One Health 19 (2024) 100909

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

A first insight into seropositivity and risk factors for *Brucella* spp. and *Coxiella burnetii* in free-roaming dogs in Ecuador

Angel Sebastian Rodriguez-Pazmiño [a,1], Carla M. Brito [b,1], Mauricio Salas-Rueda [c], Solon Alberto Orlando [d,e], Miguel Angel Garcia-Bereguiain [a,*]

[a] One Health Research Group, Universidad de las Américas, Quito, Ecuador
[b] Yachay Tech University, Urcuquí, Ecuador
[c] Universidad Politécnica Salesiana, Cuenca, Ecuador
[d] Instituto Nacional de Salud Pública e Investigación, Guayaquil, Ecuador
[e] Universidad Espíritu Santo, Guayaquil, Ecuador

ARTICLE INFO

Keywords:
Brucella abortus
Brucella
Brucellosis
Coxiella burnetii
Q fever
One health
Free-roaming dogs
Ecuador

ABSTRACT

Brucellosis and Q fever are two bacterial zoonoses caused by *Brucella* spp. and *Coxiella burnetii*, respectively. Dogs are reservoirs of these pathogens and play an important role in their spread. In this research, we determined the seroprevalence of antibodies against *Brucella* spp. and *C. burnetii* in free-roaming dogs from Ecuador and conducted a statistical analysis based on geographical variables. Serum samples were collected from 397 free-roaming dogs between November 2018 and May 2019 and analyzed with commercial ELISA tests for *Brucella* spp. and Q fever. An overall seroprevalence of 2.8 % (CI: 95 %, 0.0–6.2 %) and 1.8 % (CI: 95 %, 0.0–5.6 %) was found for *Brucella* spp. and *C. burnetii*, respectively. No statistical differences in seroprevalence values were found between geographical regions in Ecuador or between dogs from rural or urban settings, except for the association of *C. burnetii* infection with the Coastal Region. This is the first study of this kind in Ecuador and points out the need for a One Health approach for control and surveillance of zoonotic diseases like brucellosis and Q fever including feral and stray dogs as reservoirs to spread those pathogens to cattle, humans, or wildlife.

1. Introduction

Brucellosis and Q fever are bacterial zoonosis with a worldwide distribution [1,2] and affect an ample range of vertebrates [3–5]. These pathogens can be transmitted to humans by direct contact with tissue or fluids from infected animals or by consumption of raw animal products [4–6]. The genus *Brucella*, responsible for brucellosis, has twelve recognized species [7] including *B. abortus*, *B. melitensis*, *B. suis,* and *B. canis* which can infect animals and humans [8]. Brucellosis usually provokes flu-like symptoms, including fever, weakness, malaise, and weight loss, or even may present in several unusual forms [9]. On the other side, *Coxiella burnetii* is responsible for causing Q fever [10]. A severe human Q fever can trigger hepatitis or pneumonia, while a chronic condition could produce endocarditis [11]. Both diseases require mandatory notification to animal health regulatory agencies due to their socioeconomic or public health repercussions [12].

Of the 67 countries that reported human brucellosis cases in 2019, a global prevalence of 5.95 cases per 100,000 inhabitants was obtained [21]. Human brucellosis is most widespread in developing countries across South America, Central Asia, the Mediterranean, and the Middle East [9]. Countries like Argentina, Mexico, Brazil, and Colombia reported a high prevalence of human brucellosis, especially associated with occupational risk groups like veterinarians [22,23,26]. In Ecuador, human brucellosis reports are very scarce, with two studies finding seroprevalence values between 1 and 2 % in reduced convenience samples. [24,25]

A systematic review of human Q fever in 27 countries documented that most outbreaks occurred in communities outside areas traditionally considered at risk, although associated with living near livestock farms [32]. Human Q fever studies in Latin America are scarce, with no reports for many countries [33]. Interestingly, French Guiana has the highest reported incidence of Q fever globally, with an overall incidence of 223 cases per 100,000 inhabitants [34]. Also, one study from Ecuador has reported a seroprevalence of Q fever in 34 % of farm workers [35].

