



## Research article

Seroprevalence of antibodies to *Chlamydia abortus* and risk factors in cattle from Villavicencio, Colombia

Agustín Góngora Orjuela<sup>a</sup>, Leidy J. Reyes Castañeda<sup>a</sup>, Julio César Tobón<sup>b</sup>,  
 Jorge L. Parra Arango<sup>a</sup>, Blanca Guzmán-Barragán<sup>c,\*</sup>

<sup>a</sup> Escuela de Medicina Veterinaria y Zootecnia, Universidad de los Llanos, Villavicencio, Colombia

<sup>b</sup> Industria Colombiana de Productos Veterinarios (Vecol), Bogotá, Colombia

<sup>c</sup> Universidad de Ciencias Ambientales y Aplicadas (UDCA), Bogotá 111166, Colombia

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## ABSTRACT

*Chlamydia abortus* is a Gram-negative obligate intracellular bacterium, responsible for abortions and reproductive problems. The disease has a high zoonotic potential and causes great economic losses in ruminant farmers. A cross-sectional study was carried out with 514 cattle from 24 farms of Villavicencio, Colombia. The blood samples were collected from each individual animal and analyzed by Indirect Elisa for immunoglobulin G (IgG) in blood serum (Idexx Chlamydiosis total Ab test). A serum was considered positive when the optical density (OD) of the sample was  $\geq 30\%$  of that of the positive control serum. Data on potential risk factors associated with the disease were collected through a questionnaire in each farm and analyzed. The individual and herd prevalence was estimated. A risk factors analysis was performed through univariate and multivariable using the software SPSS version 20. The animal level seroprevalence was found to be 47.1% and the herd 91.6%. The prevalence in cattle aged 0–1, 1–3 and >4 years was 23.8%; 31.4% and 51.4% respectively. The risk factors associated with the prevalence of disease were female sex (OR = 2.102 CI: 1.066–4.144), age older than 4 years (OR = 2.707 CI: 1.667–4.394), presence of canines on the farm (OR = 2.556 CI: 1.560–4.189) and retention of placenta (OR = 2.678 CI: 1.670–4.295). A high prevalence was identified, suggesting natural infection where the pathogen could be transmitted to humans at the animal-human interface.

## 1. Introduction

Chlamydias are Gram-negative obligate intracellular bacteria that cause various diseases in humans, mammals, and birds; currently, 13 species of the *Chlamydiaceae* family are recognized (Everett, 2000). The species *Chlamydia abortus* and *Chlamydia pecorum* affect ruminants causing abortions and reproductive problems that produce significant economic losses (Appino et al., 2015; Godin et al., 2008). The disease has a nonspecific clinical presentation, and clinical symptoms, such as pneumonia, enteritis, polyarthritis, sporadic encephalomyelitis, abortion, vaginitis, endometritis, repeat breeding, weak calf syndrome, perinatal mortality and fertility disorders have been reported (Kaltenboeck et al., 2005; Wehrend et al., 2005). The infection occurs by inhalation or consumption of contaminated pastures with residues of aborted fetuses or placenta (Kauffold et al., 2014).

The role of *Chlamydia* in reproductive diseases in bovine is not yet clear; therefore, further studies are required (Reinhold et al., 2011). The

presence of *Chlamydias* in the bovine oviduct has raised the question about its possible participation in infertility problems in cattle (Appino et al., 2015). Chlamydial infection could also be present subclinically, which leads to the debate about whether these organisms are really or commensal pathogens. *Chlamydia abortus* has been identified in suboptimal production of the livestock in the absence of other pathogens. These observations have suggested a role for *Chlamydia* in multifactorial diseases that involve interactions among low virulence pathogens, nutritional deficiencies, poor management and hygiene and host genetics (Reinhold et al., 2011).

