UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

Brucellosis and Q Fever seroprevalence associated with free-roaming dogs from urban and rural areas of Ecuador during the year 2019

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo.

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A mi madre, familia y amigos.
La fortaleza y soporte
de mi vida
gracias.

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RESUMEN

La Brucelosis y Fiebre Q son enfermedades zoonóticas que representan un problema para la salud animal y humana. El ganado es el huésped preferencial para ambas enfermedades, pero, a través de transmisión horizontal, los perros pueden infectarse con *Brucella abortus* y *Coxiella burnetti*. Dado que, en Ecuador, la seroprevalencia de *B. abortus* y *C. burnetti* en ganado es alta, los perros pueden resultar infectados y ser un vector de transmisión a los humanos. A pesar de su relevancia para la salud, pocos estudios informan sobre la seroprevalencia de estas enfermedades en los caninos ecuatorianos. En esta investigación, se informa la seroprevalencia de Brucelosis, causada por *B. abortus*, y de Fiebre Q, causada por *Coxiella burnetii*, tanto para zonas urbanas como rurales.

Los ensayos de Rosa de Bengala (RB) y los ensayos inmunoabsorbentes ligados a enzimas (ELISA) se utilizaron para detectar la presencia de patógenos que causan las enfermedades zoonóticas, Brucelosis y Fiebre Q, en perros callejeros. Los resultados mostraron una seroprevalencia de 8.9% y 4.0% para Brucelosis causada por *B. abortus*, usando Rosa de Bengala y ELISA, respectivamente, y 2.6% para Fiebre Q causada por *C. burnetii*, usando ELISA. Se realizó una prueba de chi-cuadrado para determinar una diferencia entre la seroprevalencia de los caninos de las zonas urbanas y rurales. No se encontraron diferencias estadísticamente significativas entre las seroprevalencias. Nuestra investigación determinó que ambos patógenos causantes de enfermedades están indistintamente presentes en perros de zonas rurales y urbanas, lo que indica el potencial rol de los perros en la transmisión y propagación de los patógenos causantes de ambas enfermedades, Brucelosis y Fiebre Q, debido al contacto cercano entre perros y humanos.

Palabras clave

Zoonosis, Perros, *Brucella abortus*, *Coxiella Burnetti*, Seroprevalencia.

ABSTRACT

Brucellosis and Q fever are zoonotic diseases that represent a problem for animal and human health. Cattle are the preferential hosts for both diseases but, through horizontal transmission, dogs can result in infected with *Brucella abortus* and *Coxiella burnetti.* As in Ecuador; the seroprevalence of *B. abortus* and *C. burnetii* in cattle is high, dogs can be a vector of transmission to humans. Despite their health relevance, few studies report on the seroprevalence of these diseases in Ecuadorian canines. In this investigation, the seroprevalence of Brucellosis, caused by *B. abortus*, and Q fever, caused by *Coxiella burnetii*, are reported for both urban and rural zones.

Rose Bengal (RB) Assays and Enzyme-linked immunosorbent assays (ELISA) were used to detect the presence of pathogens that cause the zoonotic diseases, Brucellosis and Q fever, in free-roaming dogs. Results showed a seroprevalence of 8.9% and 4.0% for Brucellosis caused by *B. abortus*, using Rose Bengal and ELISA, respectively, and 2.6% for Q fever caused by *C. burnetii*, using ELISA. A chi-square test was carried out to determine any difference between the seroprevalence of canines from urban and rural zones. No statistically significant difference was found among seroprevalences as p-values were higher than 0.05. Our research determined that both diseases are indistinctively present in dogs from rural and urban zones, which indicates the potential role of dogs in the transmission and spillover of both diseases due to the close contact between dogs and humans.

Keywords

Zoonosis, Dogs, *Brucella abortus*, *Coxiella Burnetti*, Seroprevalence.

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1. INTRODUCTION-JUSTIFICATION

1.3 Brucellosis: history, taxonomy and features.

The emergence of Brucellosis can be traced back to the 19th century when an occasional fever (known as Undulant Fever, Malta Fever or Mediterranean Fever) appeared among British soldiers, causing weakness and even death. The physician David Bruce isolated the organism from a soldier suffering Malta Fever and named it as *Micrococcus melitensis* (Tan & Davis, 2011). Nowadays, the disease is mainly known as Brucellosis and, the pathogens that cause it are classified as *Brucella* spp., family *Brucellaceae*, order *Rhizobiales*, class *Alphaproteobacteria*, phylum *Proteobacteria* (Alton & Forsyth, 1996; Ficht, 2010).

Organisms of this genus are, non-sporulating, non-motile, facultative intracellular Gram-negative *coccobacilli*, with the ability to provoke infection of mammalian cells, and in some species, persist in the soils due to a plant-based molecules metabolism (Alton & Forsyth, 1996; Ficht, 2010). *Brucella* spp. have been classified as category B pathogens and potential agents for bioterrorism due to the low number of virulent propagules required for human infection and, the capacity for aerosolization. *Brucella* spp. can avoid the killing mechanisms of immune cells and proliferate inside mammalian macrophages. Virulence in Brucella spp. is mainly attributed to a non-endotoxic lipopolysaccharide (LPS) that compounds their outer membrane (Christopher et al., 2010). The LPS confer resistance to antimicrobial responses and interfere with the immune response of the host; functions needed to survive and replicate inside the host. Virulence factors of *Brucella* strains can occur either as smooth lipopolysaccharide (S-LPS) or rough lipopolysaccharide (R-LPS). Strains containing S-LPS (*B. abortus, B. suis and B. melitensi*) are known to be pathogenic to humans (Cardoso et al., 2006).