* Corresponding author.
E-mail address: magbereguiain@gmail.com (M.A. Garcia-Bereguiain).
[1] These authors have contributed equally.

https://doi.org/10.1016/j.onehlt.2024.100909
Received 25 July 2024; Received in revised form 3 October 2024; Accepted 3 October 2024
Available online 5 October 2024
2352-7714/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Livestock such as cows, goats, or pigs frequently serve as a reservoir for *Brucella* spp. and *Coxiella burnetii* [3,13]. They potentially pose an occupational transmission risk for farmers or veterinarians, so it is necessary to eliminate infected animals from the herds [14–16]. Additionally, these infections can cause abortions, infertility, and reduced milk production in livestock, generating economic losses for farmers [17–20]. A systematic review determined that the seroprevalence of bovine brucellosis in Latin America and the Caribbean was 4.0 %, ranging from free status in Chile to 16.0 % in Venezuela [27]. Recent studies carried out in Ecuador, found brucellosis seroprevalence in cattle of 2.63 % to 17 %, and 11,5 % to 45.1 % at at animal and herd level, respectively [25,28,29,31]; and 17.8 % in goats [30].

The worldwide seroprevalence of *C. burnetii* in cattle was estimated at 14.9 %; it is most prevalent in Africa at 21.7 %, followed by Asia at 16.3 %, America at 14.4 %, Europe at 12.7 %, and Oceania at only 0.2 % [36]. In Latin America and the Caribbean, a meta-analysis study determined a wide variability ranging from 0 to 67 % for the 20 countries analyzed. [37] There are three recent studies in Ecuador reporting seroprevalence values for *C. burnetii* of 12.6 %, 43 % and 52.9 % in cattle [35,38,39].

Dogs can be carriers and reservoirs of *Brucella* spp. and *C. burnetii* for the spillover of those pathogens. Brucellosis in dogs is endemic to America, Asia, and Africa, with *B. canis* the most common cause of canine brucellosis [40]. Infections with *B. melitensis*, *B. abortus*, or *B. suis* can also take place in contact with tissues or secretions of infected cattle. [41,42] Indeed, *B. abortus* infection has been described as more common in dogs that share habitats with livestock and wildlife [43]. A seroprevalence of 30.46 % for *Brucella* spp. in a convenience sample of 151 dogs from farms in Ecuador has been reported recently [44]. About Q fever, animal reservoirs, and transmission dynamics are still largely unknown [45], although seroprevalence of *C. burnetii* infection in dogs has been reported in Argentina, Brazil, and French Guiana, with values ranging from 1.8 to 21.7 % [46–49]. Notably, in Brazil and Guyana, positive dog cases have been found around cases of human Q fever, although these appear not to be the origin of those human outbreaks [46,50,51]. No previous serological study of Q fever in dogs has been conducted in Ecuador.

In low and middle-income countries like Ecuador, the role of dogs as reservoirs for the spread of zoonotic diseases is a matter of concern as there are extensive populations of free-ranging dogs either in urban or urban settings, in interaction with humans, livestock, and wildlife. [52–54] Under this scenario, we aimed to investigate for the first time the seroprevalence of *Brucella* spp. and *C. burnetii* infection in free-roaming dogs from Ecuador.

2. Methodology

2.1. Study design

This cross-sectional study included a convenience sample of free-roaming dogs recruited in 2018 and 2019 during spay and neuter campaigns. A total number of 397 dogs were included, distributed in the following provinces and cantons (Fig. 1): 80 dogs from Imbabura province (Andean Region; 49 dogs from Urcuquí canton and 31 dogs from Ibarra canton); 170 dogs from Azuay province (Andean region; 82 dogs from Santa Isabel canton and 88 dogs from Paute canton); 68 dogs from Guayas province (Coastal region; 31 dogs from Guayaquil canton and 37 dogs from Daule canton); 40 dogs from Santo Domingo province (Coastal region; 40 dogs from Santo Domingo canton); and 39 dogs from Napo (Amazon region; 39 dogs from Tena canton).

2.2. Sample collection

A veterinarian collected 5 ml of blood from the cephalic vein in a red-top tube with a serum clot activator. The samples were kept in a refrigerated container and taken to the lab. After the clotting process, 1 ml of serum was collected and transferred into a cryovial tube. Serum samples were maintained at −20 °C until their analysis.

