### Title

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VETERINARY MICROBIOLOGY - REVIEW

# *Coxiella burnetii* abortion in a dairy farm selling artisanal cheese directly to consumers and review of Q fever as a bovine abortifacient in South America and a human milk-borne disease

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## Abstract

*Coxiella burnetii* is a highly transmissible intracellular bacterium with a low infective dose that causes Q fever (coxiellosis), a notifiable zoonotic disease distributed worldwide. Livestock are the main source of *C. burnetii* transmission to humans, which occurs mostly through the aerogenous route. Although *C. burnetii* is a major abortifacient in small ruminants, it is less frequently diagnosed in aborting cattle. We report a case of *C. burnetii* abortion in a lactating Holstein cow from a dairy farm producing and selling artisanal cheese directly to consumers in Uruguay, and review the literature on coxiellosis as a bovine abortifacient in South America and as a milk-borne disease. The aborted cow had severe necrotizing placentitis with abundant intratrophoblastic and intralesional *C. burnetii* confirmed by immunohistochemistry and PCR. After primo-infection in cattle, *C. burnetii* remains latent in the lymph nodes and mammary glands, with milk being a significant and persistent excretion route. Viable *C. burnetii* has been found in unpasteurized milk and cheeses after several months of maturing. The risk of coxiellosis after the consumption of unpasteurized dairy products, including cheese, is not negligible. This report raises awareness on bovine coxiellosis as a potential food safety problem in on-farm raw cheese manufacturing and sales. The scant publications on abortive coxiellosis in cattle in South America suggest that the condition has probably gone underreported in all countries of this subcontinent except for Uruguay. Therefore, we also discuss the diagnostic criteria for laboratory-based confirmation of *C. burnetii* abortion in ruminants as a guideline for veterinary diagnosticians.

**Keywords** Abortion · Dairy production · Food safety · Milk-borne disease · Q fever · Zoonosis

## Introduction

*Coxiella burnetii* is a highly infectious, gram-negative, obligate intracellular bacterium that causes Q fever (coxiellosis), a zoonosis described worldwide, deemed as re-emerging or emerging in various countries [1], and listed as a notifiable disease by the World Organization for Animal Health (OIE) [2]. Q fever has been regarded as one of the ten most important zoonotic diseases in terms of impact on human health and livestock production, and concern because of emergence or severity in developing countries [3]. Several vertebrate and invertebrate species can host *C. burnetii*; however, domestic ruminants are the major source of human infection [4, 5]. Reproductive losses, particularly abortion, are significant clinical consequences of coxiellosis in goats and sheep, although *C. burnetii* abortion has been infrequently confirmed in cattle [6–8], in which the infection is often subclinical [9]. In addition to abortion, clinical signs in

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ruminants may include premature delivery, stillbirth, and weak offspring [9], all of which result in economic losses to the livestock sector.

Q fever is mostly an occupational disease; workers in direct or indirect contact with ruminants are at increased risk of infection [10]. It is frequently either subclinical or clinically characterized by nonspecific symptoms, this being the reason why it is commonly undiagnosed [11]. However, *C. burnetii* can cause severe illness and abortion in people; the former is especially true in patients with immunodeficiencies or cardiopathies [12]. While Q fever has long been recognized in humans in most South American countries largely by serologic evaluation [13–18], the epidemiology, sources of infection, and eventual animal reservoirs involved in most cases remain largely unknown. Free-living and captive wildlife species [14, 19], ticks, ruminants [16, 17], and companion animals [20] have been suspected to play a role in transmission.

*Coxiella burnetii* is mainly transmitted aerogenously and has an extremely low infective dose by this route [21]. It can also be persistently shed in bovine milk and survive in unpasteurized dairy products [22, 23], which raises concerns about the possibility of foodborne transmission. Despite initial findings, when neither clinical Q fever nor antibodies were detected after the deliberate human consumption of unpasteurised milk contaminated with *C. burnetii* [24], the oral route of transmission has been confirmed experimentally in mice [25]. However, discrepancies remain among different research groups about the relevance of *C. burnetii* digestive transmission under non-experimental conditions.

Here, we report a case of bovine abortion caused by *C. burnetii* in a dairy farm in Uruguay that elaborated artisanal cheese which was directly sold to consumers. This prompted us to review the literature on coxiellosis as a cause of bovine abortion in South America and as a milk-borne disease for humans. Considering the few available publications on *C. burnetii* abortion in cattle in South America, we propose that the condition has gone undiagnosed or underreported in most countries of this subcontinent. Therefore, we also discuss the diagnostic criteria for laboratory-based etiologic confirmation of abortive coxiellosis, which could prove valuable as a general guideline for veterinary diagnosticians.

