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# Shedding light on risk: Seroprevalence of Q fever among farm animals and workers in Ecuador

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ABSTRACT

Q fever, caused by the bacterium *Coxiella burnetii*, is a zoonotic disease that has been largely overlooked despite presenting significant risks to both animal and public health. Although well studied in some countries, in most countries in Latin America, there's a lack of information on *C. burnetii* infection, its prevalence, and its impact on both livestock and human populations. To address this gap, we conducted a serosurvey among farm workers, cattle, sheep, and dogs on two dairy farms in Ecuador using a commercial ELISA kit. Additionally, we conducted a case-control study in cattle to investigate the association between *C. burnetii* infection and abortion. The findings revealed that 18 % of farm workers, 30 % of dogs, 25 % of cattle and 2 % of sheep tested positive for Q fever antibodies. Interestingly, no significant association between *C. burnetii* infection and abortion was observed in cattle ($p < 0.05$) but a high *Neospora caninum* seroprevalence indicated a strong link to abortion due to this parasite infection. The results highlight the presence of Q fever in both humans and animals on the surveyed farms, with farm dogs showing the highest seroprevalence. A point of concern arises from the significant prevalence of antibodies detected among farm workers, suggesting a potential history of unconfirmed symptomatic respiratory infections caused by a *C. burnetii* infection. However, further investigations are necessary to better understand the infection dynamics and its potential implications for public and animal health.

## 1. Introduction

Q fever, caused by the intracellular bacterium *Coxiella burnetii*, is a globally recognized zoonotic disease classified as a notifiable animal disease by the World Organization for Animal Health [1]. Since its identification in 1937, it has been documented in domestic and wild mammals, birds, and arthropods [2]. Mainly transmitted through cattle, sheep, and goats, Q fever in humans can manifest as either acute or chronic disease, with symptoms ranging from self-limited febrile illness to severe cardiac pathologies [3]. Occupational exposure, particularly among farmers, slaughterhouse workers, and veterinarians, brings the highest risk of transmission, often through aerosols from infected animal fluids [2].

Following a significant outbreak in the Netherlands affecting thousands, Q fever has gained recognition as a major threat to human health, and livestock. [4]. Serological studies in countries like the USA, Ethiopia and Argentina have revealed considerable prevalence rates, underscoring the global concern [5–7]. Q fever in Latin America has been reviewed in two publications [8,9] and has been identified as an emerging pathogen in French Guiana, with a high prevalence of approximately 24 % as a cause of community-acquired pneumonia [10].

In livestock, though clinical signs may not be apparent, *C. burnetii* poses a significant veterinary health issue, especially in small ruminants [2], leading to reproductive disorders with substantial economic repercussions [11]. Seroprevalence rates in animals vary across regions, with notable differences observed in European countries like the Netherlands [12], Switzerland [13], and Albania [14], as well as in South America, including Colombia [15], Ecuador [16], and Brazil [17].

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Despite these findings, surveillance, and control measures for Q fever, especially in humans, remain limited in many developing countries. In Ecuador, for instance, the prevalence of Q fever in both farm animals and workers has not been investigated and in the whole country no laboratory diagnosis is available in the medical diagnostic laboratories. Hence, our study aimed to address this gap by assessing the prevalence of Q fever antibodies in farm workers, cows, sheep, and dogs on two farms in the Cotopaxi ang Tungurahua regions in Ecuador and investigating its potential role in bovine abortions.

## 2. Methods

### 2.1. Sample collection

#### 2.1.1. Farm workers

For this study, we selected two dairy farms, one located in Tungurahua (Farm A) and the other in Cotopaxi (Farm B) province, in the Andean Mountains of Ecuador. Farm A, which was tuberculosis and brucellosis-free, was situated at an altitude of 2800 m, while Farm B also tuberculosis and brucellosis-free was located at 3500 m above sea level. The location of the cattle farms on the map of Ecuador is shown in Fig. 1. Cattle farm workers were invited to participate, and 22 individuals agreed, providing informed consent (Ethical approval Code MB-14-2022), before a healthcare professional collected their blood samples. During the investigation period in 2022, both farms exhibited a relatively high abortion rate of 10 %, potentially associated with Q fever [18].

#### 2.1.2. Farm animals

We collected a total of 175 blood samples from the farm animals under investigation, comprising 10 serum samples from dogs and 57 from sheep. Additionally, for our case-control study aimed at determining the association between Q fever with abortion, we obtained 53 serum samples from cows that had experienced at least one abortion during mid and late gestation. Simultaneously, 55 serum samples from cows of comparable age with no history of abortion were randomly selected as the control group. For detailed data, concerning bovines in the abortion and control group, consult Table 1.