In Colombia there are few reports of *Chlamydia* in ruminants. The only report dates back to 1995, and refers to a study carried out in cattle farms with reproductive problems, where a prevalence of *Chlamydia abortus* of 50% was identified (Otte et al., 1995). In sheep, a complement-fixing antibody test against *Chlamydia* sp. yielded 2.45% of positive reactions to the test in 2681 sera (Mogollón Galvis et al., 1981). Likewise, *C. abortus* has important zoonotic potential, mainly in pregnant

\* Corresponding author.

E-mail address: [blancalissethguz@hotmail.com](mailto:blancalissethguz@hotmail.com) (B. Guzmán-Barragán).

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women who work in field activities of infected farms, leading to sepsis that triggers miscarriage or premature birth (Baud et al., 2008; Longbottom and Coulter, 2003). Cases of *C. abortus* infections have been reported in pregnant women associated with ruminant production (Pichon et al., 2020; Pospischil et al., 2002).

Reproductive diseases in Colombia have a high impact, causing significant losses in the livestock sector (Betancur and Rodas, 2008; Moreno Figueredo et al., 2017; Pulido Medellín et al., 2017). Different studies have been carried out analyzing the presence of various pathogens and its association with reproductive problems; however, there are no recent studies of chlamydiosis in Colombia. The nonspecific course of the disease and the lack of diagnosis are aspects that contribute to the under-reporting of this disease in the country (Reinhold et al., 2011). The Orinoquia region has 21% of the Colombian bovine population, being an important cattle production center for the country's economy. The objective of this study was to establish the seroprevalence of *C. abortus* in cattle from the municipality of Villavicencio and to determine the associated risk factors.

## 2. Materials and methods

### 2.1. Study area and population

A cross-sectional epidemiological study was carried out in five villages and 24 herds of the dual-purpose system in the rural area of the municipality of Villavicencio. The municipality has an average altitude of 380 m above sea level, average annual temperature and relative humidity of 27 °C and 80% respectively, with a rainfall between 3,500 and 4,000 mm per year. The Instituto Colombiano Agropecuario (ICA) census reported a bovine population of 108,109 in the Villavicencio municipality (ICA, 2016) (Figure 1).

### 2.2. Sample size calculation

The sample size was determined following the procedure reported by Dohoo et al. (2003), with a hypothetical seroprevalence of 50% (Otte et al., 1995), 95% confidence and design effect of 1.4, for a sample of 536

animals according to the formula below, using Epiinfo™ version 7 (CDC) software.

$$n = \left(1 - (1 - CL)_d^{\frac{1}{d}}\right) \cdot \left(N - \frac{d-1}{2}\right)$$

where:

*n*: required sample size.

*N*: population size

*d*: expected number of diseased individuals in the population.

CL: confidence level expressed as proportion (=1- $\alpha$ ).

The samples were collected in 2015, and a stratified random sampling was carried out in the five villages and 24 herds.

### 2.3. Collection of samples

5.0 mL of coccygeal vein blood was collected from each animal in sterile tubes without anticoagulant (Vacutainer®) and each sample was identified with the general data of the animal and the animal code. These samples were preserved under refrigeration at 4 °C and were taken in isothermal cellars to the Animal Reproduction and Genetics Laboratory of the Universidad de los Llanos for processing.

### 2.4. ELISA test

The samples were centrifuged at 5,000 g for 10 min and the sera were collected and stored at -70 °C until analysis. The commercial Kit (IDEXX Chlamydiosis Total Ab, IDEXX Switzerland AG, Liebefeld-Bern, Switzerland) was used, following the manufacturer's instructions. The samples and the positive and negative controls were diluted 1: 400, and 100  $\mu$ L of the samples and controls were added to the cells in the plates, which were covered with adhesive paper and incubated at 37 °C for 1 h. Three automatic washings were done to each cell (Biotek Instruments Inc, ELX508, Winooski, vt, USA) and the residues of each plate were removed. 100  $\mu$ L of peroxidase-labeled protein G conjugate was dispensed, covering the plate with adhesive paper and incubating it for 1 h at 37 °C in a humid chamber. The plates were washed again three times