1.4 Epidemiology, transmission mechanisms, and pathology of Brucellosis

Brucellosis is endemic in many regions of the world, including countries from the Middle East, Asia, Africa and South America (de Figueiredo et al., 2015). Due to control measures, the incidence levels of Brucellosis have decreased in Europe and North America. In contrast, increased infection rates of the disease have become a health problem in regions where the disease has become endemic and, a growing animal production occurs in precarious hygienic conditions (Abdussalam & Fein, 1976).

Little information on seroprevalence of Brucellosis in animals and humans exists for South America. In Ecuador, an extensive cross-sectional investigation was carried out in 386 farms where the prevalence of seropositivity for *B. abortus* in diary and mixed cattle cows reached 17% (Carbonero et al., 2018). In Manabí, the disease has been reported with a seroprevalence of 2,63% in slaughtered cattle (Zambrano Aguayo & Pérez Ruano, 2015). Human brucellosis has been reported in northwestern zones of Ecuador and, Manabí, reaching a seroprevalence that ranges from 1.06% to 1.88% (Ron-Román et al., 2014; Zambrano Aguayo & Pérez Ruano, 2015).

Brucellosis is a disabling zoonosis for human health caused by *Brucella spp*. In Ecuador, the only circulating strain is *Brucella abortus*, which uses cattle as preferential host but, through horizontal transfer, naturally acquired *B. abortus* in dogs associated with cattle has been reported. As in Ecuador; the seroprevalence of *B. abortus* in cattle is high, dogs can be a vector of transmission. Horizontal, dog to dog and, dog to human transfer has been demonstrated in previous studies (Luna et al., 2016; Baek et al., 2003; Xavier et al., 2009; Wareth et al., 2016). Either infected cattle or vector carries (dogs) accidentally transmit *Brucella* spp. to humans via multiple ways, such as direct contact of skin or mucous with infected tissues, infected blood, fetuses, fetal fluids, vaginal discharges, feces, and urine; from consumption of infected animal products such as unpasteurized milk and cheese; or by inhalation. Sexual contact between humans and tissue transplantation have reported as a rare way of transmission (Khan & Zahoor, 2018). In animals, the bacteria infect reproductive tissues, lymph nodes, the spleen, and udder, causing inflammation, edema, and necrosis, and in pregnant animals increase the risk of abortion. Similarly, in humans, the disease can cause abortion but also a myriad of other clinical manifestations such as irregular fever, headache, fatigue, arthritis, etc. Additionally, the bacterium can cause neurobrucellosis, affecting the central nervous system with serious clinical manifestations such as stroke, meningitis, neuropathies, etc.

1.5 Q fever: history, taxonomy and pathogenic features.

In 1935, the febrile disease known as Query (Q) fever caused the first outbreak among workers of a slaughterhouse in Brisbane, Australia. Simultaneously, an illness known as Nine Mile Fever with similar signs and symptoms occurred in Montana, USA. Trough isolation of infected tissues, Sir Frank Burnet in Australia and Dr. Herald Cox in the USA determined that both diseases were the same, and the causative agent is a *Rickettsia* bacterium, named *Coxiella burnetii* in their honor. This bacterium belongs to the family Coxiellaceae, class ɣ -Proteobacteria, phylum Proteobacteria (Hechemy, 2012).

Coxiella burnetti is a small, obligate intracellular, gram-negative *coccobacillus*, which undergoes a sporulation-like process that shields the organisms from the environment, allowing it to survive for long periods under hot and dry conditions. In mammals, the bacterium infects macrophages, rendering the immune cells unable to kill the organism. *Coxiella burnetti* presents unique LPS molecules in the outer membrane, which confers the organism its antigenicity and potential virulence. (Angelakis & Raoult, 2010; Hechemy, 2012; Million & Raoult, 2013)

1.6 Epidemiology, transmission mechanisms, and pathology of Q fever

Q fever has a worldwide distribution on five continents, including those in the tropics, but excluding New Zeeland. Underestimated cases and outbreaks of the disease occur in developing countries, especially those with limited resources (Million & Raoult, 2013). In South America and Central America, Q fever cases have been poorly reported. In Ecuador, a cross-sectional study was performed in 386 farms where the prevalence of seropositivity for *C. burnetii in* the diary and mixed cattle, reached 12.6% (Carbonero et al., 2015). In cattle from rural zones, the seroprevalence of Q fever ranges from 43% to 52.9% (Changoluisa et al., 2019). Human cases have been associated with farmworkers, who reached a seroprevalence of 34%. (Echeverría et al., 2019).

Worldwide, cattle, sheep, and goats are the most common reservoirs for *C. burnetii* but, through horizontal transfer, naturally acquired *C. burnetii* in dogs associated with cattle has been reported. As in Ecuador; the seroprevalence of *C. burnetii* in cattle is high, dogs can be a vector of transmission for the disease. Horizontal, dog to dog and, dog to human transfer has been demonstrated in previous studies (Cooper et al, 2011). Either cattle or dogs shed the pathogen in their urine, feces, amniotic fluids, milk, placentas (afterbirth remains), and aborted tissues. Human infection is caused by aerosolization of the bacteria and subsequent inhalation during slaughter and parturition of infected animals. Wind-borne transmission may also occur as bacteria are resistant to heat and drying conditions, and can cause infection by inhalation of contaminated dust. Winds can disperse contaminated dust at least 10 km away from the source soil containing C. *burnetti*. Ingestion of infected milk or dairy products can occur as a source of infection too. High-risk human groups include abattoir workers, veterinarians, and people living close to farms with infected animals (Barnham, 1991; NUSINOVICI et al., 2015).