All samples were collected in tubes containing clot activator and transported in an ice cooler to the laboratory on the same day for processing. Following blood coagulation, the samples underwent centrifugation at 2000*g* for 5 min. The resulting serum was then aliquoted and stored at −20 °C until employed in the indirect ELISA assays.

**Table 1**
Number and mean age in months of the abortion and control groups with standard deviation from the cows of the study.

| Tested cows | Abortion group and age | Control group and age |
|---|---|---|
| Farm A | $n = 44$; 71 ± 27 months | n = 44; 68 ± 31 months |
| Farm B | $n = 9$; 65 ± 29 months | $n = 11$; 62 ± 30 months |
| Total = 108 | n = 53 | $n = 55$ |

#### 2.1.3. Q fever and Neosporosis

All serum samples of human and animals were used for the detection of antibodies against *C. burnetii*. As previously described by Changoluisa and collaborators, bovine abortion in a tropical region of Ecuador is associated with *Neospora caninum* infection [16]. To determine an association between seropositivity and abortus the serum samples from both aborted cattle and the control group were therefore analyzed for Q fever and *Neospora caninum* antibodies.

#### 2.1.4. Serological tests

For Q fever and Neosporosis diagnosis, we utilized commercial indirect multi-species ELISA kits. (ID.vet, Innovative Diagnostics, France). The ELISA procedures were conducted manually by an operator, in strict accordance with the manufacturer's guidelines, and interpretation was carried out following the recommended procedures. The manufacturer provided the measured sensitivity and specificity of this ELISA, which utilizes both phase I and phase II antigens of *Coxiella burnetii*, bound to the same ELISA well. The sensitivity and specificity were 100 % (95 % CI: 89.28 %–100 % and 97.75 %–100 %, respectively). The manufacturer internal validation report (version 1117) for the ID Screen® Q Fever Indirect Multi-species is available upon request at info@innovative-diagnostics.com.

### 2.2. Statistical analysis

We conducted a chi-square test to evaluate the relationship between *C. burnetii* or *N. caninum* infection and abortion among cows on both farms. Furthermore, we calculated 95 % confidence intervals (CI) for prevalence rates. Statistical significance was defined at $p < 0.05$, and all

