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Longitudinal Monitoring of Coxiella burnetii shedding in Bulk Tank Milk and Environmental Dust Following Vaccination of Three Naturally Infected Dairy Goat Herds

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ABSTRACT

Q fever episodes on three dairy goat operations (A–C) were followed after animals received an inactivated Coxiella burnetii phase I vaccination. Prior to immunization, two phase-specific ELISAs were used on serum samples to determine the existing infection status. Shedding dynamics were assessed through vaginal swabs collected across three consecutive kidding seasons and through monthly bulk tank milk (BTM) testing. In addition, monthly dust swabs were taken from a single windowsill in each barn as well as from the milking areas to evaluate indoor contamination; all samples were examined via qPCR. Phase-oriented serology indicated that herd A was undergoing an acute infection, whereas herds B and C showed, respectively, an active and a historical infection pattern. Vaginal excretion occurred in all three herds during every monitored kidding season. Altogether, C. burnetii DNA was detected in 50%, 69%, and 15% of BTM samples from herds A, B, and C. Dust from barns contained pathogen DNA in 71%, 45%, and 50% of the corresponding swabs. The highest proportion of positive samples originated from milking parlors (A: 91%, B: 72%, C: 73%), underscoring a substantial exposure risk for humans during the milking process.

Keywords: Bulk tank milk, Dust swab, Goat, Longitudinal study, Milking parlor, Phase-specific serology, One Health, Q fever, Vaccination, Zoonosis

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Introduction

Q fever is a zoonotic infection caused by the intracellular bacterium Coxiella burnetii. The organism is notably resilient and can withstand drying, elevated temperatures, UV exposure, and various disinfectants [1,2]. Cattle, sheep, and goats serve as the primary reservoirs, shedding the pathogen mainly through birth materials during abortion or normal kidding, as well as through feces and milk [3,4]. In goats, reproductive losses—such as abortion rates reaching 90%, stillbirths, and weak neonates—are well documented [3,5,6]. In cattle, the clinical impact remains uncertain [7], while sheep typically show minor disturbances, with abortion frequencies up to 5% [8].
Human infection generally results from inhalation of contaminated dust or aerosols. The median infectious dose (ID50) of approximately 1.5 organisms highlights its remarkable aerosol infectivity [9]. In the Netherlands, airborne spread from infected dairy goat premises led to the largest known Q fever epidemic, involving over 4000 confirmed cases and an estimated 40,000 infections [10,11]. Similarly, a single infected ewe caused 299 human cases at a German farmers' market [12]. Although less common, consumption of raw contaminated milk is another potential transmission pathway [2,13]. Roughly 40% of human cases develop influenza-like symptoms, including fever, pneumonia, and hepatitis [14]. Long-term consequences include chronic fatigue syndrome in up to 20% of

patients with previous acute infection [15], and individuals with cardiovascular abnormalities may develop endocarditis or vascular complications [14,16]. Seroprevalence within the European Union ranges markedly, from about 1% in the general population to 83.8% among livestock veterinarians [17]. Reported seroprevalence is 3.1% in the United States [18] and roughly 5% in both rural and urban settings in Australia [19], illustrating that exposure risk is not limited to rural environments.

C. burnetii undergoes antigenic phase shifts, yielding phase I (PhI) and phase II (PhII), driven by lipopolysaccharide modifications [20,21]. These changes are utilized in phase-specific serology to assess infection history in goat herds. A rise in IgG PhII alone indicates a recent introduction of infection [22,23], whereas comparable IgG PhI and PhII levels denote an active, ongoing infection approximately nine weeks post-exposure [22]. Exclusive IgG PhI detection reflects a past infection [24]. Nevertheless, phase-specific serology is not routinely applied in veterinary diagnostics despite its usefulness for monitoring herd-level infection dynamics [25].