## History and diagnostic investigation to identify *C. burnetii* abortion in the affected farm

In November of 2017, a lactating dairy cow from a herd of ~ 100 Holstein cows located in San José, Uruguay, had a spontaneous abortion in the second trimester of gestation. The herd's milk was used on-farm for artisanal cheese manufacturing, and the cheese was regularly sold directly to consumers.

The aborted fetal tissues and placenta were submitted to the veterinary diagnostic laboratory of INIA for diagnostic workup. Samples of the placenta and tissues, including heart, trachea, esophagus, tongue, eyelid and conjunctiva, lymph nodes, intestines, forestomachs, kidney, liver, brain, synovial joint capsule, and skeletal muscle, were examined macroscopically and no lesions were observed. All samples were immersion-fixed in 10% buffered formalin, routinely processed, and stained with hematoxylin and eosin for histopathology. Microscopically, the chorion had severe diffuse neutrophilic and histiocytic placentitis with multifocal mineralization and necrosis of trophoblasts, as well as neutrophilic arteriolitis. Occasionally, the trophoblasts and infiltrating macrophages were swollen, rounded, and contained myriad intracytoplasmic, basophilic, ~ 1-µm-long coccobacilli (Fig. 1a); similar bacteria were found intralesionally in extracellular locations. No protozoa or fungi were identified in the chorion. The allantois showed lesions comparable to those described in the chorion, except for those involving the trophoblasts. No microscopic lesions or pathogens were found in any of the examined fetal tissues.

Based on the placental lesions, the intratrophoblastic bacteria were strongly suspected as the causative agents. Thus, serial sections of placenta were processed by immunohistochemistry for the detection of *Chlamydia* spp. and *C. burnetii* antigens, as previously described [8, 26], using placenta from two goats naturally infected by *Chlamydia* spp. and *C. burnetii*, respectively, as positive controls. Sections of placenta of the aborted cow, in which the primary antiserum was replaced by non-immune serum, were used as negative controls. The immunohistochemistry for *C. burnetii* showed strong positive immunoreaction, revealing abundant intralesional antigen, both in the cytoplasm of the trophoblasts and macrophages, and extracellularly (Fig. 1b), in the allantois and chorion. *Chlamydia* spp. immunohistochemistry was negative and so were the negative control sections.

For molecular confirmation, DNA was extracted from the placenta using a commercial kit (MagMAX Pathogen RNA/DNA kit, Life Technologies), and later used as a template for *C. burnetii* and *Chlamydia abortus* duplex PCR, based on the repetitive transposon-like region (*IS1111*) and *pmp 90/91* gene, following a previously described protocol [27]. The assay targeted two specific 687-bp and 821-bp long fragments for *C. burnetii* and *C. abortus,* respectively. The PCR was done in 25 µL final volume reactions, with a concentration of 0.8 µM of each primer (Trans-1: 5′-TATGTATCCACCGTAGCCAGT-3′, Trans-2: 5′-CCCAACAACACCTCCTTATTC-3′; pmpF: 5′-CTCACCATTGTCTCAGGTGGA-3′, pmpR821: 5′-ACCGTAATGGGTAGGAGGGGT-3′), 1.5 U of Taq polymerase (New England Biolabs®, Ispwich, MA), 1 × PCR buffer (New England Biolabs®,