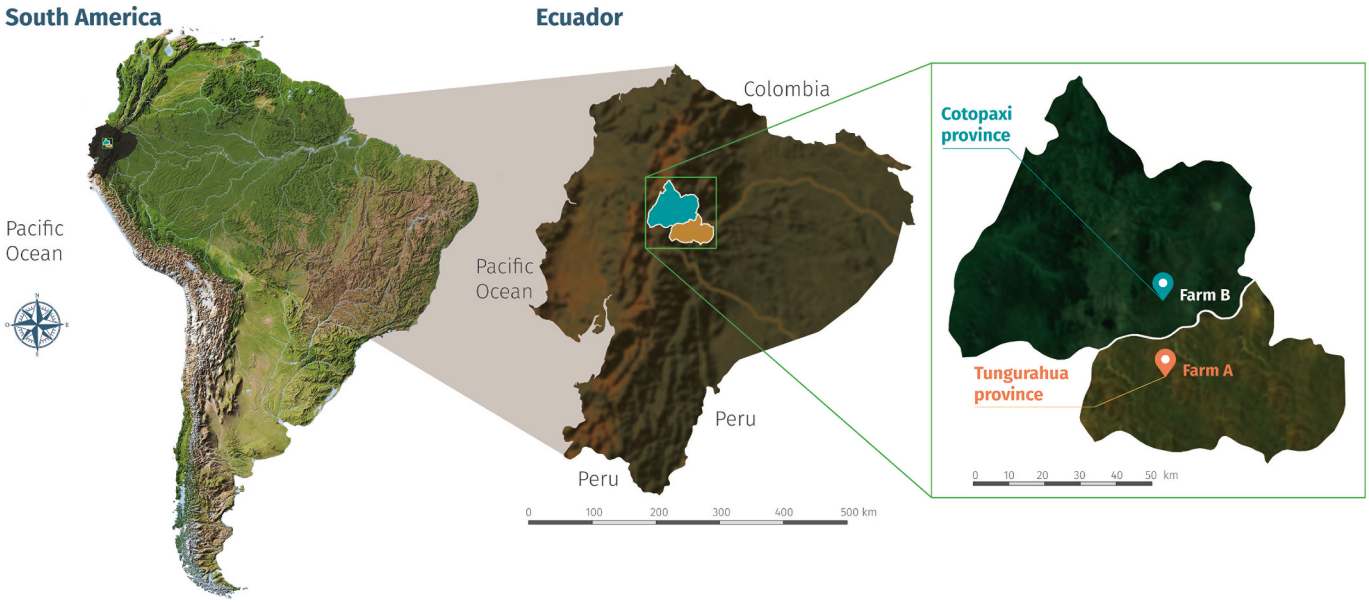


**Fig. 1.** The map of Ecuador and the ubication of the two cattle farms in the Provinces of Cotopaxi and Tungurahua.

data were analyzed using RStudio version 2023.12.1 [19].

### 2.3. Ethical considerations

Approval for this research and publication was obtained from the ethical committee of the Pontifical Catholic University in Quito, Ecuador. Ethical approval Code MB-14-2022. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki, a formal statement of ethical principles published by the World Medical Association (WMA) to guide the protection of human participants in medical research. All patient data were de-identified. While Ecuador currently lacks an established ethical committee for animal research, it's important to note that blood samples were collected by a highly skilled veterinary professional with over a decade of experience. Throughout the process, utmost care was taken to prioritize the well-being of the animals, both before, during, and after the sample collection procedure.

## 3. Results

Twenty-two cattle farm workers participated in the study, with 8 from Farm A and 14 from Farm B. The overall seroprevalence of Q fever was 18.2 % (4/22), with no significant difference in seropositivity between the two farms (Chi-square analysis, $p = 0.076$). See Table 2.

We collected 175 blood samples from animals on both farms. The seroprevalence of Q fever and *N. caninum* in sheep from Farm B was 1.8 % (1/57; [95 % CI 0.044–9.39]) and 3.5 % (2/57; [95 % CI 0.43–12.11]), respectively. In dogs, Q fever seroprevalence was 30 % (3/10) with no significant difference in seropositivity between farms (Chi-square analysis, $p = 0.259$), no *N. caninum* antibodies were found in the dogs. Q fever serodiagnosis results for animals are also summarized in Table 2, excluding the sheep from Farm B due to its presence only on this farm.

In cattle, the overall seroprevalences of Q fever and *N. caninum* were 25 % (27/108) and 15.74 % (17/108) respectively. Among cows with a history of at least one abortion, the Q fever prevalence was 32.1 %, compared to 18.2 % in control cows, while the prevalence for *N. caninum* antibodies was 26.4 % and 5.45 %, respectively. See Table 3.

Statistical analysis of Q fever prevalence rates in cows from both farms revealed no significant association between *C. burnetii* infection and abortion (Odds ratio [OR] 2.13; [95 % CI 0.88–5.13]; $p = 0.096$). Subsequent separate analyses of Farm A and Farm B also failed to identify any association between Q fever and abortion ($p > 0.05$). On the other hand, a strong association was found between *N. caninum* antibodies and abortion (Odds ratio [OR] 6.22; [95 % CI 1.80–21.46]; $p = 0.0028$). Statistical analysis also showed significant associations in Farm A (Odds ratio [OR] 4.67; [95 % CI 1.06–20.46]; $p = 0.044$) and Farm B (Odds ratio [OR] 20; [95 % CI 2.33–171.42]; $p = 0.0072$).

**Table 2**
Q fever seroprevalence with 95 % CI in cattle farm workers and dogs from the two farms of Tungurahua and Cotopaxi provinces, Ecuador.

| Subject tested | Farm | Sample size (n) | Q fever (+) | Prevalence (%) | 95 % CI |
|---|---|---|---|---|---|
| Dairy farm workers | Farm A | 8 | 3 | 37.5 | 8.52–75.51 |
| | Farm B | 14 | 1 | 7.1 | 0.18–33.87 |
| | **Total** | **22** | **4** | **18.2** | **5.19–40.28** |
| Dogs | Farm A | 4 | 2 | 50 | 6.76–93.24 |
| | Farm B | 6 | 1 | 16.7 | 0.42–64.12 |
| | **Total** | **10** | **3** | **30.0** | **6.67–65.24** |