If left unmanaged, C. burnetii can persist within goat populations for years [24,26,27]. To reduce Q fever occurrence in livestock, several European countries have approved an inactivated phase I vaccine for cattle and goats [28]. Administering this vaccine before mating markedly reduces abortions and pathogen shedding in naïve goats [29]. Although vaccination of previously infected goats does not entirely stop vaginal shedding, it can lower the quantity of bacteria excreted and may decrease the number of shedding animals [30-32]. However, information on the extended impact of phase I vaccination in naturally infected goats remains scarce—knowledge essential for public health authorities assessing human exposure risks following outbreaks.

BTM screening has proven useful for monitoring the Q fever status of dairy goat herds [33-35]. PCR studies on caprine BTM have shown variable detection frequencies across countries: 16% in Belgium [35], 32.9% in the Netherlands [34], 54% in Poland [36], and 16.1% in Iran [37]. Yet this approach applies only to dairy operations, increasing interest in dust sampling from ruminant housing to detect environmental contamination [3,6,38–41]. The bacterium has been observed in dust more than a year after outbreaks, though its viability over time remains poorly characterized [3,40]. Infective C. burnetii has been identified in dust within two months after the final kidding event [6]. Windowsills and fencing are often optimal sampling points [3,38,41,42]. Despite the high exposure risk during milking activities [43,44], milking parlors have seldom been systematically studied [45].

This field study aimed to track three Q fever outbreaks in dairy goat herds following administration of an inactivated phase I vaccine. Shedding patterns were evaluated through vaginal swabs and monthly BTM testing over three kidding seasons. Dust sampling from barn windowsills and milking parlors was performed to assess levels and persistence of environmental contamination. These long-term clinical observations complement existing modeling work [26] and support future One Health-based assessments of Q fever transmission risks.

Materials and Methods

Herd history

Dairy goat herd A

Herd A, located in Schleswig-Holstein in northern Germany, comprised 360 adult females and had been facing ongoing reproductive losses. In January 2018, 24 does aborted, and until the end of the kidding period in April 2018, more weak and stillborn kids continued to appear. No additional records describing kid mortality were available. Early in January 2018, one aborted fetus with its placenta was submitted to the state diagnostic laboratory of Schleswig-Holstein for testing against multiple abortive pathogens (*Brucella spp.*, *Campylobacter fetus* ssp. *fetus*, *Chlamydia spp.*, *C. burnetii*, *Listeria spp.*, *Salmonella spp.*). The only agent detected was *C. burnetii* (Cq 13; VetMAX™ *C. burnetii* Absolute Quant Kit, Thermo Fisher Scientific GmbH, Dreieich, Germany).

The does were housed together in a single barn made of wooden walls on all sides, with ventilation occurring solely through open doors at the front and rear. The milking parlor shared a wall with the barn but was partitioned with metal fencing. The farm followed organic practices and used a semi-intensive system, giving the goats daily access to pasture from April through November. A comprehensive account of this outbreak has previously been published by the authors [44].

Dairy goat herd B

Farm B maintained 152 dairy goats in North-Rhine Westphalia (western Germany). Kidding occurred from February to April 2018, and by the end of this period, 20 does had presented with reproductive issues—abortion, stillbirth, or weak offspring. Four fetuses with placentas from two of these goats were analyzed at the state laboratory of North Rhine-Westphalia for abortive pathogens (*Brucella spp.*, *Campylobacter spp.*, *Chlamydia spp.*, *C. burnetii*, bluetongue virus, pestivirus, Schmallenberg virus). Only *C. burnetii* was detected (Cq 11–22; VetMAX™ *C. burnetii* Absolute Quant Kit, Thermo Fisher Scientific GmbH, Dreieich, Germany).
The barn consisted of three wooden exterior walls, while the fourth side was enclosed with curtains. The milking parlor did not directly open into the barn; animals moved in and out through a flap. As an organic and semi-intensive operation, the herd grazed in nearby fields from April until October each year.