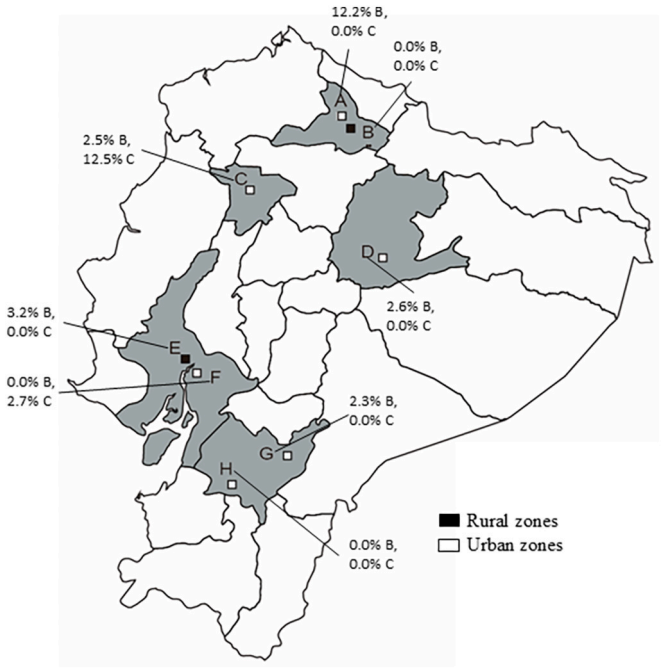

Fig. 1. Distribution of the dogs included in the study by region and canton in Ecuador, and seropositivity occurrence for *Brucella spp.* (B) and *C. burnetii* (C). Black and white boxes represent the urban and rural zones, respectively. Coastal region: C. Santo Domingo, E. Guayaquil, F. Daule; Andean region: A. Urcuquí, B. Ibarra, G. Paute, H. Santa Isabel; Amazon region: D. Tena.

2.3. Serology

The commercial indirect ELISA kits, "*ID Screen® Brucellosis Serum Indirect*" and "*ID Screen® Q Fever Indirect Multi-species*" (IDVet, France), were used to identify antibodies against *Brucella* spp. (*B. abortus, B. melitensis,* or *B. suis*) and *C. burnetii*, respectively. The procedure was carried out per the manufacturer's manual instructions, considering all 397 samples for *Brucella* spp. antibodies detection, and 339 samples for *C. burnetii* antibodies detection (here, 58 samples were discarded due to low serum availability). The kit's manufacturer provided the diagnostic test's sensitivity and specificity of 100 % (CI 95 %: 89.57–100 %) and 99.74 % (CI 95 %: 99.24–99.91 %), respectively, for *Brucella* spp. antibodies; and 100 % (CI 95 %: 89.28–100 %) and 100 % (CI 95 %: 97.75–100 %), respectively, for *C. burnetii* antibodies.

The brucellosis IDVet kit has been validated in detecting antibodies for *B. abortus* (in cattle), *B. melitensis* (in sheep and goats), and *B. suis* (pigs), and the Q fever IDV kit has been validated in detecting antibodies for *C. burnetii* in sheep, cows, and goats. However, according to information provided by the supplier, as the kits use a conjugate that recognizes anti-mammal antibodies, the antibodies of these pathogens in dogs would be detected.

2.4. Statistical analysis

We conducted a statistical analysis using the *chi-squared* and *Fisher's exact* test to compare the frequency of *Brucella* spp. and *C. burnetii* antibodies in free-roaming dogs in urban and rural areas, between regions, and at the canton level. These analyses used the number of positives and negatives to create contingency tables. The statistical significance was set at $p < 0.05$, and a confidence interval (95 %) was calculated for the overall seropositivity result, considering seropositivity percentage values from each canton according to location by region and zone. Due

to the limited size of the sample, the confidence interval was not calculated for the Amazon region (only one canton was sampled here). We used these tests because some of the comparisons involved tables with small cells (where Fisher is more appropriate), while others presented expected values more appropriate for chi-square. Both tests were chosen to ensure the validity of the results based on the structure of the data. The data were analyzed using R software (version 4.2.1.) [55].

3. Results

In this study, 397 serum samples were collected from free-roaming dogs in urban and rural areas of Ecuador across the three continental regions (Coastal, Andean, and Amazon). These samples were tested using ELISA test kits to detect antibodies against *Brucella* spp. (*B. abortus, B. melitensis,* or *B. suis*). Additionally, 339 of the samples were tested for *Coxiella burnetii* antibodies, with 58 samples from the initial group being excluded due to insufficient serum volume.

3.1. Seropositivity of Brucella spp. in free-roaming dogs and statistical analysis for geographical location and urban/rural condition

Of the 397 free-roaming dogs, eleven tested positive for *Brucella* spp. antibodies, resulting in an overall seroprevalence of 2.8 % (CI: 95 %, 0.0–6.2 %) (Supplementary Data). Table 1 presents the seropositivity values for each geographical region and canton. The seropositivity values by canton were Guayaquil 3.2 %, Daule 0.0 %, Santo Domingo 2.5 %, Ibarra 0.0 %, Urcuquí 12.2 %, Santa Isabel 0.0 %, Paute 2.3 %, and Tena 2.6 %. The seropositivity values for the Coastal, Andean, and Amazon regions were 1.9 %, 3.2 %, and 2.6 %, respectively. The chi-squared test yielded a *p*-value of 0.7727, and Fisher's exact test resulted in a p-value of 0.8947.