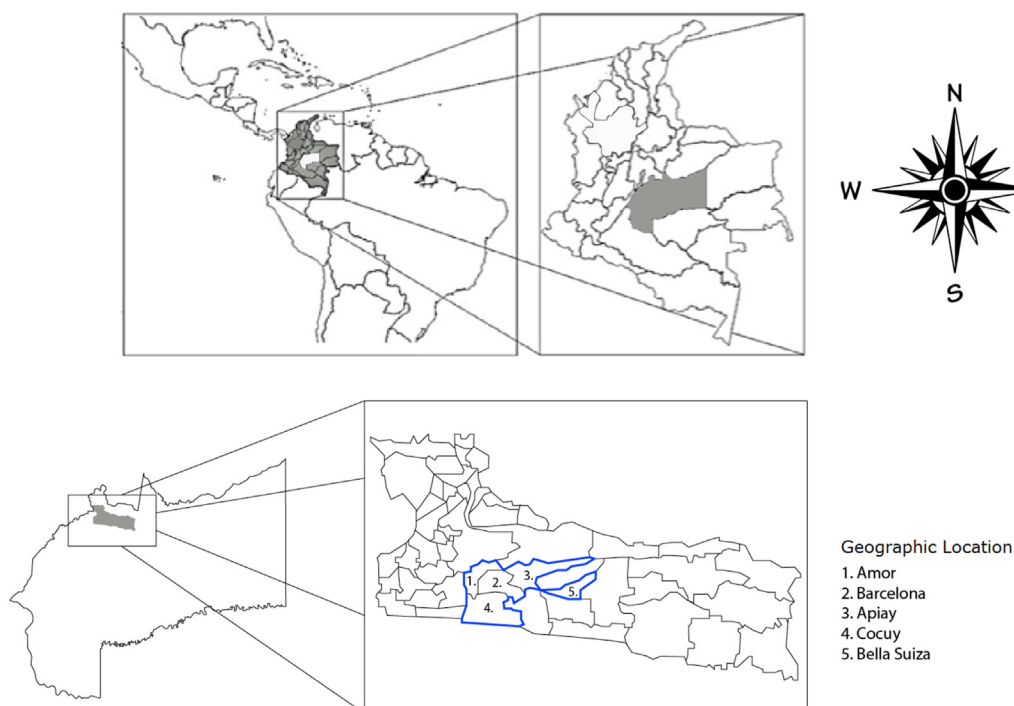


Figure 1. Locations of the study sites in municipality of Villavicencio, Colombia.

and 100  $\mu$ L of TMB substrate was added and incubated for 15 min at 18–26 °C. 100  $\mu$ L of a stopping solution was added to each well and finally reading optical density (OD) of serum samples was read at 450 nm, in a spectrophotometer (BioTeck® EON ELK 508 San Diego CA, USA). A serum was considered positive when the optical density (OD) of the sample was  $\geq$ 30% of that of the positive control serum. The cut-off point was 0.3575.

### 2.5. Epidemiological survey

Considering of the risk factors and the health conditions of the farms, an epidemiological survey was carried out to collect information on populations and domestic animal species, productive activities, technical assistance, sanitation, infrastructure and health events. Subsequently the questionnaire was evaluated and validated. The design and application of the questionnaire was carried out in coordination with the Colombian Health Authority ICA applying it to each of the studied farms with prior authorization.

### 2.6. Statistical analysis

The seroprevalence was determined by dividing the number of seropositive animals by the number of sampled animals with 95% of confidence intervals (CI) for the prevalence values, using the Epiinfo™ version 7 (CDC) software to analyze the data. The univariable analysis was performed using Pearson's Chi-square test to assess the relationship between *C. abortus* and the variables; those variables with  $p \leq 0.05$  in the univariable analysis were included in the multivariable logistic regression model. Statistical analyzes were performed using Epiinfo™ Version 7 (CDC) software.