Dairy goat herd C
Herd C, located in Bavaria in southern Germany, consisted of 85 dairy does. Although most kidding occurred in January 2018, individual animals gave birth until March. The first stillborn kids appeared in February, and in total, six goats delivered either stillborn or weak offspring. Because Q fever was suspected, vaginal swabs were taken from the final twelve does that kidded. All samples were positive for *C. burnetii* DNA (Cq 25–38; VetMAX™ *C. burnetii* Absolute Quant Kit, Thermo Fisher Scientific GmbH, Dreieich, Germany), based on analysis by the state laboratory of Lower Saxony. No other pathogens were evaluated.
The barn had three curtained sides and one brick wall; a glass door separated the milking area from the main housing. Goats in this intensively managed herd were kept primarily indoors but had access to a concrete paddock from April to November.
All three producers (A–C) contacted the Clinic for Swine and Small Ruminants at the University of Veterinary Medicine Hannover for assistance in managing their Q fever outbreaks.

Sample collection
At herd A, the first visit occurred in January 2018 while kidding was ongoing. By contrast, in herd B, only a few animals were still pregnant during the initial visit in April 2018. Herd C's peak kidding period had ended roughly two months earlier, and first interventions began in April 2018.

Blood samples and vaginal swabs
For herd A, all 24 goats that had aborted in January 2018 were sampled. In April 2018, a random selection of animals from herd B (n = 48) and herd C (n = 35) were used for serum sampling via the Vena jugularis (KABE LABORTECHNIK GmbH, Nümbrecht-Elsenroth, Germany). Vaginal swabs were taken from these same animals during the initial farm visit by the authors (B.U.B. and M.G.).
During the kidding seasons of 2019 and 2020, farmers were instructed to collect vaginal swabs from does within 48 hours after kidding. Because individual "super-shedders" can strongly influence environmental contamination [3,27], the goal was to include as many swabs as possible to capture the true extent of *C. burnetii* shedding. Consistently sampling the same animals across all seasons was not always possible due to practical farm-management constraints.

Bulk tank milk and dust swabs
Bulk tank milk (BTM) was sampled once per month throughout the observation period (from the initial visit until September 2020) to track herd-level shedding. Monthly dust specimens were also obtained from each milking parlor and from a designated windowsill in every barn, following the procedure described earlier [3]. In short, a dry swab (Sarstedt AG & Co. KG, Nümbrecht, Germany) was rolled across a one-meter section of the same windowsill on each farm to gather accumulated dust. An identical technique was applied to collect dust from the milking areas. On farm A, the swab was moved along the milk and vacuum pipeline, whereas on farms B and C, the chosen site was a windowsill in the milking-parlor room, since the respective pipelines were difficult to access. The sampling points in both barns and parlors remained constant during the entire study. None of the barns or milking parlors underwent any form of cleaning or disinfection during the period of data collection.

Vaccination schedules and breeding management
All three herds received an initial dose of the inactivated *C. burnetii* phase I vaccine (Coxevac®, Ceva, Libourne, France) according to label instructions. Herds B and C continued with yearly boosters, and replacement females

were primed four weeks prior to mating. Herd A had already administered a booster in September 2018, prior to breeding, which included the first vaccination of female kids born that year. Beginning in 2019, only the young stock on farm A received the two-dose schedule before mating, while older multiparous does were no longer vaccinated due to financial constraints.

All producers were instructed to remove aborted materials promptly from bedding and to store them securely until rendering. No sanitation or disinfection procedures were performed in any of the facilities at any point during the study.

Farms A and B kept roughly half of their goats in milk continuously after the 2018 Q fever outbreak, while the other does were bred and dried off about six weeks before kidding. Farm C dried off the entire herd for the same interval. As a consequence, BTM samples were intermittently unavailable for herd C.

Supplementary Figure S1 summarizes the timing of blood sampling, vaginal swab collection, kidding periods, and vaccination events for all herds.

Laboratory analysis

Serum samples were analyzed using two phase-specific ELISAs (EUROIMMUN AG, Lübeck, Germany) as previously described [46]. Results were expressed in relative units (RU), calculated via a standard curve; values ≥ 22 RU were defined as positive.