Table 1 also summarizes the results of the seropositivity comparison between dogs from urban and rural areas. The seropositivity rate in urban areas was 1.6 %, compared to 3.0 % in rural areas. The chi-squared test gave a p-value of 0.854, and Fisher's exact test gave a p-value of 1.0.

3.2. Seropositivity of C. burnetii in free-roaming dogs and statistical analysis for geographical location and urban/rural condition

Of the 339 free-roaming dogs considered here, six tested positive for *C. burnetii* antibodies, resulting in an overall frequency of 1.8 % (CI: 95 %, 0.0–5.6 %) (Supplementary Data). Table 2 shows the seropositivity values for each geographical region and canton. The seropositivity values by canton were Guayaquil 0.0 %, Daule 2.7 %, Santo Domingo 12.5 %, Ibarra 0.0 %, Urcuquí 0.0 %, Santa Isabel 0.0 %, Paute 0.0 %, and Tena 0.0 %. The seropositivity values for the Coastal, Andean, and Amazon regions were 5.6 %, 0.0 %, and 0.0 %, respectively. The chi-squared test yielded a *p*-value of 0.001456, and Fisher's exact test gave a p-value of 0.003791.

Table 2 summarizes the seropositivity results comparing dogs from urban and rural areas. The seropositivity rate in urban areas was 0.0 %, while in rural areas it was 2.2 %. The chi-squared test yielded a p-value of 0.524, and Fisher's exact test gave a p-value of 0.597.

4. Discussion

This serological analysis represents the first report from a country-wide sample of free-roaming dogs in Ecuador for *Brucella* spp. (*B. abortus, B. melitensis,* or *B. suis*) and *C. burnetii* infection. The overall seropositivity of *Brucella* spp. was 2.8 %, a relatively low value compared to other reports in dogs published for countries of the region that were mainly focused on dog populations from farms or rural areas [40]. For instance, the seroprevalence values reported for *Brucella* spp. in farm and rural dogs range from 26 to 31 % in Argentina [56,57], and 8–23 % in Brazil [43,58]. Likewise, a conference paper reported a 30.46 % seropositivity for *B. abortus* in dogs from farms in Ecuador [44]. In contrast, surveillance studies done for urban dogs or owned dogs inhabiting islands and coastal areas in Brazil did not find *Brucella* spp. [59,60] On the other hand, our overall seropositivity of *C. burnetii* was 1.8 %, which is also a low value compared to the results of the few similar studies conducted in the region. In Argentina, Brazil, and French Guiana, dogs were seropositive for *C. burnetii* in a range of 0 to 21.7 % [46–49,61].

Although we recruited a large sample size of 397 free-roaming dogs, the main limitation of our study was the sampling approach done at convenience; geographical bias cannot be ruled out, particularly in the case of the Amazon region, where only a location contributed 39 dogs to the sampling. Therefore, further research would be required to address the zoonotic risk for brucellosis and Q-fever associated with free-roaming dogs in different regions of Ecuador. Also, further studies including owned dogs are necessary to confirm the higher risk associated with stray dogs for those zoonotic diseases [62]. For these reasons,

Table 1
Occurrence values on seropositivity of *Brucella* spp. in dogs from Ecuador according to location. Confidence intervals (CI) at 95 % are also included when these were possible to calculate. The chi-squared test yielded a *p*-value of 0.7727, and Fisher's exact test resulted in a p-value of 0.8947 for the comparison of regions. The chi-squared test produced a p-value of 0.854, and Fisher's exact test yielded a p-value of 1.0 for the comparison of urban and rural areas.

Region	Canton	*Brucella* spp. dogs' seropositivity		
		Urban % (n/total)	Rural % (n/total)	Total % (n/total; CI 95 %)
Coastal	Guayaquil	3.2 % (1/31)	–	1.9 % (2/108; 0.0–6.1 %)
	Daule	–	0.0 % (0/37)	
	Santo Domingo	–	2.5 % (1/40)	
Andean	Ibarra	0.0 % (0/31)	–	3.2 % (8/250; 0.0–12.9 %)
	Urcuquí	–	12.2 % (6/49)	
	Santa Isabel	–	0.0 % (0/82)	
	Paute	–	2.3 % (2/88)	
Amazon	Tena	–	2.6 % (1/39)	2.6 % (1/39; xx-xx%)
Total % (n/total; CI 95 %)		**1.6 % (1/62; 0.0–21.9 %)**	**3.0 % (10/335; 0.0–8.0 %)**	**2.8 % (11/397; 0.0–6.2 %)**

Table 2
Occurrence values on seropositivity of *C. burnetii* in dogs from Ecuador according to location. Confidence intervals (CI) at 95 % are also included when these were possible to calculate. The chi-squared test yielded a p-value of 0.001456, and Fisher's exact test resulted in a p-value of 0.003791 for the comparison of regions. The chi-squared test produced a p-value of 0.524, and Fisher's exact test yielded a p-value of 0.597 for the comparison of urban and rural areas.

