invertebrate species can also be infected.[6] Q fever is a zoonotic disease; people who work with animals or who drink raw milk are most at risk.[32] In humans, *C. burnetii* infection can produce hepatitis, pneumonia, endocarditis, and abortions, mainly in immunocompromised individuals,[45] but a large proportion of human infections are either subclinical or undiagnosed because clinical signs are usually nonspecific.[44] In cattle, coxiellosis is mainly subclinical, but anorexia, stillbirth, and late-term abortion have been described.[2] Herein we present a cluster of 4 bovine abortions caused by *C. burnetii* in a dairy cattle farm during a period of 2 mo, and review the literature on *C. burnetii* infection in cattle.

The 4 cases studied (cases 1–4) originated in a pasture-based, *Brucella abortus*–free, dairy cattle farm in the department of Colonia, Uruguay, between April and June 2017. Case 1 consisted of a placenta from an aborted American Holstein biotype cow; cases 2–4 included fetuses and the placentas of 2 American Holstein biotype cows (cases 2 and 4) and 1 New Zealand biotype cow (case 3). The affected dairy herd was composed of 356 Holstein cows, including 279 of the American biotype and 77 of the New Zealand biotype. The latter had been introduced to the herd between March and May 2017 from 3 different commercial dairy farms in Uruguay. The gestational age of the 3 fetuses examined was estimated to be 240–270 d based on crown-rump length; presence or absence of hair on lips, eyebrows, and muzzle; eruption of incisor teeth; and presence or absence of horn pits. In case 1, the abortion occurred in the third trimester of gestation, although the fetus was not available to estimate the gestational age more

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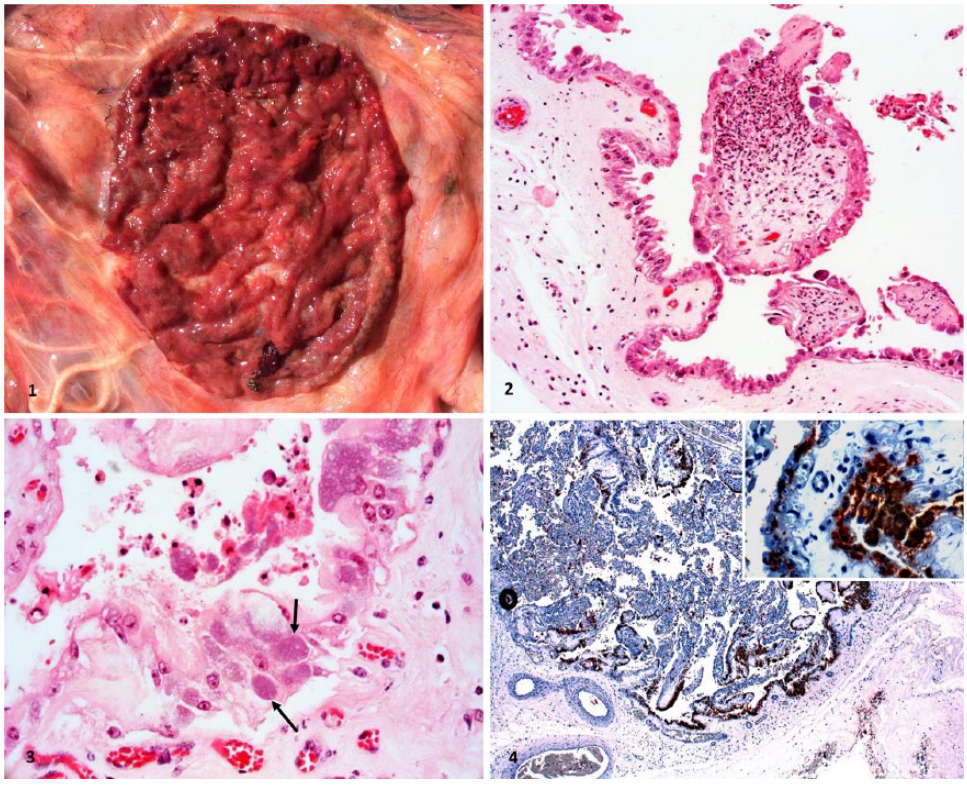