Detection of *C. burnetii* DNA in vaginal swabs was based on qPCR amplification of IS1111. According to Frangoulidis *et al.* [47], samples with Cycle Quantification (Cq) ≤ 45 were classified as positive, and >45 as negative. The BTM and dust specimens were tested with a commercial qPCR assay (LSI VetMAX™ *C. burnetii* Absolute Quant Kit, Thermo Fisher Scientific GmbH, Dreieich, Germany), which also targeted IS1111 and used the same Cq ≤ 45 threshold for positivity.

Statistical analyses

Normality of serum values was assessed using the Shapiro–Wilk test. Depending on data distribution, either a t-test or a Mann–Whitney test was employed to compare phase-specific IgG levels within each herd. A significance level of $p < 0.05$ was applied.

Vaginal-swab results were analyzed descriptively, considering both DNA quantity and the total number of qPCR-positive swabs.

All statistical evaluations were conducted using SAS software (SAS Institute Inc., Cary, NC, USA).

Results

Serology

In herd A, IgG PhII values were significantly higher than IgG PhI **(Figure 1)**. No significant difference between the two antibody classes was observed in herd B. In herd C, however, the median IgG PhI level surpassed the IgG PhII median value.

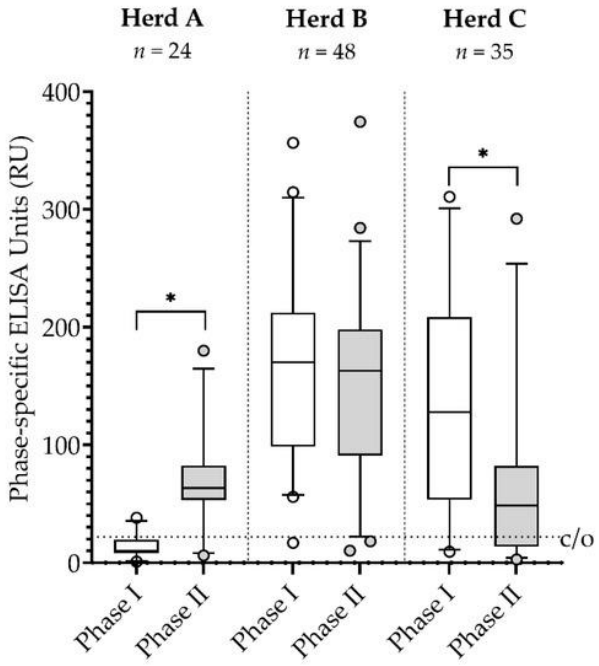

Figure 1. Median IgG phase I and phase II antibody levels for *C. burnetii* in the three naturally infected herds (A–C) at the beginning of the 2018 investigation. * $p < 0.05$; c/o = ELISA cut-off

Vaginal swabs

In herd A, every one of the 24 goats sampled in 2018 released C. burnetii, and approximately half of those samples contained very high DNA loads (Cq ≤ 20). In the later kidding periods, shedding persisted but only at low concentrations (Cq ≥ 33).

For herd B, all swabs were positive at the first sampling and showed Cq ≤ 33. During 2019–2020, both the number of shedding does and the detectable DNA levels declined, with remaining samples containing minimal pathogen quantities (Cq ≥ 37).

Within herd C, 25 out of 35 animals emitted small amounts of DNA (Cq ≥ 34) in 2018. Over the next seasons, fewer goats continued shedding, and when present, only low-level DNA (Cq ≥ 36) was found.

A detailed summary of shedding prevalence and corresponding Cq values can be found in **Figure 2**.