Region	Canton	*C. burnetii* dogs' seropositivity		
		Urban % (n/total)	Rural % (n/total)	Total % (n/total; CI 95 %)
Coastal	Guayaquil	0.0 % (0/31)	–	5.6 % (6/108; 0.0–21.4 %)
	Daule	–	2.7 % (1/37)	
	Santo Domingo	–	12.5 % (5/40)	
Andean	Ibarra	0.0 % (0/31)	–	0.0 % (0/192; 0.0–0.0 %)
	Urcuquí	–	0.0 % (0/49)	
	Santa Isabel	–	0.0 % (0/44)	
	Paute	–	0.0 % (0/68)	
Amazon	Tena	–	0.0 % (0/39)	0.0 % (0/39; xx-xx%)
Total % (n/total; CI 95 %)		**0.0 % (0/62; 0.0–0.0 %)**	**2.2 % (6/277; 0.0–7.8 %)**	**1.8 % (6/339; 0.0–5.6 %)**

the association of *C. burnetii* infection with dogs from the Coastal region found in our study should be taken very cautiously. Moreover, a striking lack of statistical differences for *Brucella* spp. and *C. burnetii* seropositivity between rural and urban dogs was found in our study. As a high prevalence of these pathogens has been described in rural locations and farm dogs, [28–31,35,38,39] further studies with larger sample sizes would be needed in Ecuador to confirm or exclude this association in free-roaming dogs in Ecuador.

The large number of free-roaming dogs in the context of low and middle-income countries like Ecuador represents a challenge for public health, animal production, and wildlife conservation that needs to be addressed from a One Health perspective [52–54]. Like many countries in the region, rural communities in Ecuador significantly base their economy on livestock production not only for business but also to meet domestic demand [63]. Small, medium, and large production farms are usually located on the outskirts of cities or in rural areas, where dogs often roam freely in search of food. Recent reports have highlighted the occupational risk of brucellosis and Q fever for farmers and slaughterhouse workers in Ecuador. [38,68] In addition, climatic and geographical factors can also facilitate the spread of these infections in the context of tropical settings like Ecuador. For instance, a strong correlation between rainfall and positive cases of Q fever in humans has been found in French Guiana [46]. So far, animal health policies for surveillance and control of zoonotic diseases should consider the role of dogs as reservoirs.

Furthermore, *Brucella* spp. and *C. burnetii* infection have been described in a wide variety of wild mammals, including wild boars, feral pigs, rodents, rabbits, foxes, bears, opossums, foxes, raccoons, seals, sea lions, or dolphins [63,64]. Given the high biodiversity of wild mammals in Ecuador, feral dog populations pose a risk of zoonotic transmission to wildlife and threaten the conservation of endangered species [65–67]. This is especially relevant for pathogens like *Brucella* spp. and *C. burnetii* that affect the fertility and reproduction of livestock and wild mammals [17–19,65].

In conclusion, this study reports the exposure of *Brucella* spp. and *C. burnetii* in free-roaming dogs from Ecuador through antibody detection against those pathogens. Sentinel surveillance of zoonotic diseases should consider free-roaming dogs as a reservoir and a proxy to monitor the spillover to humans, livestock, and wildlife.

Funding statement

Universidad de Las Américas funded this work (MED. MGB.23.13.01).

Ethics approval statement

The study was conducted following national regulations for animal care. Veterinarians collected samples. According to Ecuador's regulations for animal research, studies including blood sample collection for laboratory diagnosis do not require Institutional Review Board approval.

CRediT authorship contribution statement

Angel Sebastian Rodriguez-Pazmiño: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Carla M. Brito:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation. **Mauricio Salas-Rueda:** Writing – review & editing, Methodology, Investigation, Data curation. **Solon Alberto Orlando:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation. **Miguel Angel Garcia-Bereguiain:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, 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.

Data availability

All the data used in this study are included either in the main text or in the supplementary material.