### 2.7. Ethical statement

The animals used in this study received handling and treatment under qualified veterinary supervision following the animal experimentation rules described in the International Guiding Principles for Veterinary Research Involving Animals. The owners of the animals signed an informed consent before their inclusion in the study, and the personal or farm information was treated according to the Habeas Data Colombian laws. The study was approved by the Ethics Committee of the Faculty of Agricultural Sciences of the Universidad de Ciencias Aplicadas y Ambientales (UDCA) Nr. 001–2019.

## 3. Results

536 samples were collected, of which 514 were viable for serological testing. The population consisted in 472 females and 42 males, considering the age of 42 animals under 1 year, 54 between 1 and 3 years and 418 over 4 years of age (Table 1). The general *C. abortus* seroprevalence was 47.1% (95% CI:41.4–53.3%) and the herd was 91.6%. The seroprevalence in both females and males was 48.5% (95% CI:42.5–53.7%) and 30.9% (95% CI:17.2–51.6%), respectively. Prevalence was estimated by age group: 23.8% prevalence was identified in animals under 1 year (95% CI:12.0–42.4%), 31.4% in animals between 1 to 3 years (95% CI:18.9–49.3%), and 51.4% in animals over 4 years of age (95% CI: 44.9–58.8%). The village with the highest seroprevalence was the one in the neighborhood of Cocuy with 64.2.2% (95% CI: 47.0–85.6%) and Amor with 51.0% (95% CI: 38.9–68.3%) (Figure 2).

At animal level, the risk factors considered were female sex (OR = 2.102 CI:1.066–4.144), age older than 3 years (OR = 2.707 CI: 1.667–4.394), presence of dog on the farm (OR = 2.556 CI:1.560–4.189) and retention of placenta (OR = 2.678 CI:1.670–4.295). Male sex (OR = 0.476 CI:0.491–0.925), animals younger than 1 year (OR = 0.549 CI:0.410–0.734), Angus breed (OR = 0.549 CI:0.410–0.734), and Gyr breed (OR = 0.549 CI:0.410–0.734) were considered as protective factors (Table 2). Among the symptoms, diarrhea (OR = 0.590

**Table 1.** Seroprevalence of *Chlamydia abortus* in cattle according to population characteristics from Villavicencio, Colombia.

General variables	Total animals	Positive Animals	Prevalence (%)	IC 95%
<b>Sex</b>				
Female Sex	472	229	48.52	42.4–55.1
Male sex	42	13	30.95	17.2–51.6
<b>Age ranges</b>				
Under 1 year old	42	10	23.81	12.0–42.4
Between 1 to 3 years	54	17	31.48	18.9–49.3
Over 4 years old	418	215	51.44	44.9–58.6
<b>Village</b>				
Amor	94	48	51.06	38.9–68.3
Barcelona	122	57	46.72	36.9–61.5
Apiay	38	10	26.32	13.3–46.9
Bella suiza	193	84	43.52	34.9–53.6
Cocuy	67	43	64.18	47.0–85.6

CI:0.416–0.837) and respiratory diseases (OR = 0.494 CI:0.278–0.879) showed no association with the disease. In the analysis of the relation between biosecurity practices and the disease, risk factors considered were natural riding bull (OR = 1.995 CI:1.282–3.102), poor disposal of dead animals (OR = 2.001 CI: 1.207–3.402), inadequate food storage (OR = 2.009 CI:1.404–2.885), sale and purchase of animals (OR = 1.913 CI:1.761–2.079). The corrals in the farm (OR = 0.233 CI:0.085–0.638) and individual needle usage (OR = 0.417 CI:0.25–0.680) were considered as protection factors (Table 3) (see Table 4).

The multivariate analysis identified bovine over 4 years of age (OR = 1.872 CI:0.365–2.649), placenta retention (OR = 2.348 CI:1.406–3.856), bad disposition of dead animals (OR = 2.956 CI:1.477–10.058) and improper food storage (OR = 1.896 CI:0.285–2.923) as risk factors. The estimated protective factor was the Gyr breed (OR = 0.347 CI:0.046–2.604) (Table 4).