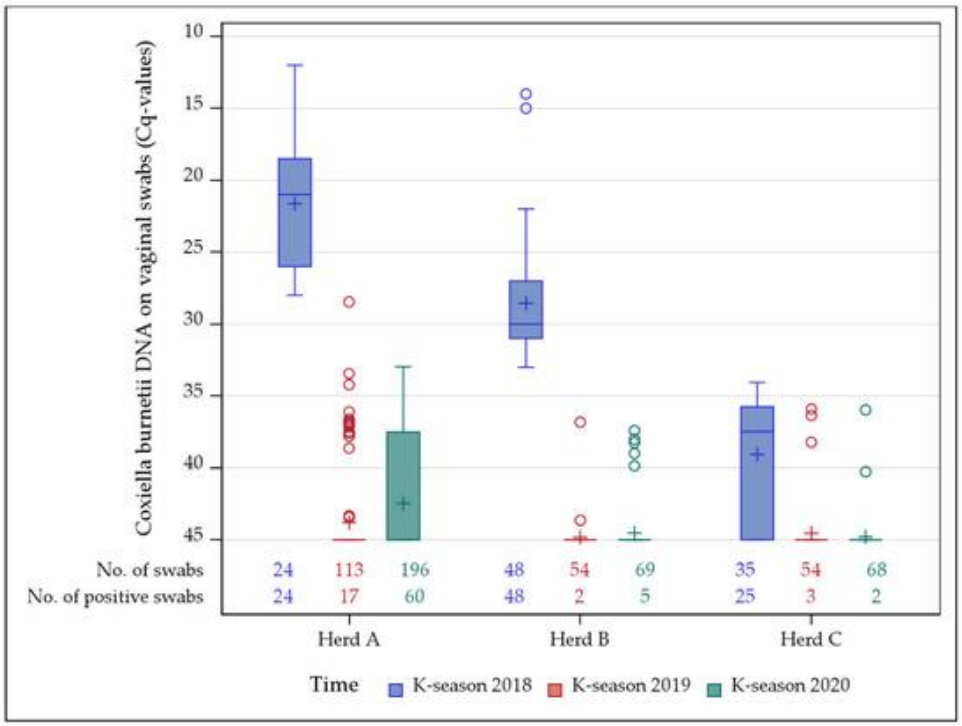

Figure 2. Detection of C. burnetii in vaginal swabs via qPCR during three kidding periods (2018–2020) across herds A–C. All goats received vaccination following the identification of infection in 2018

Bulk tank milk

After C. burnetii was first confirmed, DNA from the organism remained detectable in bulk tank milk for 9 months in herd A and 16 months in herd B. After these periods, detection was intermittent. In herd C, BTM samples were positive at the beginning of monitoring and again once in August 2019 **(Figure 3)**. Overall, the proportion of positive BTM samples was 50% (16/32) for herd A, 69% (20/29) for herd B, and 15% (4/26) for herd C.