**Table 3**
Q fever and *N. caninum* seroprevalences with 95 % CI in cows of the two farms of study in Tungurahua and Cotopaxi provinces, Ecuador. We tested cows of the same age with a history of at least one abortion ($n = 53$) and a control group that had never aborted ($n = 55$).

| | Seropositive cows | | | |
|---|---|---|---|---|
| | Q fever ELISA | | *N. caninum* ELISA | |
| | Abortion group | Control group | Abortion group | Control group |
| Farm A | 14 (31.81 %) [18.61–47.58] | 9 (20.45 %) [9.80–35.30] | 8 (18.18 %) [8.19–32.71] | 2 (4.55 %) [0.56–15.47] |
| Farm B | 3 (33.3 %) [7.49–70.07] | 1 (9.09 %) [0.23–41.27] | 6 (66.67 %) [29.93–92.51] | 1 (9.09 %) [0.23–41.28] |
| Total = 108 | 17 (32.08 %) [19.92–46.32] | 10 (18.18 %) [9.08–30.90] | 14 (26.42 %) [15.26–40.33] | 3 (5.45 %) [1.14–15.12] |

## 4. Discussion

The serological status for Q fever among farm workers and animals on two farms in Ecuador was evaluated. Antibodies against *C. burnetii* were detected in all study groups including humans, cattle, sheep, and dogs, and a relatively high seroprevalence was found. Sheep were tested only on Farm B, as Farm A did not have any sheep.

### 4.1. Farm workers

Among cattle farm workers, 18 % tested seropositive for Q fever, with no clinical cases detected upon medical interrogation. The transmission to these workers may have occurred from animals to humans due to proximity and contact. To confirm seropositivity in human subjects, we conducted two rounds of testing, including a retest after three months, and consistently obtained positive results.

In Ecuador, only two other reports of Q fever in humans exist. The first, a longitudinal observational study by Manock et al., found that 4.9 % of patients with fever of unknown origin in a hospital in the Ecuadorian Amazon were seropositive for Q fever [20]. The second study, conducted in 2019, included surveillance of cattle farm workers and veterinarians, revealing an overall seroprevalence of 34 % in these farm workers [21]. Notably, there are no known records of humans with active Q fever in Ecuador, likely attributed to the lack of routine screenings and absence of diagnostic capacity for *C. burnetii* infection. In contrast, in French Guiana, where an active monitoring system is in place, Q fever is hyperendemic, with over 24 % of community-acquired pneumonia (CAP) cases attributed to *C. burnetii* infections [22,23]. In Chile, between 2017 and 2019 an outbreak investigation of undiagnosed human atypical pneumonia of 357 cases demonstrates that 71 (20 %) of the cases were Q fever [24]. And recently in 2021, an outbreak of Q fever was a reported among slaughterhouse workers in Argentina, symptomatically affecting 11 workers, out of a total exposed population of 49 individuals, indicating transmission within the slaughterhouse environment [5].

Regarding chronic Q fever infection, well documented for example in the Netherlands [25], there is limited knowledge about cases in Latin America. Chronic Q fever develops in an estimated 1 %–5 % of all infected humans and can become manifest even years after primary infection [26]. In French Guiana, a retrospective study from 2008 to 2016 identified 51 confirmed or probable cases. During the same period, approximately 6 % of microbiologically documented endocarditis cases in French Guiana were attributed to *C. burnetii* [9]. Concerning our farm workers, no clinical manifestations of Q fever were present in the seropositive workers. Moreover, we were unable to differentiate between acute and chronic Q fever infections using IgM and IgG antibodies against *C. burnetii* phase I and phase II due to the unavailability of human diagnostic kits for this disease. [21].

### 4.2. Farm animals

To the best of our knowledge, this is the first report of the presence of Q fever antibodies in sheep and farm dogs in Ecuador. A recent review of literature from Latin America and the Caribbean determined that reports of Q fever in animals are scarce, serological prevalence patterns differ, and its challenging to make broad generalizations about the prevalence of antibodies on the continent in animals [27]. For example, in sheep, prevalence rates oscillate between 0 % and 66.6 % [28,29], while in dogs, prevalences ranges from 1.8 % to 15.4 % [30,31]. The transmission of *C. burnetii* between ruminant hosts is primarily via the airborne route and thus uncontrolled animal movement and cross-border trade may act as a catalyst for the spread of the bacterium. In pets, the origin of infection is poorly understood. Nonetheless, dogs and cats may get infected by ticks, consumption of placenta, raw milk, and raw meat from infected livestock [32].