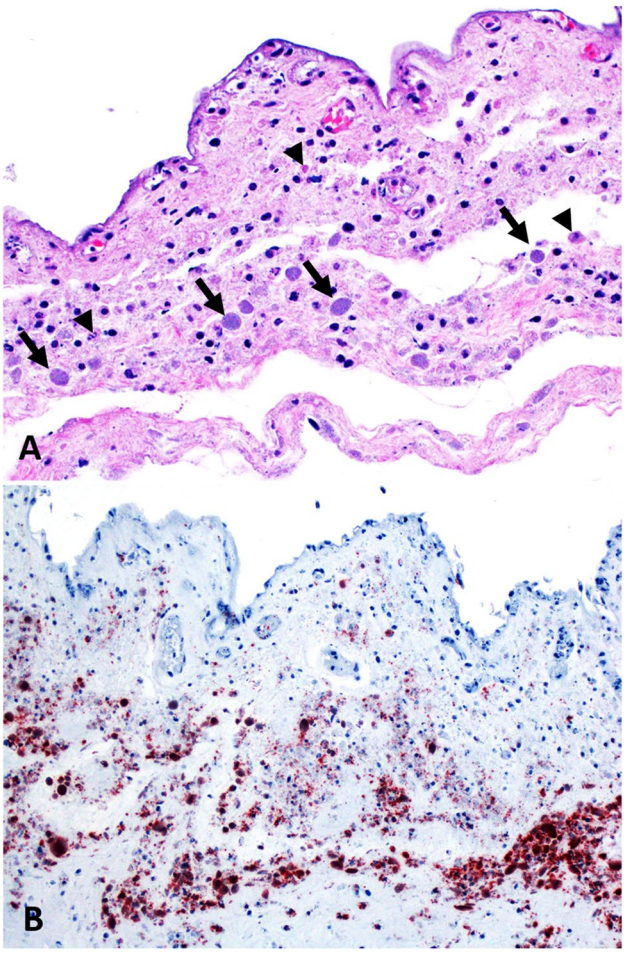


**Fig. 1** Microscopic lesions in the placenta of the aborted Holstein cow. **a** The intercotyledonary chorionic stroma is infiltrated by neutrophils and macrophages that contain myriads of intracytoplasmic basophilic coccobacilli (arrows); pyknotic and karyorrhectic hypereosinophilic cellular debris (arrowheads) are indicative of necrosis. H&E. **b** In a serial section of **a**, the bacteria are strongly immunoreactive with *C. burnetii* antiserum, which is depicted as intracytoplasmic and extracellular granular brown chromogen deposition. Immunohistochemistry for *C. burnetii*, hematoxylin counterstain

Ispwich, MA), 3 mM of $MgCl_2$, 0.2 mM of dNTPs, and 2 µL of template. The PCR was run in a ProFlex™ PCR System (Applied Biosystems, Foster City, CA). The *C. burnetii* Nine Mile phase II strain and *C. abortus* reference strain S26/3 were used as positive controls. Ultrapure water was used as negative control. The PCR products were visualized by electrophoresis in 1.2% agarose gel stained with Good View® dye using a Bio-Rad GelDoc EZ imager (Bio-Rad Laboratories GmbH-Munich, Germany). Amplification revealed *C. burnetii* DNA in the placenta, with negative results for *C. abortus*.

To investigate other possible causes of abortion, fetal liver and placenta were routinely cultured at 37 °C for 7 days aerobically on MacConkey and blood agars, as well as microaerobically on Skirrow agar (Oxoid, Basingstoke, Hampshire, England) using sealed jars and commercial sachets (CampyGenTM, Oxoid, Basingstoke, Hampshire, England), for the simultaneous detection of *Campylobacter* spp. and *Brucella* spp. [28]. Kidney and liver were inoculated into Ellinghausen-McCullough-Johnson-Harris medium for *Leptospira* spp. culture [29]. No bacterial pathogens were isolated by these methods.

Additionally, *Campylobacter fetus* and *Leptospira* spp. were investigated by direct immunofluorescence assays on acetone-fixed impression smears of liver and placenta (*C. fetus*), and kidney and liver (*Leptospira* spp.), using pure cultures of these bacteria as positive controls. Samples were incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-*C. fetus* antibody (Biotandil, Tandil, Buenos Aires, Argentina), and with a polyclonal rabbit FITC-conjugated antibody (LEP-FAC, NVSL, Ames, IA, USA) for *Leptospira* spp., and examined using a fluorescence microscope (AxioLab.A1, Carl-Zeiss, Germany). The placenta was also examined under dark-field microscopy to assess for trichomonads, spirochetes, or curved bacilli with darting motility. Lastly, the placenta was cultured on a medium for *Tritrichomonas foetus* (CM0161, Oxoid, Basingstoke, UK) supplemented with 1% chloramphenicol and inactivated bovine serum. *Leptospira* spp., *C. fetus*, and *T. foetus* were not detected by these methods.

In summary, we found placentitis with intratrophoblastic bacteria that were reactive with *C. burnetii* immunohistochemistry and identified *C. burnetii* DNA by PCR, while other abortifacients were not detected. Altogether, the results of the diagnostic investigation supported an etiologic diagnosis of *C. burnetii* placentitis and abortion.