In mammals, *C. burnetii* resides in the uterus and mammary glands of females but multiplying mostly in the placenta. No clinical manifestations are seen in animals; nevertheless, many abortions have been linked to the bacterium. In humans, Q fever can remain asymptomatic; however, it can manifest itself in two ways, an acute phase, which is characterized by flu-like symptoms, high temperatures, headaches, myalgia, abdominal and chest pain, development of pneumonia in certain patients, and a chronic phase associated with endocarditis (Sandrock, 2016 ; Kersh et al., 2012).

1.1 The "One Health" approach for zoonotic diseases: an historical overview

Over the last couple of centuries, many experts in the medical and veterinarian community have pointed out that that human health partially depends on animal health as well as the environment (Kahn et al., 2007). The first interaction between animal and human health professionals was reported in the18th century in the attempts to improve human health by enhancing the animal-based food supply (Kahn et al., 2008). Later, in the 19th century, the pathologist Rudolf Virchow introduced to the concept of zoonosis as a disease that is transmissible among animals and humans (Monath et al., 2010). His experiments led him to suggest the need for incorporating veterinary medicine into human health care.

In the 20th century, Dr. Calvin Schwabe coined the term "One Medicine" as a unifying approach combining the efforts of veterinary and human health professionals to combat zoonotic diseases or zoonoses. Nowadays, the term "One Medicine" has been replaced by "One Health," an integration of human, animal and environmental health (Monath et al., 2010). Agencies, such as the Food and Agriculture Organization and World Health Organization, support the One Health model as a promising approach to better understand the ecology of diseases and thus to implement better prevention strategies that hopefully will reduce disease risk and outbreaks in animals, humans, and environment (Kahn et al., 2007).

1.2 The role of zoonoses, wildlife and domestic animals in the health system.

The relationship between humans and animals is becoming deeper through the years and involves social, biological, and physic-chemical factors (Woolhouse & Gowtage-Sequeria, 2005). The most intense interaction between humans and animals occurs with companion animals, also referred to as pets (Tarazona et al., 2019). The main concern regarding this interaction lies with the growing rates of endemic and emerging infectious

diseases (EIDs), due to close human contact with domestic and wild animal populations (Woolhouse & Gowtage-Sequeria, 2005). Zoonoses are infectious diseases caused by a variety of pathogens such as viruses, bacteria, protozoa, and fungi. On a global scale, zoonotic pathogens cause highly transmissible infectious diseases with high lethality rates (Patz et al., 2004).

The World Health Organization, in collaboration with the World Bank, determined infectious diseases to rank at position 29 out of 96 as a major cause of human mortality and morbidity. Infectious diseases, including human diseases and zoonoses, are responsible for 25% of all deaths globally per year (Woolhouse et al., 2001). Zoonotic pathogens are the major source for emerging infectious diseases (EIDs) that affect humanity, being twice as likely to be associated with EIDs than non-zoonotic pathogens (Woolhouse et al., 2001). At present, there are approximately 1,415 species of infectious organisms that are pathogenic for humans, 60% of them are zoonotic (Woolhouse & Gowtage-Sequeria, 2005). Not only are zoonoses a threat to public health care, but they also play an important role for domestic and wild animals which may become important zoonotic pools from which new and unknown pathogens may spill over to human populations (Patz et al., 2004).

2. PROBLEM STATEMENT

Brucellosis and Q fever are threateningly zoonosis for human health and an economically unfavorable disease in cattle. In Ecuador, current reports regarding the presence of both, Brucellosis and Q fever in cattle, indicate high seroprevalences, reaching up to 17% and 52.9%, respectively (Carbonero et al., 2015; Carbonero et al., 2018). Even though cattle are the preferential host for both diseases, dogs can result in infected. In Ecuador, the presence of free-roaming dogs in the entire country has not been estimated, however, according to the Instituto Nacional de Salud Pública e Investigación (INSPI or National Institute of Public Health and Investigation in English) there are approximately 23455 free-roaming dogs in Guayaquil (n.d.). For both zoonotic diseases, it has been demonstrated that human infection can accidentally occur as a result of horizontal transmission (from dogs to humans).

The presence of *Brucella abortus* and *Coxiella burnetti* in dogs indicates the potential role of dogs in the transmission and spillover of both diseases to humans (Wareth et al., 2016). This risk is especially problematic for people who are immunocompromised (Kahn, 2006). This work aims to estimate the seroprevalence of Brucellosis and Q fever in free-roaming dogs from rural and urban areas of Ecuador.

3. OBJECTIVES

3.1 General Objective

To determine the seroprevalence of *Brucella abortus* and *Coxiella burnetii* in free-roaming dogs.

3.2 Specific Objective

- To identify a potential difference in seroprevalence between free-roaming dogs living in rural zones from those living in urban zones.

4. METHODOLOGY

4.1 Sample collection

Two hundred twenty-seven blood samples were taken from Ecuadorian free-roaming dogs. Dogs were classified as belonging to urban or rural zones, giving a total of 62 and 165, respectively. The samples were obtained during sterilization campaigns, between January and August of 2019 and, with the previous consent of dog owners. Campaigns were organized in collaboration with the animalist group, *Personas Unidas por el Bienestar Animal* PUBA (People United for Animal Welfare) and the Municipal Autonomous Government of each location. The sterilization process required dogs to be anesthetized and, only after the sterilization was completed, samples were taken by veterinarians. Approximately 5 mL of blood was collected from the cephalic vein in a red-top tube with a serum clot activator. Samples were stored in a refrigerant box and transported to the laboratory. After the clotting process, 1 ml of serum was collected and transferred into a cryovial tube. Serum samples were maintained at -20 ˚C until analyzed with Rose Bengal Test kit and ELISAs kits.