Figures 1–4. Placentas from aborted cows infected with *Coxiella burnetii*. **Figure 1**. The cotyledon in case 1 has moderate and diffuse reddening, and the peripheral intercotyledonary area exhibits loss of translucency. **Figure 2**. The stroma of the chorionic villus in this cotyledon in case 4 is expanded by abundant neutrophilic infiltrate and necrotic debris. H&E. **Figure 3**. Clusters of basophilic bacteria in case 4 expand the cytoplasm of numerous trophoblasts (arrows), some of which have a pyknotic nucleus displaced to the periphery of the cell and are detached from the basement membrane. There is also cell debris in the lumen. H&E. **Figure 4**. Diffuse intralesional immunoreactivity for *C. burnetii* in the chorionic villi epithelium in case 4. Inset: higher magnification showing strong granular intracytoplasmic immunoreactivity in numerous trophoblasts. Immunohistochemistry for *C. burnetii*, hematoxylin counterstain.

precisely. Gross examination of the placentas and autopsies of the fetuses were performed.

All fetuses and placentas were in a mild state of postmortem decomposition. Grossly, the placenta from case 1 had moderate, diffuse reddening of the cotyledons and loss of translucency of the intercotyledonary areas (Fig. 1). No gross lesions were observed in the other placentas or any of the fetuses. Samples of placentas and fetal tissues, including lung, liver, kidney, brain, spleen, thymus, lymph nodes, heart, tongue, skeletal muscle, and gastrointestinal tract, were fixed in 10% neutral-buffered formalin (pH 7) for 48 h, dehydrated through graded alcohols to xylene, embedded in paraffin, cut at 4–6 µm, and stained with hematoxylin and eosin. Selected sections were also stained with Gram stain.

Microscopically, the 4 placentas had fibrinonecrotizing placentitis characterized by transmural infiltration of the chorioallantois with abundant viable and degenerate neutrophils, fewer lymphocytes, plasma cells, and macrophages, necrotic debris, and fibrin. Many trophoblasts were necrotic. In 2 of the placentas, myriad ~1-µm long basophilic coccobacilli were present multifocally in the cytoplasm of trophoblasts (Figs. 2, 3). Diffuse, transmural edema was also observed in the intercotyledonary area. The fetus in case 3 had mild neutrophilic alveolitis with multinucleate syncytial cells within the alveolar spaces. No other significant microscopic lesions were observed in other tissues of any of the other fetuses examined or in the placentas.

Selected sections of the 4 placentas and lungs from the 3 fetuses were processed by immunohistochemistry (IHC) for *C. burnetii* and *Chlamydia* spp. as described previously.[18,21] Briefly, antigen retrieval was performed in a decloaking chamber, following quenching the endogenous peroxidase with 3% hydrogen peroxide. Mouse monoclonal antibodies (AB-COX-MAB; U.S. Department of Defense, Critical Reagents Program; anti-*Chlamydia* lipopolysaccharide antibody; Virostat, Westbrook, ME), were used for *Coxiella* spp. and *Chlamydia* spp. IHC.[18,21] Then, the anti-species horseradish peroxidase (HRP)-labeled polymer (Biocare, Pacheco, CA) and a detection system, with 3-amino-9-ethylcarbazole (Thermo Scientific, Fremont, CA) as the chromogen substrate solution, were applied. Samples of caprine placentas

that were PCR-positive or -negative for these microorganisms were used as positive and negative controls, respectively.

Sections of the 4 placentas and brains from the 3 fetuses were processed by *Neospora caninum* IHC as described previously.[15] Briefly, a goat polyclonal antibody against *N. caninum* (VMRD, Pullman, WA) was used as a primary antibody, followed by anti-goat IgG HRP-labeled polymer (Enzyme polymer detection kit; Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbazole (Dako, Santa Clara, CA). Brain from a calf that had been experimentally inoculated with *N. caninum* was used as a positive control; brain from a healthy calf with no brain lesions was used as a negative control.