Acknowledgments

We thank all our colleagues who contributed to the blood sample collection in different provinces of Ecuador. Special thanks to the *Spay and Neuter Ambato Program*, *Club de Bienestar Animal de Yachay Tech, Animal Vet*, and *Personas Unidas por el Bienestar Animal.*

References

[1] M. Abdussalam, D.A. Fein, Brucellosis as a world problem, Dev. Biol. Stand. 31 (1976) 9–23.
[2] H. Honarmand, Q fever: an old but still a poorly understood disease, Interdiscip. Perspect. Infect. Dis. 2012 (2012).
[3] I.D. Aitken, Clinical aspects and prevention of Q fever in animals, Eur. J. Epidemiol. 5 (1989) 420–424.
[4] I.T. Paulsen, et al., The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts, Proc. Natl. Acad. Sci. USA 99 (2002) 13148–13153.
[5] A. Unver, H. Erdogan, H. Atabay, M. Sahin, O. Celebi, Isolation, Identification, and Molecular Characterization of *Brucella Melitensis* from Aborted Sheep Fetuses in Kars, Turkey, 2006.
[6] Transmission, Q Fever, CDC, 2024. https://www.cdc.gov/qfever/transmission/index.html.
[7] A.M. Whatmore, et al., Extended multilocus sequence analysis to describe the global population structure of the genus Brucella: Phylogeography and relationship to biovars, Front. Microbiol. 7 (2016) 242018.
[8] S. Zvizdić, et al., *Brucella melitensis* review of the human infection case, Bosn. J. Basic Med. Sci. 6 (2006) 15.
[9] WHO, Brucellosis, World Health Organization, 2020.
[10] E.I. Shaw, D.E. Voth, Coxiella burnetii: a pathogenic intracellular Acidophile, Microbiol. (NY) 165 (2019) 1.
[11] Signs and Symptoms, Q Fever, CDC, 2024. https://www.cdc.gov/qfever/symptoms/index.html.
[12] Agrocalidad, Resolución N° 214 - Lista de enfermedades de notificación obligatoria para las diferentes especies animales en todo el territorio nacional, 2013.
[13] S.K. Khurana, et al., Bovine brucellosis – a comprehensive review, Vet. Q. 41 (2021) 61–88.
[14] J. Zinsstag, et al., Human benefits of animal interventions for zoonosis control - volume 13, number 4—April 2007 - emerging infectious diseases journal - CDC, Emerg. Infect. Dis. 13 (2007) 527–531.
[15] P.-E. Fournier, D. Raoult, Q. Fever, Hunter's Tropical Medicine and Emerging Infectious Diseases, 2020, pp. 599–601, https://doi.org/10.1016/B978-0-323-55512-8.00071-5.
[16] M.E. Hensel, M. Negron, A.M. Arenas-Gamboa, Brucellosis in dogs and public health risk - volume 24, number 8—August 2018 - emerging infectious diseases journal - CDC, Emerg. Infect. Dis. 24 (2018) 1401–1406.
[17] M.N. Seleem, S.M. Boyle, N. Sriranganathan, Brucellosis: a re-emerging zoonosis, Vet. Microbiol. 140 (2010) 392–398.
[18] H. To, et al., Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders, J. Vet. Med. Sci. 60 (1998) 859–861.
[19] M. Ibarra, et al., Financial losses associated with bovine brucellosis (*Brucella abortus*) in Carchi-Ecuador, Open J. Anim. Sci. 13 (2023) 205–216.
[20] R.L. Santos, T.M. Martins, Á.M. Borges, T.A. Paixão, Economic losses due to bovine brucellosis in Brazil, Pesqui. Vet. Bras. 33 (2013) 759–764.
[21] Z. Liu, L. Gao, M. Wang, M. Yuan, Z. Li, Long ignored but making a comeback: a worldwide epidemiological evolution of human brucellosis, Emerg. Microbes Infect. 13 (2024).
[22] A.S. Dean, L. Crump, H. Greter, E. Schelling, J. Zinsstag, Global burden of human brucellosis: a systematic review of disease frequency, PLoS Negl. Trop. Dis. 6 (2012) e1865.
[23] T.S. Lemos, et al., Outbreak of human brucellosis in southern Brazil and historical review of data from 2009 to 2018, PLoS Negl. Trop. Dis. 12 (2018) e0006770.
[24] J. Ron-Román, et al., Human brucellosis in Northwest Ecuador: typifying Brucella spp., seroprevalence, and associated risk factors, Vector Borne Zoonotic Dis. 14 (2014) 124–133.
[25] M. Zambrano, M. Pérez, Seroprevalencia de brucelosis en ganado bovino y en humanos vinculados a la ganadería bovina en las zonas norte y centro de la provincia Manabí, Ecuador, Rev Salud Anim. 37 (2015) 164–172.