## Diagnostic criteria and challenges of laboratory-based diagnosis of *C. burnetii* bovine abortion

The examination of the placenta is the keystone in the diagnostic investigation of *C. burnetii* abortion [6, 8, 9]; thus, it is critical that the placenta is submitted to the laboratory when attempting to investigate coxiellosis. Obtaining placenta of aborted cattle under field conditions suitable for laboratory investigation, i.e., before significant autolysis and post-mortem contamination occur, is challenging, particularly in extensive pasture-based production systems such as those prevalent in South America. In fact, most submissions to veterinary diagnostic laboratories include the aborted fetuses, but the placenta is much less frequently included [30–33], reducing the chances of reaching an etiologic diagnosis.

Placental lesions caused by *C. burnetii* can be severe enough to be appreciated grossly as intercotyledonary and

cotyledonary placentitis, although in some cases the infection can induce subtle macroscopic placental alterations, while in others the placenta may look unremarkable [6, 8, 34]. Because *C. burnetii* targets mainly the placenta with high tropism toward trophoblasts, the histologic examination of this tissue is critical and perhaps the single most informative laboratory investigation. *Coxiella burnetii* colonization frequently induces a neutrophilic or mixed inflammatory reaction and necrotizing placentitis, which along with the visualization of abundant intracytoplasmic coccobacilli within distended trophoblasts, guide toward the diagnosis of coxiellosis [6, 8, 9]. Because *C. burnetii* does not usually cause lesions in the fetal tissues, even when severe placentitis is present, the examination of the fetal tissues is usually unrewarding [8]. Although fetal pneumonia has been described as an accompanying lesion in a few confirmed cases of *C. burnetii* abortion in cattle [6, 8], this is a non-specific lesion that can be caused by many bacterial, fungal, or protozoal infections, such as *T. foetus* [35]. As an association between lesions and the presence of the bacterium has been regarded as mandatory to confirm *C. burnetii* abortion in cattle [9], and lesions are mostly restricted to the placenta, laboratory submissions not including the placenta should be considered unsuitable for the assessment of *C. burnetii* abortion.

Once a histologic diagnosis of necrotizing placentitis with intratrophoblastic bacteria has been established, the identification of *C. burnetii* is the next step in the diagnostic investigation. This can be achieved through PCR-based tests, immunohistochemistry [6, 8], fluorescent in situ hybridization (FISH) [36], or combinations thereof. Besides *C. burnetii*, other intracellular bacteria that can cause placentitis and invade the trophoblasts including *C. abortus* and *Brucella abortus* [35] should be considered as differential diagnoses. In the case described here, abundant *C. burnetii* antigen was detected intralesionally by immunohistochemistry, and the presence of the agent was further confirmed by PCR, while *C. abortus* and *B. abortus* were ruled out by specific testing (immunohistochemistry and PCR for *C. abortus*, and selective culture for *B. abortus*). Thus, the identification of typical placental lesions in conjunction with the detection of *C. burnetii*, along with the exclusion of other abortifacients that can cause similar placental lesions, fulfilled the diagnostic criteria for etiologic confirmation of *C. burnetii* placentitis (Fig. 2) [9].

When attempting to identify *C. burnetii* infection and abortion either through direct or indirect laboratory methods, the use of single laboratory tests may be misleading. The mere detection of *C. burnetii* DNA in the placenta or fetal tissues does not necessarily imply disease causality,