IHC for *C. burnetii* revealed abundant intralesional antigen in the 4 placentas (Fig. 4.) The immunoreaction was mainly within the trophoblasts of the chorionic villi, seen as granular intracytoplasmic staining. The sections of lung of the 3 fetuses were negative for this test. *Chlamydia* spp. and *N. caninum* IHC were negative in all of the tested samples.

DNA from 3 formalin-fixed, paraffin-embedded placentas (cases 1, 3, 4) was extracted using a commercial kit (QIAamp DNA FFPE tissue kit; Qiagen, Hilden, Germany), concentrated (DNA clean & concentrator kit; Zymo Research, Irvine, CA) following the manufacturer's instructions, and used as template for PCR. The PCR for *C. burnetii* was performed as described previously[29] in 25-µL final volume reactions, using a master mix (Green master mix; Promega, Madison, WI), 800 nM of each primer (forward: TATG TATCCACCGTAGCCAGTC; reverse: CCCAACAA CACCTCCTTATTC), and 5 µL of template. An initial denaturation period of 5 min at 95°C was done, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 1 min. This ended with a final extension step at 72°C for 3 min. The PCR products were visualized on 2% agarose gel (UltraPure; Invitrogen, Carlsbad, CA). The reactions were purified (Invitrogen quick gel extraction and PCR purification combo kit; Thermo Fisher, Waltham, MA). The gel extraction products were sent for Sanger sequencing (Genewiz, South Plainfield, NJ), and sequences were also aligned by a kit (SeqMan Pro 13, Lasergene 13; DNASTAR, Madison, WI). *C. burnetii* DNA was amplified by PCR, and the amplicon sequenced in cases 1, 3, and 4.

Placenta, liver, lung, and abomasal fluid were inoculated onto blood and MacConkey agar and incubated at 37°C under aerobic conditions. The same specimens were also inoculated into Skirrow medium and incubated at 37°C in an atmosphere of 5–10% carbon dioxide, for 4 d.[12] Direct immunofluorescence for *Campylobacter* spp. was done on impression smears from placenta, abomasal fluid, lung, and liver, fixed in acetone at room temperature, and incubated with an anti-*Campylobacter* conjugate.[20] Placenta, liver, kidney, and abomasal fluid were also inoculated into *Leptospira* medium base EMJH agar and incubated in aerobiosis at 29°C for up to 6 mo.[48] The abomasal fluid was examined under dark-field microscopy for *Campylobacter* spp. and *Tritrichomonas foetus*. No aerobic bacterial pathogens or *Leptospira* spp. were isolated, and no *Campylobacter* spp. or *T. foetus* were observed in any of the samples.

PCR tests for bovine parainfluenza virus 3 (BPIV-3; species *Bovine respirovirus 3*) and bovine viral diarrhea virus 1 (BVDV-1; species *Pestivirus A*) were performed on pools of liver, lung, spleen, kidney, and heart of the 3 fetuses, and the 4 placentas, as described previously.[30,33] PCR tests for BPIV-3 and BVDV-1 were negative.

A commercial indirect ELISA (IDEXX Laboratories, Westbrook, ME) for IgG antibodies against *C. burnetii* was performed on serum from the dams of the 4 aborted fetuses. This test was positive on serum from the dam of case 4 but negative for the other 3 dams. Sera from the 4 aborted dams and pericardial fluid from the 3 fetuses were analyzed for *Leptospira* serovars Grippotyphosa, Icterohaemorrhagiae, Pomona, Canicola, Hardjo-bovis, Hardjo-prajitno, and Wolfii using the microagglutination test (MAT).[48] The MAT was negative at 1:10 for fetal samples and negative at ≥200 for the dams.[48]

C. burnetii can be transmitted to the fetus either hematogenously, affecting mainly the liver, or transplacentally, causing pneumonia and enteritis following the ingestion of contaminated amniotic fluid.[2,11] Among post-natal animals, C. *burnetii* is transmitted by inhalation of aerosols from contaminated fluids and tissues shed by parturient or post-parturient cows.[17,24] Infected dams shed large amounts of bacteria in the feces, milk, and fetal membranes, regardless of the infection status of the birthed calf.[2] The placenta is the most frequent source of infection because the bacterial load can be as high as 10^9 cells/g of tissue, and the infective dose can be as low as 1 cell.[25] It has been postulated that *C. burnetii* can also be transmitted by ticks.[19]