4.2 Sample sites

Two hundred twenty-seven samples from urban and rural zones were collected in the three geographic regions of continental Ecuador, namely Coast, Sierra/Andes, and Amazon. A total of 62 dogs from urban zones were sampled on the Coast and in the Sierra regions, 31 dogs from Guayaquil, Guayas, and 31 dogs from Ibarra, Imbabura. A total of 165 dogs from rural zones located on the Coast, in the Sierra, and the Amazon regions were also sampled; 37 dogs in Daule, Guayas; 49 dogs at Yachay Tech University and Urcuquí, Ibarra; 40 dogs at farms located around Santo Domingo, Santo Domingo de los Tsáchilas; and 39 dogs at IKIAM University in Tena (Fig.1).

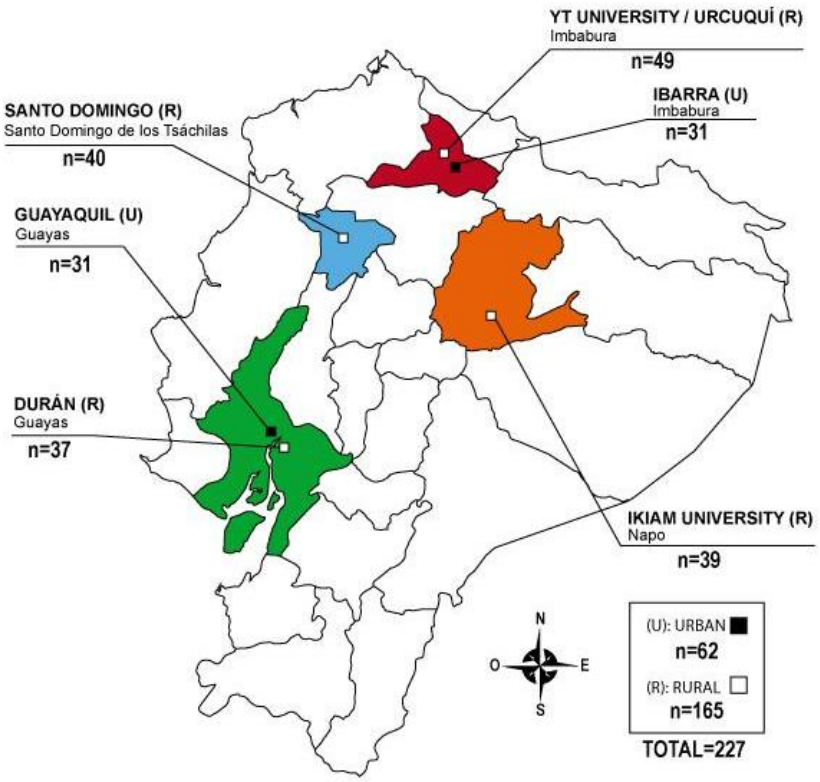

Figure 1. *Sampling sites including rural and urban zones. The total number of samples collected from free-roaming dogs are shown in each sampling zone.*

4.3 Serological testing: Rose Bengal assay, and enzyme-linked immunosorbent assay.

Serological techniques are broadly used in seroepidemiological studies to determine the presence of a specific infectious disease in an animal population (Campos, 2002). The basis of a serological diagnosis lies in the detection of specific antibodies in the blood and other body fluids. The preferred technique in laboratories for the diagnosis of infectious diseases is the enzyme-linked immunosorbent assay (ELISA; Campos, 2002). However, qualitative tests including agglutination techniques can be used first as a screening method and, other serological tests, such as ELISA, must be used to confirm seropositivity. The production of unique antibodies occurs when infectious agents, such as zoonotic pathogens, elicit an immune response by the animal. Therefore, the presence of specific antibodies is an indicator of exposure to it (Fierz, 1999). Because of the impact of zoonotic diseases on both animal and human health, it is necessary to consider the use of reliable, accurate, and sensitive serological techniques, as well as fast and economical options for small and large-scale surveys (Ochoa-Azze, 2018).

Commercial kits were used for all assays. In the present study, 227 serum samples were tested for antibodies against *B. abortus* and *C. burnetii* by indirect multispecies enzyme-linked immunosorbent assays (ELISAs). Additionally, 157 serum samples were tested for antibodies against *B. abortus* by the agglutination Rose Bengal assay (RB). The missing 70 samples, corresponding to Santo Domingo and, Yachay Tech and Urcuquí, were not tested, since the serum was not available. All the tests were performed following the manufacturer's instructions. Specific details of the ELISA kits and Rose Bengal assays, along with the sensitivities and specificities of the assays, are shown in Table 1.

Table 1. Commercially available ELISAs tests and Rose Bengal Plate Test kits were used for detecting antibodies against B. abortus and C. burnetii. The sensitivity and specificity of the diagnostic kits were provided by the manufacturer.

Infectious Agent	**Test kit**	**Antigen**	**Sensitivity**	**Specificity**
Coxiella burnetii	ELISA IDVet ID Screen	Phase I and Phase II antigens *Coxiella burnetii*	100% (CI95%: 89.28– 100%)	100% (CI95%: 97.75–100%)
Brucella abortus	ELISA IDVet ID Screen	S-LPS of purified *Brucella abortus*	100% (IC95%: 89.57– 100%)	99.74% (IC95%: 99.24– 99.91%)
Brucella abortus	Rose Bengal	S-LPS inactivated brucella cells	99 %	97.6%

4.3.1 Diagnosis of Brucellosis caused by B. abortus according to the Rose Bengal Assay

The Rose Bengal kit consists of a concentrated suspension of inactivated *Brucella* cells (R-LPS strain) suspended in acid buffer and stained with Rose Bengal. Equal quantities of serum from the samples and antigen (30 µL) were added on each circle of a card. Drops from serum and *Brucella* cells, were mixed and then shaken on a rocking shaker for 4 minutes. The formation of agglutination indicates the presence of anti-*Brucella* antibodies in the samples. In contrast, the lack of agglutination indicates the absence of antibodies (Díaz et al., 2011). Any degree of agglutination was considered like a positive reaction (See Appendix A)