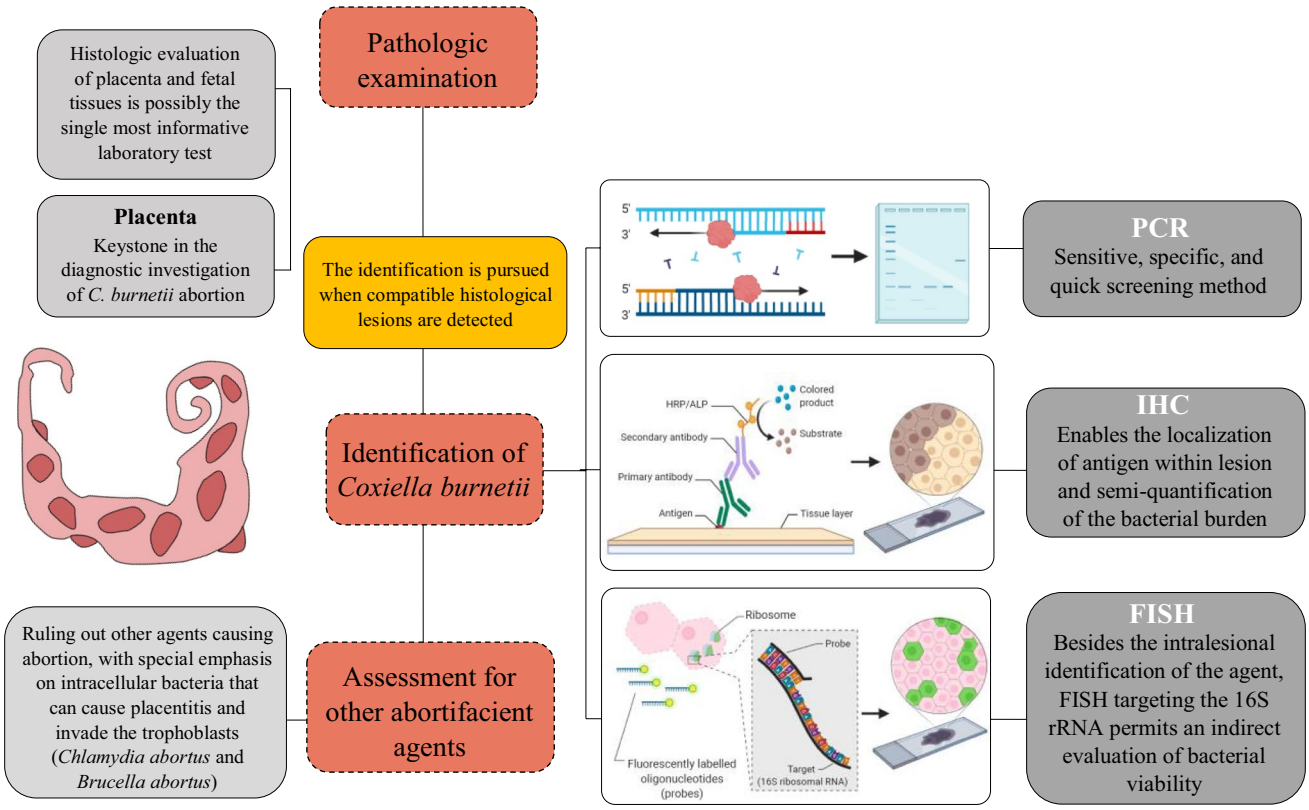


**Fig. 2** Diagnostic workflow for laboratory-based confirmation of abortion caused by *Coxiella burnetii* in ruminants. PCR, polymerase chain reaction; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization

considering that subclinical infections are common [9] and the high molecular prevalence in dairy herds [37]. Similarly, serologic approaches at the individual level are not informative enough, as seroconversion can occur without detectable lesions or bacterial shedding, animals can remain seropositive long after they have overcome the infection, shed *C. burnetii* before the development of detectable antibodies, and even shed the agent without ever seroconverting [38]. Attempting the isolation of *C. burnetii* poses an unnecessary risk and requires level III biosecurity laboratories. Both PCR and immunohistochemistry are valuable tools for *C. burnetii* detection in diagnostic settings [6, 8]. PCR-based assays are sensitive, specific, and quick screening methods used in a wide variety of samples. Quantitative PCR targeting the *IS1111* gene has been used to quantify the bacterial load in placenta of aborted cattle [34, 39]. Immunohistochemistry enables the colocalization of *C. burnetii* antigen within lesioned tissues, which is a powerful indicator of causality [6]. Interestingly, in the case described here, although the chorion showed strong positive immunoreactivity by immunohistochemistry, the signal was even stronger in the allantois, which represents an unusual localization of bacterial antigen. FISH targeting the 16S ribosomal RNA of *C. burnetii* has been used experimentally for the intralesional identification of the agent in formalin-fixed paraffin-embedded placenta of aborted cattle, obtaining results comparable to those of immunohistochemistry [36], although this technique has not been broadly adopted in diagnostic settings. FISH targeting the 16S rRNA is a promising marker for intact and metabolically active bacterial cells, representing an alternative to evaluate *C. burnetii* viability when bacterial isolation or inoculation in experimental animals are not available options [40, 41]. The lack of veterinary diagnostic laboratories offering histology and validated immunohistochemical, FISH, and PCR-based tests for the identification of *C. burnetii* placentitis is a major limitation for the diagnosis of coxiellosis in cattle and other ruminants in South America.