Two microscopic forms of *C. burnetii* are recognized based on their pathogenicity (i.e., large-cell variant and small-cell variant). The large-cell variant is the vegetative form in infected cells. The small-cell variant is the extracellular form, which is shed in milk, urine, feces, placenta, and amniotic fluid.[45] The small-cell variant is resistant to high temperature and desiccation, characteristics that confer to this form of *C. burnetii* the capacity for airborne transmission and to survive in the environment for years.[32]

Although there seems to be little doubt about the role of *C. burnetii* in bovine abortion, the role that this microorganism may have in other reproductive disorders of cattle, such as infertility, premature delivery, endometritis, metritis, and mastitis is controversial.[1,16] *C. burnetii* DNA has frequently been detected in cases of endocarditis in cattle at slaughter[3]; however, the clinical significance of this finding remains undetermined. *C. burnetii* DNA and its antigen have also been detected in endometrial biopsies of cows with repeat breeding failure.[16] Although these findings suggest an association between *C. burnetii* and a reproductive disorder in

cattle, the findings have not been compared to those of healthy cows, and final conclusions can therefore not be drawn.[16]

Differences in the clinical presentations of coxiellosis in cattle could be the result of differences in bacterial genotype. Analysis of the genetic diversity of *C. burnetii* from different sources (bulk-tank milk and surface dust) by multi-locus variable number tandem repeats analysis showed high genotypic diversity.[38] However, the presence in cattle of genotypes closely related to those identified in humans does not seem to be common.[38]

C. burnetii has been considered an opportunistic pathogen associated with sporadic abortion in cattle.[1,4,8,41] An outbreak of coxiellosis in cattle in southeast Poland affected 220 dairy cows and at least 1,300 people.[7] Although there were no bovine abortions reported in this particular outbreak, seroconversion was reported in cows that had stillborn calves in later outbreaks in this region.[27,43] This was the largest outbreak of coxiellosis in humans linked to cattle. In The Netherlands, there was also another significant epidemic of Q fever in people, but it was linked to dairy goats and dairy sheep.[40]

In cattle, shedding of *C. burnetii* is higher during the peripartum period and can then decrease to barely detectable levels.[22] *C. burnetii* can infect and remain latent in the mammary gland and lymph nodes, but shedding of the bacteria can occur during subsequent calving seasons and lactation.[32]

The diagnosis of *C. burnetii* abortion in cattle is confirmed by detection of the agent in association with placental lesions, coupled with ruling out other causes of bovine abortion.[1,2] However, other factors should be considered, such as type and severity of the lesions in the placenta and abundance of *C. burnetii*. The latter is an important consideration to establish the role of *C. burnetii* in cases of bovine abortion because this microorganism can be found in tissues of clinically healthy cattle.[1] In a prospective study of ovine and caprine abortion using real-time PCR,[23] the authors concluded that the use of a single positive PCR result used for diagnosis of *C. burnetii* abortion can be misleading because the mere presence of nucleic acids of this agent does not confirm the disease. That study[23] suggested that determination of the amount of *C. burnetii* DNA in tissues provides additional value to real-time PCR results, given that increased copies of DNA correlated with the presence of lesions and the results of other tests for *C. burnetii.* Unfortunately, quantitative PCR was not available during the diagnostic workup of our 4 cases.