### 4.3. Abortion in bovines

Infertility, abortions, metritis, and mastitis are factors linked to chronic Q fever in cattle [11]. The abortion rate in Ecuadorian cattle is estimated to be between 3 and 5 %, although the cause remains poorly studied [16]. Globally reported bovine abortion rates range from approximately 0.5 % to 10 %. However, underreporting is a significant issue affecting the accurate assessment of bovine abortion rates internationally.

Concerning Q fever as the cause of abortion and although our study observed a high prevalence of infected cattle, no association was found between the presence of antibodies of *C. burnetii* and abortion. However, other studies have demonstrated a strong association with late abortions in cattle and seropositivity for a *C. burnetii* infection [33,34]. An example is Northern Cyprus [18], where significantly high occurrence of *C. burnetii* abortions of 37 % was reported in cattle. A previous study in Ecuador has also found that seropositivity for *C. burnetii* infection, was not associated with abortion and that a *Neospora caninum* infection is most probably a more important cause of abortion in our country [16].

### 4.4. One health approach and Q fever

The lack of clinical suspicion and diagnostic testing in Ecuador and much of Latin America has led to Q fever being neglected and underreported in both humans and animals. Bailly et al. emphasize the need for a One Health approach, integrating studies on the environment and both domestic and wild animals with human health [22,23]. However, the One Health concept remains unfamiliar to many physicians, highlighting the importance of raising awareness to strengthen public health efforts [35].

Although the World Organization for Animal Health (OIE) recommends reporting Q fever in animals, Ecuador has never officially reported any cases. Human cases are also not considered endemic, as no animal cases have been identified. Consultations with public and private health laboratories revealed limited knowledge of Q fever, resulting in a lack of diagnostic capacity [21]. This study's detection of Q fever in both humans and animals underscores an urgent need to improve awareness and establish diagnostic capabilities in Ecuador. Such efforts would support the inclusion of Q fever in differential diagnoses for community acquired pneumoniae (CAP) and endocarditis cases with negative blood cultures.

## 5. Conclusion

Our study highlights the presence of Q fever antibodies in both humans and domestic animals, underscoring the need for a deeper understanding of the epidemiology of Q fever in Ecuador [36]. To address this knowledge gap, it is imperative to employ microbiological and molecular approaches to characterize the circulating *C. burnetii* strains in both humans and animals. While Q fever has been detected in dairy herds throughout the country, there is no evidence suggesting a negative impact on reproduction or the overall health status of cattle [16]. This observation may be attributed to the virulence of the circulating strains in Ecuador. Notably, virulent strains belonging to genomic groups GG-I, GG-III, and GG-IV have been identified in countries such as French Guiana, Argentina, and Brazil [37]. Among these, group I strains are associated with the most severe disease outcomes, while group II-V and group VI strains demonstrate intermediate and low virulence, respectively [38]. Therefore, the isolation of the bacterium should be prioritized to determine which strains are circulating in Ecuador, providing critical insights for effective control and management strategies.

Additionally, ELISAs or indirect fluorescent antibody assay (IFA), capable of detecting antibodies against phase I and II antigens can provide valuable insights into the transmission dynamics of *C. burnetii* in cattle herds and serving as valuable tools for both active and chronic Q fever detection in humans [39]. These investigations will serve as the cornerstone for unraveling the dynamics of infection and evaluating its potential impact on human and animal health in our country.

## 6. Limitation of the study

This small-scale study included a limited number of farm animals and human subjects from two farms located 20 km apart, meaning our findings may not be representative of Ecuador as a whole. Additionally, the lack of human diagnostic kits for Q fever in Ecuador prevented separate serological testing for phase I and phase II IgG and IgM antibodies, which would have offered valuable insights into distinguishing acute from chronic Q fever in human patients.

## Authors contribution statement

The study was conceived and designed by JHdW, FB and GE, while MSGF and YL were responsible for fieldwork and collecting blood samples. MSGF and YL performed the laboratory assays and MSGF drafted the initial version of the manuscript. YL, GE, FB, and JHdW critically revised the manuscript. All authors have reviewed and approved the final version for submission.

## CRediT authorship contribution statement

**Mónica Salomé Guerrero-Freire:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Yanua Ledesma:** Writing – review & editing, Methodology, Formal analysis. **Gustavo Echeverría:** Writing – review & editing, Methodology. **Federico Carlos Blanco:** Writing – review & editing, Supervision, Conceptualization. **Jacobus H. de Waard:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

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