## 4. Discussion

This study is the first report in Colombia of *Chlamydia abortus* seroprevalence with a prevalence of 47.0% and the herd 91.6%. The presence of *C. abortus* in cattle has been studied in several countries; however, its role in reproductive problems and its impact in economic losses is still unknown (Borel et al., 2018). The present study identified seroprevalence in bovines that coincides with reports in other countries. In Taiwan a seroprevalence of 51.3% for healthy cows and 71.4% for aborted cows was reported (Wang et al., 2001); in Poland, 1,333 animals were studied using CTF and ELISA techniques, identifying a seroprevalence of 19.3% (Niemczuk, 2005). In Bosnia and Herzegovina, the identification of etiological agents associated with reproductive problems was studied in 1,970 animals, identifying *C. abortus* with a seroprevalence of 52.1% (Softic et al., 2018) as the most frequent; in Sweden, 525 animals from 70 dairy herds were studied, finding a seroprevalence of 28% (Godin et al., 2008); in Jordan a 19.9% prevalence in individuals was determined and 66.3% of the herds were positive (Talafha et al., 2012) from which it is deduced that the bacterium is ubiquitous in cattle, as suggested by Kaltenboeck et al. (2005). In Latin America, a similar seroprevalence has been determined in Peru with 40.34% (Loli-Pin et al., 2021); in Brazil a 34.0% seroprevalence in individuals and 79.8% in herd was reported (da Silva Neto et al., 2021); on the contrary, in Costa Rica a study of 608 samples in bovines identified a seroprevalence of only 0.5% in (Fonseca et al., 2015). In Ireland a low prevalence was also reported in a study where a value of 4.4% was determined in 100 herds (20 samples/herd) (Wilson et al., 2012).

The high seroprevalence found in Colombia suggests natural infection, given the absence of a vaccination history or commercial