Gross examination of the placenta of aborted cattle coupled with histopathology[46] and IHC are key for the diagnosis, given that placentitis is the primary lesion caused by *C. burnetii*, with a strong statistical association between intralesional detection of the agent by IHC and placentitis.[8] In laboratory submissions that include the fetus but not the placenta, diagnosis of *C. burnetii* abortion is challenging because fetal lesions are usually absent or scarce.[8] When present, lesions are mainly restricted to the lungs.[8] Nevertheless, fetal pneumonia is a nonspecific lesion that frequently accompanies infectious placentitis of a variety of causes. Even though molecular testing is adequate for rapid identification of *C. burnetii* in tissues and body fluids, the sole detection of this agent is of no diagnostic significance, given that *C. burnetii* can be carried and excreted by healthy cattle.[1,2]

Two of the aborted fetuses examined in our study had no gross or microscopic lesions; the third fetus (case 3) had only mild neutrophilic alveolitis with multinucleate syncytial cells. However, *C. burnetii* antigen was not detected by IHC in association with these lesions, and their etiologic role remains, therefore, undetermined. In our 4 cases, the presumptive diagnosis of *C. burnetii* infection was based on the histologic lesions observed in the placenta and confirmed by IHC and PCR. These results are similar to another report of lesions in placentas but not in fetuses.[2]

Caprine placental specimens were used as positive and negative controls for *C. burnetii* IHC. Although bovine control tissues would have been preferred, the IHC for *C. burnetii* in our laboratory has been validated with caprine specimens. Histologic lesions compatible with coxiellosis should be complemented by pathogen detection analysis to improve the possibility of arriving at an etiologic diagnosis of bovine abortion. In a study from California of 709 aborted bovine fetuses, *C. burnetii* was identified as the causal agent in only 1 (0.14%).[14] Similarly, an 11-y study from Canada revealed that only 10 (1.4%) of 722 dairy cattle abortions were attributable to *C. burnetii*, and concluded that the agent was infrequently associated with bovine abortion.[8] Furthermore, in several case series from Argentina,[10] Brazil,[5] Uruguay (Easton C. Pathologic study of the main infectious causes of bovine abortion in Uruguay [Master's thesis]. Montevideo, Uruguay: Universidad de la República, 2006) and the United States,[4,26] *C. burnetii* was not reported as a cause of bovine abortion. This could be because of a low frequency of infection coupled with the difficulty of reaching an etiologic diagnosis of *C. burnetii* abortion.[14]

Several studies have confirmed the presence of antibodies against *C. burnetii* in human and animal populations in South America, indicating that the organism is present on the continent.[13,35] One study from Brazil established the presence of the agent in a human clinical case associated with PCR-positive animals.[31] In Uruguay, the presence of antibodies against *C. burnetii* in slaughterhouse workers has been associated with a history of clinical signs, and a clinical case of endocarditis,[35] showing that this agent is an occupational hazard for slaughterhouse personnel.

Healthy cows can carry and excrete *C. burnetii*,[2] suggesting that in the cluster of cases presented here, carrier healthy cows might have been the source of infection. In this farm, before the onset of the disease, a herd of 77 Holstein cows of the New Zealand biotype had been introduced into the group. Although it was not possible to conclude that this cattle

movement was responsible for the introduction of *C. burnetii*, reports have mentioned that dairy cow herds that contain purchased cows from abroad have a 2.68 greater chance of *C. burnetii* infection than closed herds.[9] Nevertheless, the potential role of wildlife, other domestic animals such as dogs and cats, or people, in the transmission of the infection in our cases cannot be ruled out.

C. burnetii has been transmitted experimentally by several species of ticks, including *Rhipicephalus* spp., *Ornithodoros* spp., and *Ixodes* spp.[19,37] Even though these 3 genera of ticks are present in Uruguay,[36] they were not present in the dairy herd in our study, which is in an official tick-free zone.[34]

Q fever should be considered a potential hazard for dairy farm workers. In our cluster and following Uruguayan Public Health Department guidelines, 27 farm and laboratory workers were examined for *C. burnetii* by an indirect fluorescent antibody test,[47] and 10 (37%) of them had IgG phase II and IgM phase II antibody titers against *C. burnetii* (data not shown). It is speculated that these individuals had contact with the aborted animals and fetal tissues. However, conclusions about the sources of the human antibody response are difficult to make because the test used does not allow determination of the duration of infection, and the previous serologic status of these patients was unknown.[39,42]

C. burnetii can cause clusters of abortions in cattle. Tests for the identification of this agent should be included in the routine bovine abortion test panel. The placenta is essential for the diagnosis of *C. burnetii* abortion.

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