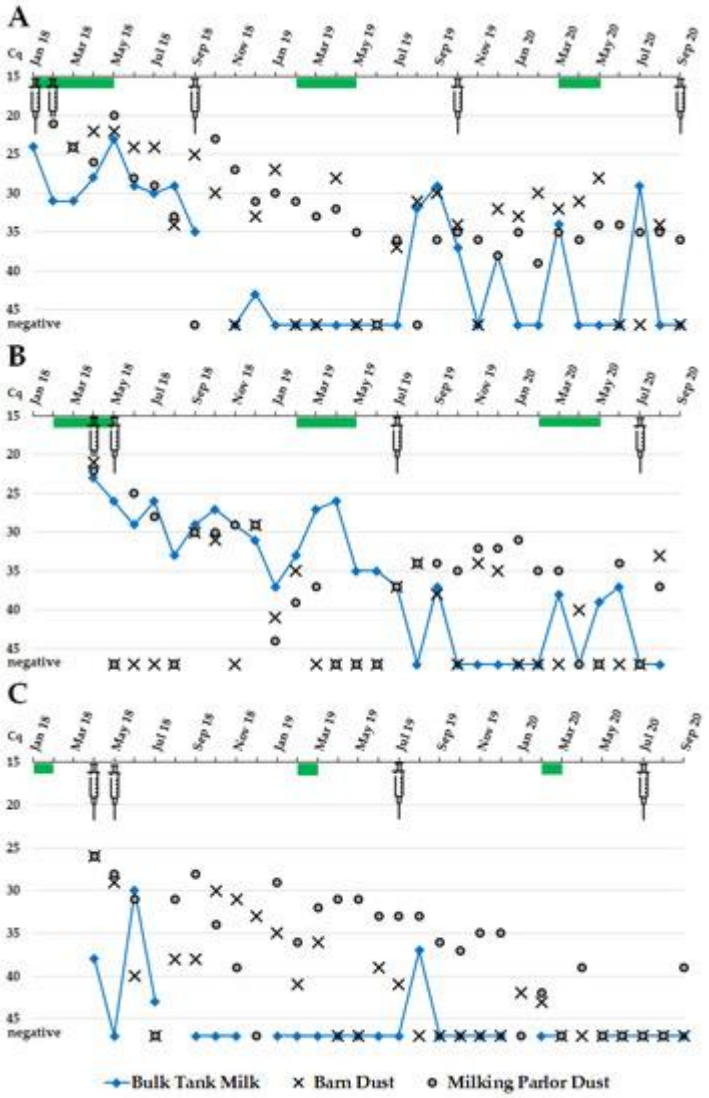

Figure 3. qPCR findings (positive defined as Cq ≤ 45) in monthly BTM samples (◆) and dust swabs from barn windowsills (✕) and milking parlors (●) from the three infected, vaccinated dairy goat herds (A–C). BTM samples were occasionally unavailable for farms A and C. Green bars show kidding periods; syringes mark vaccination events

Dust from barns and milking parlors

Across all farms, dust samples from barns and milking areas showed sporadic positivity for C. burnetii (Figure 3). In barn samples, the proportion of positives reached 71% (22/31) on farm A, 45% (13/29) on farm B, and 50% (15/30) on farm C. Detection rates were higher in milking parlors, with 91% (29/32), 72% (21/29), and 73% (22/30) positives for farms A, B, and C, respectively. The level of environmental contamination at both sampling locations fluctuated throughout the study.

Discussion

Previous outbreaks linked to shedding by small ruminants have resulted in localized human Q fever cases in Germany [2,12], and one individual from farm A also developed acute Q fever [44]. In the absence of intervention, C. burnetii can persist within a goat population for extended periods [24,26,48]. A single heavily shedding animal can produce substantial environmental contamination and trigger numerous human infections [12]. Such high-level shedders were identified in both herds A and B during 2018. For ethical and public health reasons, unvaccinated control groups were not included, as this would have increased exposure risk and could obscure vaccine performance estimates [30,49]. Moreover, excluding controls aligns with the broader goal of containing Q fever outbreaks. Little is known about outcomes in entirely vaccinated herds post-outbreak, yet such insights

are needed by public health authorities implementing or lifting restrictions. These observations support a One Health framework by providing information relevant to managing fully vaccinated dairy goat herds following Q fever events.

Phase-specific ELISAs revealed distinct infection stages across the three herds. In herd A, high IgG Phase II levels indicated a recent acute outbreak, reinforced by the large pathogen loads (Cq ≤ 20) from half the sampled goats in early 2018. In herd B, similar values for both antibody phases pointed to an active but not acute infection, corresponding to sampling shortly after kidding. Conversely, herd C showed significantly higher IgG Phase I levels, suggesting a past infection, which fits with sampling that occurred roughly two months after peak kidding, and with 71% of swabs showing only low DNA content (Cq ≥ 34). These patterns match reports from naturally or experimentally infected goats [22,23,24]. The phase-specific ELISAs, therefore, allowed the assignment of the herds to three distinct infection stages, making them useful tools for field assessment of Q fever status.

The absence of control groups has already been explained earlier, and therefore, the potential impact of vaccination on vaginal shedding can only be inferred indirectly. Still, considering that many non-vaccinated herds continue to shed C. burnetii during later kidding periods [48,50], it appears likely that the vaccine helped to lower both the number of shedding animals and the bacterial load in the following parturitions. Despite this, none of the herds were completely free of shedding in later seasons, which agrees with previously published work [3,29,30,31]. Overall, the observations suggest that three consecutive years of vaccination are insufficient to clear C. burnetii from infected goat farms and reinforce modeling predictions recommending at least six years of annual immunization [26].