[26] I. Alberto Méndez, et al., Brucella spp seroprevalence in veterinary medicine students, Bogota, Colombia 45, Revista de la Universidad Industrial de Santander. Salud, 2013, pp. 39–48.
[27] D.K. Bonilla-Aldana, et al., A systematic review and meta-analysis of bovine brucellosis seroprevalence in Latin America and the Caribbean, New Microbes New Infect. 54 (2023) 101168.
[28] A. Garrido-Haro, et al., Seroprevalence and risk factors related to Bovine Brucellosis in continental Ecuador, Pathogens 12 (2023) 1134.
[29] A. Carbonero, et al., Seroprevalence and risk factors associated with Brucella seropositivity in dairy and mixed cattle herds from Ecuador, Trop. Anim. Health Prod. 50 (2018) 197–203.
[30] K.P. Poulsen, et al., Brucellosis in dairy cattle and goats in northern Ecuador, Am. J. Trop. Med. Hyg. 90 (2014) 712.
[31] R.L. Vinueza, et al., Farm prevalence of bovine brucellosis, farmer awareness, and local practices in small-and medium-scale cattle farms in a tropical region of Ecuador, Transbound. Emerg. Dis. 2023 (2023).
[32] T. Tan, J. Heller, S. Firestone, M. Stevenson, A. Wiethoelter, A systematic review of global Q fever outbreaks, One Health 18 (2024) 100667.
[33] L. Epelboin, et al., Q fever in French Guiana: tip of the iceberg or epidemiological exception? PLoS Negl. Trop. Dis. 10 (2016).
[34] S. Bailly, et al., Transmission dynamics of Q fever in French Guiana: a population-based cross-sectional study, Lancet Region. Health Am. 16 (2022).
[35] G. Echeverría, et al., Serological evidence of *Coxiella burnetii* infection in cattle and farm workers: is Q fever an underreported zoonotic disease in Ecuador? Infect. Drug Resist. 12 (2019) 701.
[36] P. Gisbert, Q Fever in cattle: what is its real prevalence?. https://ruminants.ceva.pro/q-fever-in-cattle, 2023.
[37] L. Epelboin, et al., *Coxiella burnetii* infection in livestock, pets, wildlife, and ticks in Latin America and the Caribbean: a comprehensive review of the literature, Curr. Trop. Med. Rep. 10 (2023) 94–137.
[38] A. Carbonero, et al., *Coxiella burnetii* seroprevalence and associated risk factors in dairy and mixed cattle farms from Ecuador, Prev. Vet. Med. 118 (2015) 427–435.
[39] D. Changoluisa, et al., Serology for Neosporosis, Q fever and brucellosis to assess the cause of abortion in two dairy cattle herds in Ecuador, BMC Vet. Res. 15 (2019) 1–5.
[40] M.M. Wanke, Canine brucellosis, Anim. Reprod. Sci. 82–83 (2004) 195–207.
[41] B.K. Baek, et al., *Brucella abortus* infection in indigenous Korean dogs, Can. J. Vet. Res. 67 (2003) 312.
[42] M. Woldemeskel, M. Woldemeskel, Zoonosis due to Bruella suis with special reference to infection in dogs (carnivores): a brief review, Open J. Vet. Med. 3 (2013) 213–221.
[43] A.L.B. de Oliveira, et al., Detection of Brucella spp. in dogs at Pantanal wetlands, Braz. J. Microbiol. 50 (2019) 307–312.
[44] F. Benitez Capistros, et al., Dogs as Potential Mechanical Vector of Transmission of Brucella. Spp., in Dairy Cattle in the Sierra of Ecuador, 2011.
[45] M. Rezaei, M. Rezaei, M. Khalili, B. Akhtardanesh, S. Shahheidaripour, Q fever in dogs: an emerging infectious disease in Iran, J. Med. Bacteriol. 5 (2016) 1–6.
[46] J. Gardon, et al., Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir, J. Infect. Dis. 184 (2001) 278–284.
[47] M. Navarro, F. Gury, G. Cicuttin, Estudio serológico de fiebre Q en felinos y caninos de la ciudad de Buenos Aires y alrededores, Rev Argent Zoonosis Enferm Infec. Emerg. 124–127 (2007).
[48] G.L. Cicuttin, B. Lobo, P. Anda, I. Jado García, Seropositividad a Coxiella burnetii (agente de la fiebre Q) en caninos domésticos de la *Ciudad Autónoma* de Buenos Aires, InVet 15 (2013) 131–136.
[49] G.M.B. de Oliveira, et al., Tick-borne pathogens in dogs, wild small mammals and their ectoparasites in the semi-arid Caatinga biome, northeastern Brazil, Ticks Tick Borned. Dis. 11 (2020).