4.3.2 Diagnosis of Brucellosis caused by B. abortus and, Q fever caused by C. burnetii according to the Enzyme-Linked Immunosorbent Assays (ELISA)

For both ELISAs kits, ID Screen® Brucellosis Serum Indirect Multi-species, and ID Screen® Q Fever Indirect Multi-species, microwells were coated with the antigen of interest (see Table 1). Samples and controls were added to the wells. If antibodies are present in the samples, antigen-antibody complexes will be formed. After washing, wells were supplied with an anti-multi-species peroxidase (HPR) conjugate. Antibodies are fixed to it and, antigen-antibody-conjugate-HRP complexes are formed (See Appendix B). Optical Densities (OD) of ELISA microplates were measured and recorded using a plate reader at 450 nm.

For each sample, the S/P% (Sample to Positive ratio; calculated as $\frac{OD\ sample - OD\ negative\ control}{OD\ positive\ control - OD\ negative\ control}$) was estimated. Depending on the S/P% values, samples were classified as positive, negative, and doubtful. The values and status of the diagnostic kits were provided by the manufacturer of the kits. For Brucellosis, S/P% values higher or equal to 120 are positive; S/P% values lower to 120 and higher than 110 are doubtful, and; S/P% values lower or equal to 110 are negative (Table 2). For Q fever, S/P% values higher than 50 represent positives; S/P% values lower or equal to 50 and higher than 40 are doubtful, and; S/P% values lower or equal to 40 are negative (Table 2).

Table 2. Values and status of the diagnostic kits were provided by the manufacturer of the kits.

Possible Results		**Status**
Q fever	**Brucellosis**	
S/P % > 50	S/P % ≥ 120	Positive
40 <S/P % ≤50	110 <S/P % <120	Doubtful
S/P % ≤ 40	S/P % ≤ 110	Negative

4.4 Statistical Analysis

The Software RStudio was used to carry out all statistical tests. The chi-square test of independence test was used to compare seroprevalence data of free-roaming dogs from rural areas and urban areas. For all tests, the significance level was set at 0.05.

5. RESULTS

A total of 227 Stray dogs (rural n=165, urban n= 62) were tested against Q fever and Brucellosis (See table 3).

Table 3. Summary showing serodiagnosis for each sampling zone as well as the overall seroprevalence (expressed as percentage) of the diseases in rural and urban zones, and sample kits used in this study.

	Brucella abortus		*Coxiella Burnetii*
Location	**Brucellosis + (RB)**	**Brucellosis + (ELISA)**	**Q fever + (ELISA)**
Guayaquil	3.2 % (1/31)	3.2 % (1/31)	0% (0/31)
Ibarra	16.1 % (5/31)	0 % (0/31)	0% (0/31)
Urban zones Total	**9.7 % (6/62)**	**1.6 % (1/62)**	**0% (0/62)**
Daule	10.8 % (4/37)	0 % (0/37)	2.7% (1/37)
YT University and Urcuquí	15.7 % (3/19)	12.2 % (6/49)	0% (0/49)
Santo Domingo	No data	2.5 % (1/40)	12.5% (5/40)
IKIAM University	2.6 % (1/39)	2.6 % (1/39)	0 % (0/39)
Rural zones Total	**7.3% (8/95)**	**4.8 % (8/165)**	**3.6% (6/165)**
Overall Total	**8.9% (14/157)**	**4.0 % (9/227)**	**2.6 % (6/227)**

5.1 Seroprevalence of Brucellosis caused by B. abortus according to the Rose Bengal Assay

A total of 187 serum samples from Ecuadorian free-roaming dogs, 62 from urban zones, and 165 from rural zones were tested with the Rose Bengal Test for the presence of antibodies against Brucellosis. The overall prevalence of Brucellosis, as determined by the Rose Bengal assay, is 8.3% (13/157). The prevalence of Brucellosis for urban zones and rural zones is 9.7 % (6/62) and 8.3 % (13/95), respectively. The prevalence of Brucellosis caused by *B. abortus* in dogs according to each sampling zone is 3.2 % (1/31) in Guayaquil; 16.1 % (5/31) in Ibarra; 10.8 % (4/37) in Daule; 10.5 % (2/19) at YT University and in Urcuquí, and 2.6 % (1/39) at IKIAM University, respectively.

5.2 Seroprevalence of Brucellosis caused by B. abortus according to the Enzyme-Linked Immunosorbent Assay

A total of 227 serum samples from free-roaming dogs, 62 from urban zones, and 165 from rural zones were tested with commercially available ELISAs for the presence of antibodies against Brucellosis (*B. abortus*; Figure 3). The overall prevalence of Brucellosis is 4% (9/227). The overall prevalence of Brucellosis for urban zones and rural zones is 1.6 % (1/62) and 4.8 % (8/165), respectively. The prevalence of Brucellosis in dogs according to each sampling zone is 3.2 % (1/31) in Guayaquil; 0 % (0/31) in Ibarra; 0 % (0/37) in Daule; 12.2 % (6/49) at YT University and in Urcuquí; 2.5 % (1/40) in Santo Domingo; and 2.6 % (1/39) at IKIAM University, respectively.