## *Coxiella burnetii* as a bovine abortifacient in South America

Scientific publications providing confirmatory evidence of spontaneous bovine abortions caused by *C. burnetii* are scarce not only in South America, where confirmed cases have only been reported in Uruguay [8, 42], but also globally [6, 7, 34]. *Coxiella burnetii* has been generally linked to sporadic abortion in cows, exhibiting infection rates that resemble those of opportunistic bacteria [6, 7, 9]. A recent study from Uruguay reported a cluster of four cases of abortion due to *C. burnetii* in Holstein cows in one dairy farm, based on gross and microscopic examination of the placentas, coupled with the identification of the agent by immunohistochemistry and PCR [8]. These four cases occurred between April and June of 2017; a fifth case was confirmed in August of the same year in the same farm [42]. This indicates that *C. burnetii* abortion in cattle can occur in clusters affecting several animals in a herd, as is usually the case in small ruminants. *Coxiella burnetii* was not identified as a cause of abortion in various case series aiming at assessing abortion causality in beef and dairy cattle in Argentina [30, 31], Brazil [32, 33], Uruguay [43], and Chile [44]. Collectively these studies analyzed 2080 aborted bovine fetuses, although none of them specifically tested for *C. burnetii* and only a minor subset of submissions included placentas; thus, the pathogen and disease may have been easily overlooked.

Other studies from South America aimed at investigating *C. burnetii* infection in aborted cattle. A retrospective survey conducted in Brazil, where pools of organs, gastric content, and brain from aborted bovine fetuses and stillborn calves were analyzed by PCR for the identification of *C. burnetii* DNA, found an infection rate of 10.7% (3/28) [45]. Whether these cases were examined histologically to assess for lesions of coxiellosis, as would have been required for attributing causality, was not reported.

In Ecuador, a case–control serologic study assessed the role of *C. burnetii* as a cause of bovine abortion in two large-scale dairy herds, each with approximately 2000 cows and abortion rates of 3–5%. Sera of 172 cows were screened for anti-*C. burnetii* antibodies using a commercial ELISA. The overall seroprevalence was high (52.9%), but no association with abortion was established as the seroprevalence was higher in the 77 non-aborted (57.1%) than in the 95 aborted (49.5%) cows [46].

The lack of scientific reports on bovine abortions caused by *C. burnetii* in other South American countries in which the agent is known to be present suggests that the disease may have gone undetected or underreported. This might in part reflect the difficulties and challenges associated with *C. burnetii* diagnostic confirmation and the limited availability of appropriate laboratory assays in veterinary laboratories in the region. However, the significance of *C. burnetii* as an abortifacient of cattle in the region should not be underestimated.

## Q fever as a milk- and dairy-borne human disease and risk of Q fever through consumption of dairy products

The inhalation of contaminated aerosols, following normal parturition or abortion of domestic ruminants, is the major path of *C. burnetii* infection in people [9, 47]. However, after the initial infection in cattle, the bacterium remains

latent in the lymph nodes and mammary glands, and bacterial shedding (presumably within macrophages) can occur in subsequent calving seasons and lactations, with milk shedding being a significant and persistent excretion route of *C. burnetii* [22]. As *C. burnetii* is an obligate intracellular bacterium, it is assumed that bacterial replication does not occur in milk and dairy products [48]; however, the agent is highly resistant to chemical and physical stressors [49], and can remain viable for long periods in the environment and in bovine milk at room temperature [50]. Studies that quantified *C. burnetii* shedding by qPCR have been conducted in individual milk samples of goats and cattle, although this molecular approach cannot distinguish between viable and non-viable bacteria. Goat samples presented concentrations in the range of $1 \times 10^2$ to $1 \times 10^6$ *C. burnetii* cells per ml when targeting the single copy gene *com1* [51], whereas cow samples showed similar concentrations varying from $1 \times 10^1$ to $1 \times 10^4$ *C. burnetii* cells per ml when targeting the *IS1111* [52, 53]. Differences on the *C. burnetii* load in milk among studies may suggest a heterogeneous bacterial shedding by this route. Further evaluation investigated the mean level of viable *C. burnetii* per ml of unpasteurized milk in shedding cows. This was estimated using the guinea pig (GP) intraperitoneal (IP) infectious dose (ID) 50% per ml (GP IP $ID_{50}$/ml), which is the dose intraperitoneally administered to all members of a group of GP that results in 50% of them being infected. This mean level was approximately 98.8 GP IP $ID_{50}$/ml; each GP IP $ID_{50}$ presumably representing between 2 and 112 bacteria per ml of milk [22, 48, 54]. Simulations based on these data suggest that the daily exposure to viable *C. burnetii* through unpasteurized milk in people can be high [48], although given the lack of dose–response data in humans, it is unknown whether this translates into a risk of infection through the oral route, which would have implications in food safety and public health.