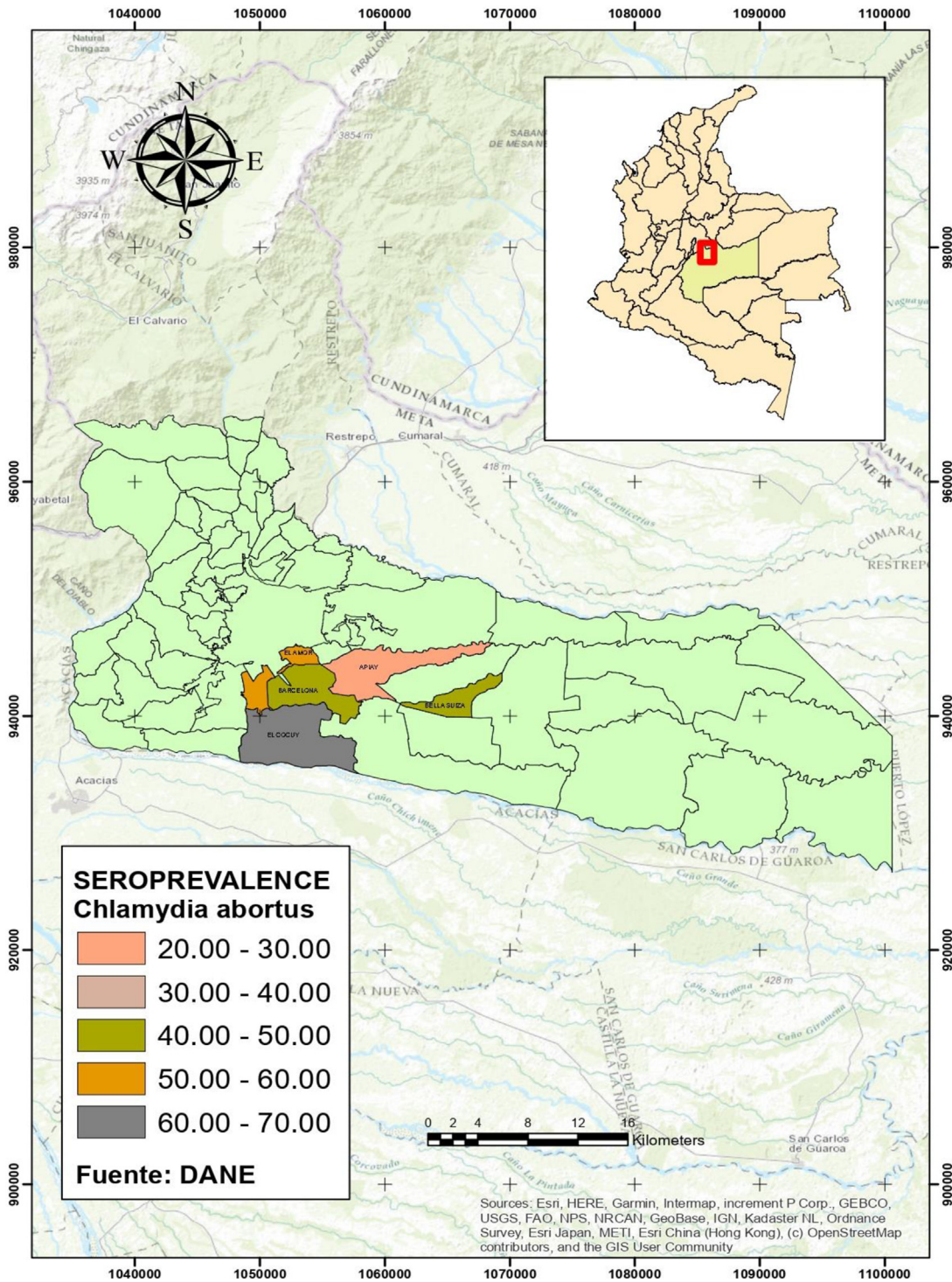


Figure 2. Seroprevalence of antibodies to *Chlamydia abortus* in cattle of Villavicencio, Colombia.

availability of vaccines. The role of *C. abortus* in reproductive problems in bovines has been underestimated (Reinhold et al., 2011); therefore, yet it is not considered a causal agent of reproductive problems object of official health programs and interventions in the country. This study highlights the need to advance in the knowledge of *C. abortus* and its involvement in reproductive problems. Considering the increase in the number of reports about the participation of this bacterium in bovine abortion syndrome, it is worth noting that 50% of the cases ended without a definitive diagnosis (Kauffold et al., 2007; Rojas et al., 2018). Additionally, this is a serious public health problem due to the

participation of *C. abortus* in women abortion (Essig and Longbottom, 2015; Longbottom and Coulter, 2003; Pospischil et al., 2002).

In the context of the present study, it is important to consider the eventual presence of cross-reactions and co-infections with other *Chlamydia* species such as *C. pecorum* and *C. psittaci* (Barati et al., 2017; Wang et al., 2001). In Germany, the presence of *Chlamydia* sp. was detected in samples of vaginal secretions from cows, where the highest prevalence was *C. psittaci* (56%), followed by *C. abortus* (37%) and *C. pecorum* (8%) (Kemmerling et al., 2009). In another study, coinfection between *C. abortus* and *C. psittaci* was reported in 30% of the samples (Barati et al.,

**Table 2.** Analysis of risk factors associated with the seroprevalence of *Chlamydia abortus* in cattle from Villavicencio, Colombia.