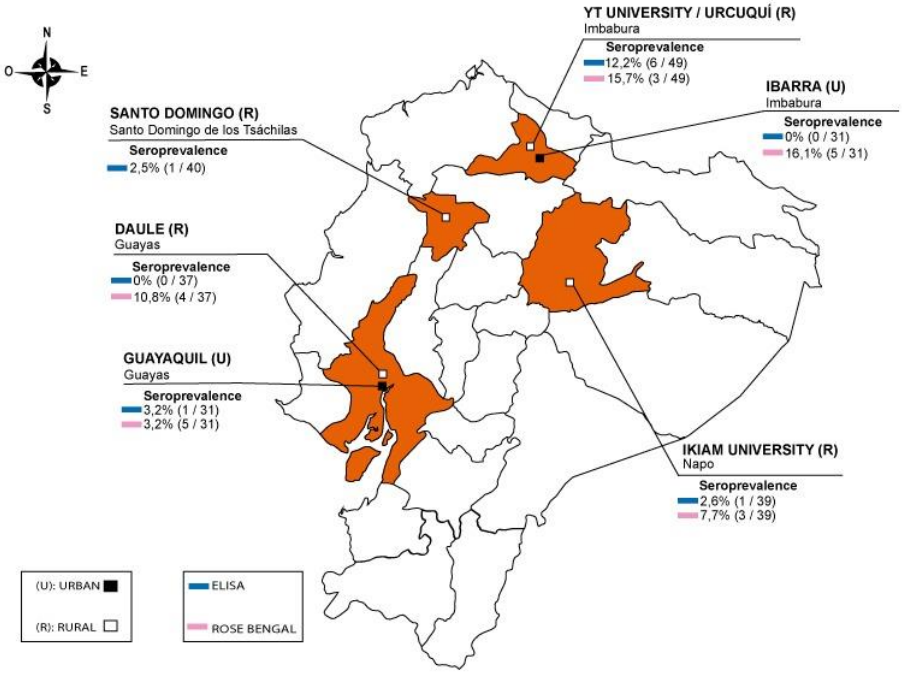

Figure 2. *Seroprevalence of Brucellosis (Brucella abortus) for each rural and urban sampling zone. Indirect multispecies enzyme-linked immunosorbent assays (blue) and Rose Bengal Plate Test (pink) were used.*

5.3 Seroprevalence of Q fever caused by C. burnetii according to the Enzyme-Linked Immunosorbent Assay

A total of 227 serum samples from Ecuadorian Stray dogs, 62 from urban zones, and 165 from rural zones were tested with commercially available ELISAs for the presence of antibodies against Q fever (*C. burnetii*; Figure 4). The overall prevalence of Q fever is 2.6 % (6/227). The overall prevalence of Brucellosis for urban zones and rural zones is 0% (0/62) and 3.6% (6/165), respectively. The prevalence of Q fever in dogs according to each sampling zone is 0% (0/31) in Guayaquil; 0% (0/31) in Ibarra; 2.7% (1/37) in Daule; 0 % (0/49) at YT University and in Urcuquí; 12.5 % (5/40) in Santo Domingo and; 0 % (0/39) at IKIAM University, respectively.

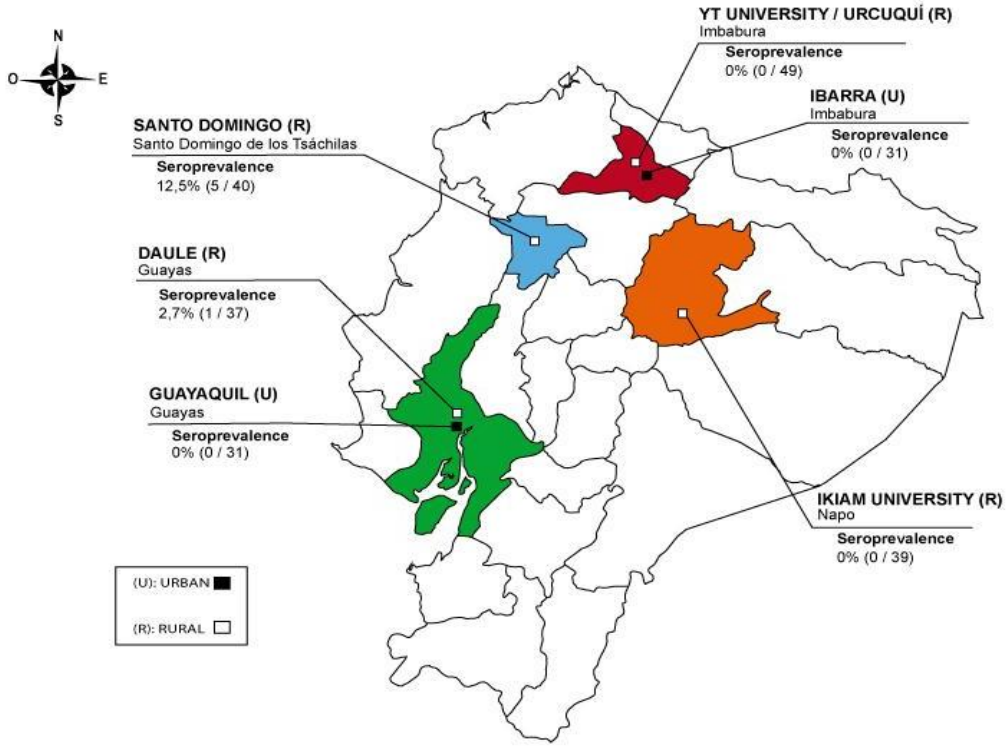

Figure 3. *Seroprevalence of Q fever (Coxiella burnetii) for each rural and urban sampling zone. Indirect multispecies enzyme-linked immunosorbent assay was used.*

5.4 Statistical results

There was not a statistically significant difference (p-value > 0.05) between seroprevalence of both diseases in free-roaming dogs from rural zones compared to those from urban zones. Estimated p-value determined by the Chi-square test were 0.16 for Brucellosis data (determined by RB test), 0.28 in Brucellosis data (determined by ELISA test), and 0.14 in Q fever data (determined by ELISA test).