[50] M.A. de Mares-Guia, et al., Molecular identification of the agent of Q fever – *Coxiella burnetii* – in domestic animals in state of Rio de Janeiro, Brazil, Rev. Soc. Bras. Med. Trop. 47 (2014) 231–234.
[51] E.R.S. Lemos, et al., Q Fever as a Cause of Fever of Unknown Origin and Thrombocytosis: First Molecular Evidence of *Coxiella burnetii* in Brazil 11, 2011, pp. 85–87. https://home.liebertpub.com/vbz.
[52] M. Cárdenas, C.J. Grijalva, S. de la Torre, Free-roaming dog surveys in Quito, Ecuador: experiences, lessons learned, and future work, Front. Vet. Sci. 8 (2021) 766348.
[53] J. Calderón, S. Poveda, A.L. Sosa, N. Mora, M. López Bejar, S.A. Orlando, M. A. Garcia-Bereguiain, Dog bites as a zoonotic risk in Ecuador: need for the implementation of a one health approach, One Health 16 (2023) 100544, https://doi.org/10.1016/j.onehlt.2023.100544.
[54] A.S. Rodriguez-Pazmiño, C.M. Brito, M. Salas-Rueda, S.A. Orlando, M.A. Garcia-Bereguiain, A first insight into seropositivity of Neospora caninum and associated risk factors in free-roaming dogs from Ecuador, Acta Trop. (2024) 256–107245, https://doi.org/10.1016/j.actatropica.2024.107245.
[55] R Core Team, R: A Language and Environment for Statistical Computing, Preprint at, 2023.
[56] E. Mortola, G.S. Miceli, L.P. Meyer, Brucella Abortus in dog population: an underestimated zoonotic disease, Biomed. J. Sci. Tech. Res. 15 (2019) 1–3.
[57] G.S. Miceli, L. Perez Meyer, L.M. Peralta, E. Mortola, Detection of antibodies against Brucella abortus in dogs in contact with rural areas: zoonotic aspects of the infection, Analecta Veterinaria 39 (2) (2019).
[58] M. Bernardino, das G. da S., et al., Zoonotic smooth and rough *Brucella* in dogs: seroprevalence and associated factors in an Atlantic rainforest area of the state of Paraíba, northeastern Brazil, Ciência Rural 51 (2020) e20200374.
[59] G.S. da Paz, et al., Seroprevalence for brucellosis and leptospirosis in dogs from Belém and Castanhal, State of Pará, Brazil, Acta Amazon. 45 (2015) 265–270.
[60] N.L. Costa Barros, et al., Serological and Molecular Survey of Brucella Species in Owners and Their Dogs Living on Island and Mainland Seashore Areas of Brazil. https://home.liebertpub.com/vbz.
[61] M. Boni, B. Davoust, H. Tissot-Dupont, D. Raoult, Survey of seroprevalence of Q fever in dogs in the southeast of France, French Guyana, Martinique, Senegal and the Ivory Coast, Vet. Microbiol. 64 (1998) 1–5.
[62] D. Otranto, et al., Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis, Trends Parasitol. 33 (2017) 813–825.
[63] Brucellosis, Cornell Wildlife Health Lab. https://cwhl.vet.cornell.edu/disease/brucellosis, 2024.
[64] B. B, Q fever : a zoonosis, Adv. Vet. Sci. 5 (1959) 81–154.
[65] S.A. Orlando, A. Perez, E. Sanchez, C. de la Cruz, O. Rugel, M.A. Garcia-Bereguiain, High seroprevalence of anti-Leptospira spp. antibodies in domestic and wild mammals from a mixed use rescue center in Ecuador: lessons for "one health" based conservation strategies, One Health 10 (2020) 100140, https://doi.org/10.1016/j.onehlt.2020.100140.
[66] M.S. Zambrano-Mila, B. Freire-Paspuel, S.A. Orlando, M.A. Garcia-Bereguiain, SARS-CoV-2 infection in free roaming dogs from the Amazonian jungle, One Health 14 (2022) 100387, https://doi.org/10.1016/j.onehlt.2022.100387.
[67] P. Vega-Mariño, J. Olson, B. Howitt, R. Criollo, L. Figueroa, S.A. Orlando, M. Cruz, M.A. Garcia-Bereguiain, A recent distemper virus outbreak in the growing canine populations of Galapagos Islands: a persistent threat for the endangered Galapagos Sea Lion, Front. Veterin. Sci. 10 (2023), https://doi.org/10.3389/fvets.2023.1154625.
[68] Primicias, Cómo prevenir la brucelosis? Brote se registra en Ecuador. https://www.primicias.ec/noticias/sociedad/prevencion-brucelosis-contagio-bacteria-ganado-perros/, 2024.