Early studies conducted in people ingesting bovine milk naturally contaminated with undetermined concentrations of viable *C. burnetii* suggested that subjects exposed through the oral route did not develop clinical signs of Q fever [24, 55]. Results on post-ingestion serology were variable, while in one study all 34 exposed individuals remained seronegative [24], in another 35% (42 of 120) turned seropositive and 10% (12/120) showed a fourfold or greater increase in antibody titers (seroconversion) [55]. These different serologic outcomes were speculated to result from differences in the *C. burnetii* strains involved in the studies, although it should be considered that the load of viable bacteria may have also differed between studies. A recent experimental study in immunocompetent BALB/c mice (considered of intermediate sensitivity to *C. burnetii*) demonstrated that after gastric inoculation of $1 \times 10^6$ genome equivalents of *C. burnetii*, the agent can colonize and persist in the digestive tract, penetrate the intestinal barrier, colonize the mesenteric lymph nodes, and invade the blood and peripheral tissues including the liver and lungs [25]. More data are needed to understand the consequences of ingesting viable *C. burnetii* in people considering the infective doses and bacterial strains.

The risk of Q fever transmission through consumption of dairy products has been reviewed fairly recently [48]. While considered much lower than the risk of airborne transmission, the risk of oral transmission after the ingestion of contaminated raw milk or unpasteurized dairy products, including cheese, was regarded as not negligible [48]. Serological evaluations conducted in France linked the consumption of contaminated unpasteurized milk with seroconversion in people [56]. A serologic survey of a cohort of goat farmers, workers, and their contacts, involved in an outbreak of Q fever in the Canadian province of Newfoundland, identified the consumption of cheese made with pasteurized goat milk as a significant independent risk factor for infection [57]. Likewise, a 2-year epidemiological evaluation conducted in 1200 hospitalized children in Greece found that eating raw cheese coming from rural areas enhanced the risk of Q fever ($p = 0.04$, OR = 6, 95% CI = 1.1–33.2) [58]. Clusters of Q fever cases in which the ingestion of unpasteurized bovine milk was considered the most likely source of infection have been reported in the UK and USA [59, 60].

Numerous investigations revealed *C. burnetii* DNA in milk and derived products, including cheese, cream, butter, and yoghurt from cows, goats, and sheep [23, 61–63]. A molecular investigation performed on the most traditional and oldest type of raw-milk cheese in Brazil, known as Minas artisanal cheese and manufactured with bovine milk, revealed a high prevalence of *C. burnetii* in this ready-to-eat product, and estimated that 1.62 tons of cheese produced daily is contaminated with this bacterium [63]. *Coxiella burnetii* has been isolated from unpasteurized bovine milk [24, 55], including milk commercialized in the USA [64]. Molecular studies suggest that the *C. burnetii* genotypes predominating in dairy products are the same that infect dairy cattle [65]. However, only a few studies took a step further toward the investigation of its viability and hazard. Viable *C. burnetii* was proven in raw cheese by culture in Vero cells and inoculation in mice [23]. The potential inactivating effect of cheese ripening was dismissed as viable *C. burnetii* was detected in samples of unpasteurized hard cheeses after 8 months of maturing [23]. There is little evidence that any of the processes used to produce butter or cream with unpasteurized milk would significantly inactivate the pathogen [48].