6. DISCUSSION

This serological analysis of exposure to Brucellosis and Q fever was the first report from a broad and diverse population-based sample of dogs in Ecuador. The study showed a seroprevalence of Brucellosis, as determined by RB and ELISA, of 8.9% and 4.0%, respectively. Moreover, results showed that Q fever seroprevalence, determined by ELISA, was 2.6%. No statistically significant difference was found between the seroprevalences of Brucellosis and Q fever in dogs from rural and urban zones.

Reports from the literature suggest a variety of Brucella seroprevalences in dogs, ranging from 5.46 % to 26.8 % when performed using RB and ELISA tests, the assays recognized as the gold standard by the OIE (World Organization for Animal Health). In this study, Brucella seroprevalence determined by RB and ELISA test were 8.9% and 4.0 %, respectively. These values were lower when compared to other countries from Latin America and Africa. Seroprevalence estimations are of 5.46 % in Sudan, 26,8 % in Argentina, and 24.24% in Brazil (Cadmus et al., 2011; Miceli et al., 2019; Vieira et al., 2016). In Ecuador, there are no previous studies of *B. abortus* available in dogs. However, the high seroprevalence of *Brucella abortus* in cattle and dogs compared to relatively few reports of human seroprevalence leads to speculate that most of the human cases remain misdiagnosed in Ecuador (Ron-Román et al., 2014; Zambrano Aguayo & Pérez Ruano, 2015).

Worldwide, there are only a few published reports regarding Q fever seroprevalence in dogs, which ranges between 5 and 22%. In the present study, Q fever seroprevalence in dogs was 2.6 %, which was lower compared to those reported in the bibliography. The estimated seroprevalences are 7.7% in Iran, 5.5% in Iraq, and, 21.8% in Queensland. So far, there are no previous studies of *C. burnetii* available in dogs in Ecuador (Esmailnejad & Hasiri, 2017; Havas & Burkman, 2011; Cooper et al., 2011).

No statistically significant difference was found between the seroprevalence of dogs from rural and urban zones (p-values > 0.05). This result was somewhat unexpected, since a difference between the seroprevalences of urban and rural zones cattle is well documented, and some interaction takes place between cattle and dogs. However, in Ecuador there is not a clear delimitation of the distribution of cattle so it can indistinctively be found in both areas. Cattle to dog's transmission of both diseases can occur indistinctly in rural and urban zones.

The sampling model was useful to determine whether both diseases were present in dogs from rural and urban zones. One of the clear implications of this study is that no sampling model design was proposed as sampling sites were chosen according to the availability to collect samples in each location. This approach allowed us to obtain a cross-sectional estimation of seroprevalence of Brucellosis and Q fever in free-roaming dogs.

Our results indicate that dogs have been exposed to Brucellosis and Q fever, possibly as a result of interaction with infected cattle secretions or ingestion of abortive tissues as in Ecuador, high seroprevalences of Brucellosis and Q fever exist in cattle (Carbonero et al., 2015; Carbonero et al., 2018). The large number of free-roaming dogs in the entire country and seroprevalence of *B. abortus* and *C. burnetii* in dogs indicates the potential role of dogs in the transmission and spillover of both diseases. As dogs are vectors for human transmission, close contact with infected pets or their secretions represent a risk factor for diseases in humans (Wareth et al., 2016). In Ecuador, Brucellosis and Q fever appear to be occupational diseases in humans. Farmworkers and people working in slaughterhouses present higher seroprevalence than those of people in the general population (Echeverría et al., 2019; Ron-Román et al., 2014).

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The large number of free-roaming dogs in the entire country and the presence of zoonotic diseases is a public health problem since humans can result infected due to the close contact. This research aimed to determine the seroprevalence of *Brucella abortus* and *Coxiella burnetii* in free-roaming dogs. Based on quantitative and qualitative methods we determined that both diseases are present in dogs. It can be concluded that dogs are transmission vectors for zoonotic diseases in Ecuador, in this case for Brucellosis and Q fever.

By statistically analyzing and comparing seroprevalences between free-roaming dogs living in rural zones from those living in urban zones, we determined that no statistically significant difference exist between both groups. Ecuador does not have clear delimitation between urban and rural zones. Thus, dogs can indistinctly acquire *B. abortus* and *C. burnetii* associated with cattle in urban and rural areas.

7.2 Recommendations

Based on the knowledge acquired during this research, the following recommendations can be drawn to increase the impact of this type of studies:

- To use a gold standard diagnostic test to estimate the prevalence of both diseases in the dog and human populations to prioritize strategies of control. Understanding patterns in prevalence helps us understand associated risk factors of transmission among cattle and dogs, and dogs and humans, which is necessary to avoid outbreaks.

- To slaughter infected dogs is highly recommended to prevent disease transmission to both healthy dogs and humans. Of special concern are the infected dogs at Yachay Tech University, where dogs have a very intimate relationship with students in a limited area. Many dogs live and are feed in the same area where food is prepared and served (personal observation).