The sporadic appearance of C. burnetii DNA in bulk tank milk from herds A and B indicates the presence of occasional milk shedders [51], a phenomenon described in cattle [52,53] but not yet verified in goats. Although the risk of contracting Q fever from unprocessed milk or dairy products is considered low, it cannot be completely ruled out. Moreover, infected goats may excrete other zoonotic pathogens in milk, including Toxoplasma gondii, Brucella melitensis, Listeria monocytogenes, and tick-borne encephalitis virus [54–57]. With raw milk becoming increasingly popular and new distribution pathways such as vending machines and online sales expanding [58], raising awareness about potential food-borne hazards in raw dairy products is essential.

Eliminating goats that shed the pathogen irregularly in milk has been shown to produce BTM-negative herds [51]. However, this “test-and-cull” strategy is expensive and challenging to apply consistently, and by itself will not eradicate Q fever [26]. Another complicating factor is that vaccination with an inactivated phase I vaccine can produce qPCR-positive milk samples for up to nine days after administration [59]. This may explain the single BTM-positive result (Cq 37) in herd C observed 31 days after a booster dose. Consequently, careful timing of sampling relative to vaccination is required when BTM is used for surveillance.

Dust collected from barns and milking areas on all three farms initially contained high DNA levels (Cq < 30) following detection of C. burnetii in 2018. Such heavy contamination is linked to the substantial pathogen excretion during abortion or kidding events [3,6,38]. Over time, detection rates fluctuated. Many variables influence recovery of C. burnetii from environmental samples, including sampling technique, matrix, location, type of PCR assay, on-farm activities, abortion history, number of pregnant animals, frequency of vaginal shedders, ventilation, and species differences [3,38,40–42,45,60,61]. These factors underline the importance of standardized, reproducible sampling protocols for obtaining reliable environmental data.

Across all three goat farms, milking parlor dust exhibited the highest rate of positive results. Milking activities generate airborne dust particles [45], meaning farm workers engaged in daily milking are at particular risk for exposure to C. burnetii [43,44]. This should be incorporated into occupational risk assessments. Nonetheless, detection of DNA alone does not confirm that the pathogen is viable. Current assumptions suggest C. burnetii may remain infectious in dust for up to two months after parturition [6], but the significance of very low DNA concentrations in the environment remains uncertain. The persistence of contamination for more than two years across the farms aligns with findings from other small-ruminant operations [3,40]. These long-term patterns stress the importance of effective decontamination strategies to reduce human exposure.

The authors acknowledge the limitations of this field investigation. Differences in herd size, housing layout, ventilation systems, management practices, vaccination routines, and infection status restricted direct comparisons among the three farms and complicate the interpretation of the longitudinal data. Additionally, the C. burnetii strains on these farms may differ in their IS1111 copy numbers [62], making direct comparison of qPCR quantification across farms inappropriate. Together, these factors illustrate the practical difficulties of monitoring and controlling Q fever outbreaks.

Conclusions

Phase-specific serological testing provides a useful means of categorizing the Q-fever status of goat herds and helps identify acute infections, which pose serious risks to humans. The use of an inactivated phase I C. burnetii vaccine did not prevent vaginal shedding in naturally infected goats over two subsequent kidding seasons, supporting the recommendation to vaccinate positive herds for at least six years [26]. The continuous detection of C. burnetii in dust from milking parlors highlights the exposure risk associated with milking procedures. Future work should prioritize routine methods for determining viability in dust samples containing low DNA levels and assess the diagnostic sensitivity of dust swabs for identifying infected herds. Collectively, the results underline the value of combining phase-specific serology with molecular testing across multiple sample types to monitor infection dynamics and shedding patterns in goat herds. These insights provide important input for Q fever risk assessments and align closely with the One Health perspective.

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Ethics Statement: None

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