*Coxiella burnetii* in milk is successfully inactivated by pasteurization, which is fundamental for the prevention of milk-borne infectious illnesses, some of which, such as tuberculosis and brucellosis, are endemic in dairy cattle

in South America. The oral route of infection and eventual foodborne transmission of *C. burnetii* should not be neglected and farmers, particularly those producing artisanal cheese on-farm instead of selling the milk to the dairy industry, as well as consumers, ought to be aware of the importance of pasteurization. In herd-level studies, *C. burnetii* was screened for by real-time quantitative PCR in bulk tank milk of 105 bovine dairy herds as part of the epidemiologic investigation of an outbreak of Q fever among dairy farm workers in Chile in 2017. Although only two farms tested positive, both sold milk directly to the local community that was consumed either raw or boiled, which was considered a potential source of infection to humans [66]. In 2017 in Brazil, *C. burnetii* DNA was found by the same technique in 4 of 112 samples of raw bovine milk that were being sold illegally for human consumption without official inspection at grocery stores, bars, farmers' markets, and small farms, which was identified as of public health concern [67]. In 2012, a random sampling conducted in Montería, Colombia, showed that 5 of 11 bulk tank milk samples collected from commercial cattle farms presented *C. burnetii* DNA, and 37 out of 61 (60.7%) apparently healthy farm workers at risk had specific IgG phase II antibody titers ≥ 1/64, suggestive of recent bacterial exposure [68].

In Uruguay, raw milk trade was first regulated in 1984, and its commercialization for direct consumption by humans is currently banned; however, the consumption of raw milk and milk products in rural areas is difficult to quantify, and therefore, to control. Of the nearly 18000 tons of cheese consumed yearly in the country, ~ 50% represents artisanal cheese produced in ~ 1000 dairy farms, most of which are in the departments of San José and Colonia. Artisanal cheese is largely commercialized internally directly to consumers at the manufacturing farms or farmers' markets, or at larger scales through intermediaries, but international contraband of Uruguayan artisanal cheese has also been documented [69]. It has been estimated that up to 50% of artisanal cheesemakers produce under informal conditions, implying that they do not necessarily comply with regulations established by the Uruguayan Ministry of Livestock, Agriculture, and Fisheries [69]. A survey conducted among local artisanal cheesemakers revealed that only a minority use pasteurized milk [69]. This practice may embody a hazard for Q fever transmission to consumers, considering the high stability of *C. burnetii* in final dairy products even with acidic pH or reduced water activity [23]. Due to its indigenous microbiota, the cheeses made with unpasteurized milk have specific organoleptic characteristics of gastronomic value, such as a strong flavor and a peculiar texture, much appreciated by consumers [23, 70]. The consumers' preferences toward raw milk products are emerging as a growing global trend, which could be of public health concern as this implies a higher risk of acquiring milk-borne diseases.

## Conclusions

Our investigation expands the evidence supporting *C. burnetii* as a significant cause of bovine abortion in Uruguay and represents the first report of *C. burnetii* abortion in a dairy farm producing and selling artisanal cheese directly to consumers. The scant scientific literature on *C. burnetii* abortion in cattle from South America suggests that this notifiable and zoonotic disease may have gone undetected or underreported in most countries of this subcontinent. Laboratory investigations for the etiologic confirmation of *C. burnetii* abortion should rely on the observation of typical placental lesions in aborting dams, coupled with the identification of *C. burnetii* by immunohistochemistry, FISH, and/or PCR. The existing literature supports that raw milk and derived dairy products represent potential sources of *C. burnetii* transmission to humans, although further investigations are needed to assess the risk of digestive transmission to humans considering exposure, infective doses, and bacterial strains. The threat to public health posed by *C. burnetii* through dairy products should not be neglected, and the need for on-farm milk pasteurization by artisanal cheesemakers should be emphasized. Further epidemiologic investigations are needed to better understand the role of *C. burnetii* as a cause of abortion in cattle in South America, and the risk and impact of Q fever transmission through the ingestion of unpasteurized dairy products in the region.

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**Author contribution** Ana Rabaza conceptualized the study, conducted laboratory work, wrote the initial manuscript draft, reviewed, edited, and approved the manuscript. Melissa Macías-Rioseco conducted laboratory work, reviewed, edited, and approved the manuscript. Martín Fraga conducted laboratory work, reviewed, edited, and approved the manuscript. Francisco A. Uzal conducted laboratory work, reviewed, edited, and approved the manuscript. Mark C. Eisler reviewed, edited, and approved the manuscript. Franklin Riet-Correa acquired funding, reviewed, edited, and approved the manuscript. Federico Giannitti conceptualized the study, conducted laboratory work, acquired funding, wrote, reviewed, edited, and approved the manuscript.

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**Data availability** Data and material are available (without disclosing the farm information) from the corresponding author on reasonable request.

**Code availability** Not applicable

## Declarations

**Ethics approval** The study was not conducted on live animals; therefore, no ethical approval is required.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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