General variables	X <sup>2</sup>	P	OR	IC
Female Sex	4,776	0,029*	2,102	1,066–4,144
Male sex	4,776	0,029*	0,476	0,241–0,938
Under 1 year old	9,942	0,002*	0,323	0,155–0,673
Between 1 to 3 years	3,259	0,071	0,554	0,289–1,060
Over 4 years old	17,03	0,000*	2,707	1,667–4,394
Jersey breed	1,257	0,262	0,657	0,314–1,375
Holstein breed	0,000	0,984	0,995	0,593–1,670
Pardo Brown breed	0,724	0,395	1,180	0,806–1,726
Simental breed	0,055	0,814	0,936	0,538–1,626
Angus breed	3,804	0,051*	0,547	0,296–1,010
Gyr breed	9,436	0,002*	0,497	0,317–0,781
Zebu breed	1,601	0,206	0,786	0,542–1,142
Criollo breed	0,534	0,465	0,858	0,569–1,294
Presence of goats on the property	2,626	0,105	2,588	0,787–8,514
Presence of pig on the property	1,293	0,255	0,756	0,466–1,226
Presence of equines on the property	0,002	0,969	1,012	0,555–1,846
Presence of canines on the property	14,494	0,001*	2,556	1,560–4,189
Abortion	0,891	0,345	0,830	0,563–1,222
Placenta retention	17,48	0,001*	2,678	1,670–4,295
Dystocic births	0,167	0,683	1,075	0,759–1,523
Diarrhea	8,789	0,003*	0,590	0,416–0,837
Fever	0,098	0,754	1,059	0,741–1,512

Animals 514; Herds: 24.

OR: Odds Ratio; CI: Confidence Interval (95%), \* statistically significant.

**Table 3.** Biosafety practices factors associated with the seroprevalence of *Chlamydia abortus* in cattle from Villavicencio, Colombia.

General variables	X <sup>2</sup>	P	OR	IC
Manual milking	0,074	0,786	0,944	0,620–1,435
Mechanical milking	3,406	0,065	0,645	0,404–1,030
The property does not have corrals	9,395	0,002*	4,286	1,626–13.1
Individual needle	12,77	0,001*	0,417	0,255–0,680
Direct riding bull	9,582	0,002*	1,995	1,282–3,102
Artificial insemination	1,128	0,288	0,828	0,585–1,173
Entry of vaccinated animals	0,320	0,572	0,900	0,623–1,298
Bad disposition of dead animals	7,331	0,007*	2,001	1,207–3,402
Improper food storage	6,124	0,013*	1,637	1,106–2,415
Hay feeding	3,584	0,058	0,676	0,450–1,015
Feeding with concentrate	1,109	0,292	0,801	0,530–1,211
Pasture lease	2,272	0,132	0,315	0,065–1,533

Animals 514; Herds: 24.

OR: Odds Ratio; CI: Confidence Interval (95%), \* statistically significant.

**Table 4.** Multivariable logistic regression analysis of variables associated with *Chlamydia abortus* seroprevalence in cattle from Villavicencio, Colombia.

General variable	$\beta$	Exp(B)	p	C.I (95%)
Over 4 years old	0.627	1.872	0.067*	0.365–2.649
Placenta retention	0.845	2.328	0.001*	1.406–3.856
Gyr breed	-1,057	0.347	0.040*	0.046–2.604
Bad disposition of dead animals	0.307	2.956	0.006*	1.477–10.058
Improper food storage	0,593	1.896	0.058*	0.285–2.923

Potential risk factors ( $P < 0.1$ ) were selected for inclusion in the multivariable model.

OR: Odds Ratio; C.I: confidence interval (95%). \*statistically significant.

2017). It is necessary to conduct further research, in order to discriminate the levels of antibodies attributable to each species of *Chlamydia* involved in cross-reactions, as well as to integrate studies using molecular tests, such as the polymerase chain reaction (PCR) technique, especially in tissues, as suggested by Berri et al. (2009) and Opota et al. (2015).

The results of the risk factors analysis suggest higher seropositivity according to age, in agreement with the findings of Hireche et al. (2014) and Talafha et al. (2012), who reported a higher probability of infection with increasing age. A higher risk was observed in females; however, to the best of our knowledge there is no study addressing this aspect, so further research is required in this regard. This study identified the Angus and Gyr breeds as protective factors possibly associated with better management and sanitary practices frequently used in high value breed animals. In Bosnia and Herzegovina Softic et al. (2018) identified the association between seropositive animals and the Red Angus breed, stating that these animals are kept for several months in overcrowded stables, which promoted the contact between animals and their exposure to contamination; therefore, the handling conditions of the animals is a fundamental aspect that can influence the transmission of the disease.