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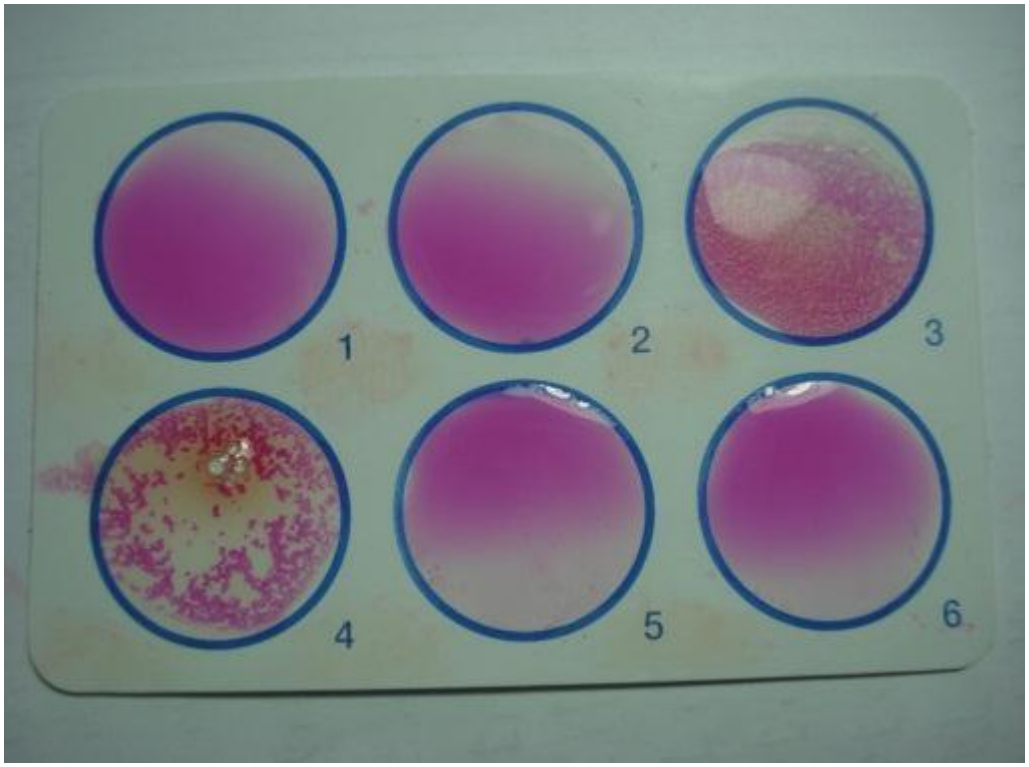
APENDIXXES

Appendix A

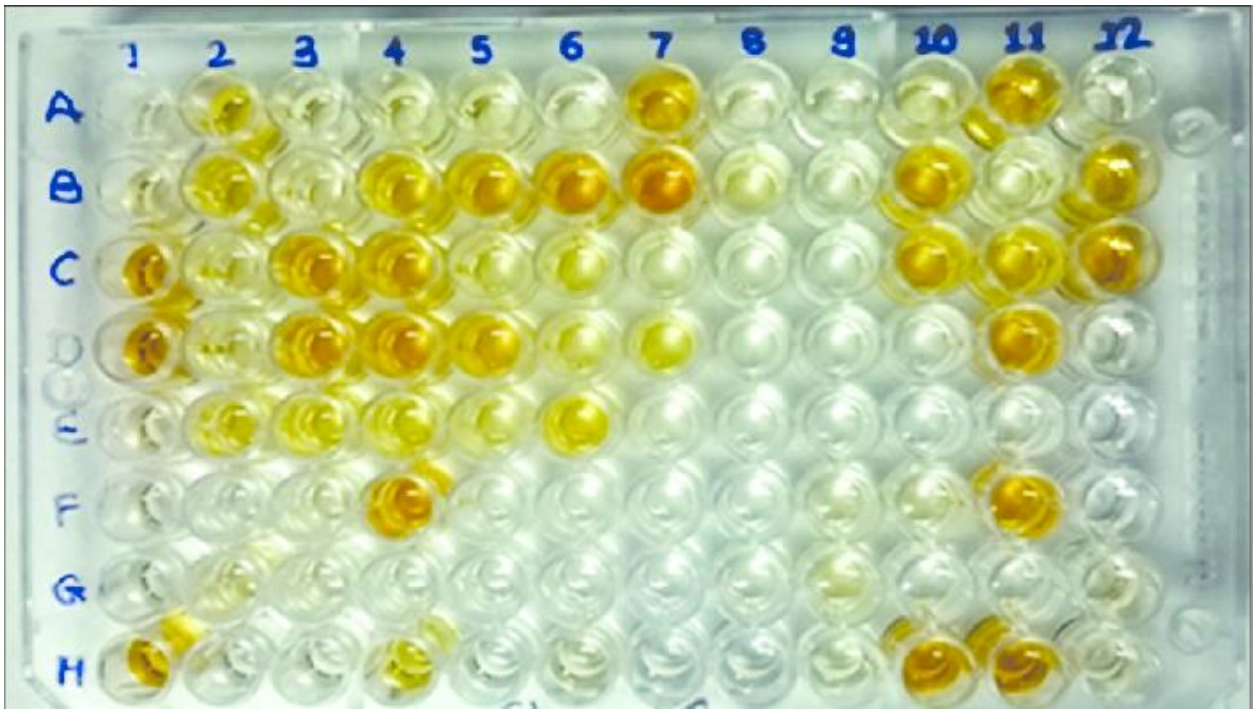
Rose Bengal Plate Test; Positive test showing agglutination in sample no.3 and 4.



Source: Seroprevalence of ovine brucellosis in Bangladesh - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Rose-Bengal-Plate-Test-Positive-test-showing-agglutination-no3-and-4_fig2_303239433 [accessed 15 February, 2020]

Appendix B

Microtiter plate showing the results of Indirect ELISA



Seroprevalence and comparison of different serological tests for brucellosis detection in small ruminants - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Figure-3-Microtiter-plate-showing-the-results-of-I-ELISA-Well-A1-and-B1-Conjugate_fig3_276501450 [accessed 28 Jul, 2020]