The present exploratory study established an association between *C. abortus* and retention of the placenta, but the association with the presence of abortion was not significant. There are reports in Brazil that relate the serological response to clinical problems, where the prevalence of antibodies in cows with a history of abortions (N = 3,102) in 373 farms analyzed by the complement fixation test was 8.82% (Silva-Zacarias et al., 2009). In Sweden, cows with reproductive disorders (N = 525) of 70 dairy farms analyzed using the Chekit ELISA were studied reporting that in 28% of them no differences between cases (history of abortion) and controls (Godin et al., 2008) were found. In the Pampa-Argentina in samples of abortions and stillbirths (N = 252), *C. abortus* DNA was detected in 12% of the samples (4.78%) of which 83.3% (10/12) corresponded to abortions and 16.7% (2/12) to stillbirths (Rojas et al., 2018). A study of diagnosis of *C. abortus* in clinical samples of abortions in southern Iran, using PCR, reported 66 (56.4%) positive samples, of which 36.6% were positive for *C. abortus* and 30% were coinfecting with *C. abortus* and *C. psittaci* (Barati et al., 2017). In Taiwan, 71% of aborted cows had IgG antibodies, although only 22.7% of them were confirmed by PCR in vaginal discharge samples (Wang et al., 2001).

*Chlamydia* affect various mammals and birds causing a wide spectrum of diseases that are transmissible among species (Longbottom and Coulter, 2003). The presence of canines was a risk factor in this study; similar results were observed in Ethiopia (Alemayehu et al., 2021). Different studies have shown the presence of *C. abortus* in horses, goats, pigs, foxes and birds (Origlia et al., 2019; Rubio-Navarrete et al., 2017; Schautteet and Vanrompay, 2011). More recently, its presence was reported in wild animals in captivity in the suburbs of the city of Changchun, China (Li et al., 2018), these findings evidencing the possibility of multiple transmission routes. Ovines are usually an important source of herd infection; however, their presence was not a significant risk factor in this study, maybe because their presence was scarce (4%).

The present study showed natural breeding as a risk factor. This factor has been discussed in several studies pointing out to a possible venereal transmission of the disease. In Germany, a study in bulls detected the three species of *Chlamydia*: *C. psittaci*, *C. pecorum* and *C. abortus* in the semen and foreskin washes (Kauffold et al., 2007). Also in Germany, in an epidemiological study of risk it was found that the use of bull was a risk factor (Kemmerling et al., 2009). In Sweden, artificial insemination was considered a low risk factor (Karlsson et al., 2010). In addition, it is believed that, to produce infection, a high bacterial load is required, which can be affected by the dilution of semen during the production process (Kauffold et al., 2007).

Other variables associated with sanitary and hygienic conditions were statistically significant; the lack of corrals, improper food storage and inadequate disposal of corpses were identified as risk factors, while the use of individual needles was considered as a protection factor. These results are similar to those reported by Godin et al. (2008); Hireche et al.

(2014), Kemmerling et al. (2009) and Talafha et al. (2012), reaffirming the influence of the sanitary conditions in the transmission of the disease.

## 5. Conclusions

The present study shows the presence of *C. abortus* antibodies in bovines in Colombia, with a high seroprevalence, suggesting natural infection, where the pathogen could be transmitted to humans at the animal-human interface. The presence of *C. abortus* antibodies was associated with risk factors at the animal level, namely, female sex, age over 4 years, presence of canines on the farm and retention of placenta. Management and biosecurity practices were shown to be protective factors associated with *C. abortus*. It is important to advance in the knowledge of the role of this bacterium in abortions and reproductive problems, as well as in its zoonotic potential.

## Declarations

### Author contribution statement

Agustín Góngora Orjuela, Leidy J Reyes Castañeda, Julio César Tobón: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Blanca Guzmán-Barragán: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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