Low Seroprevalence Of *Brucella* Spp. Among Remote Colombian Communities From The Sierra Nevada De Santa Marta

Regina Oakley[1,*], Simone Kann[2], Michèle Plag[1], Gustavo Concha[3], Anou Dreyfus[4] and Daniel H. Paris[1]

[1]Swiss Tropical and Public Health Institute, Allschwil, Switzerland; University of Basel, Basel, Switzerland

[2]medmissio, Institute for Global Health, Würzburg, Germany

[3]Organización Wiwa Yugumaiun Bunkuanarua Tairona, Valledupar, Colombia

[4]Vetsuisse Faculty, University of Zürich, Zurich, Switzerland

Running title: Seroprevalence of *Brucella* spp. in Colombian tribes

Abstract

Brucella species cause a high burden of disease globally, infecting both humans and animals; however, One Health has been under-appreciated in Colombia. This study aimed to determine the seroprevalence of *Brucella* spp. in two remote indigenous communities from the Sierra Nevada de Santa Marta, Colombia. These communities live in close contact with their livestock, indicating a potential susceptibility to zoonotic pathogens. The livestock routinely kept by these communities include cattle, small ruminants and pigs, the known hosts of human pathogenic *Brucella* spp.. A low level of exposure to *Brucella* spp. was documented, with only one positive participant among 539 participants (0.2%; 95% CI 0.0 – 1.0). Nevertheless, due to the high risk that zoonoses pose, we recommend discussions with the community for the potential establishment of One Health surveillance studies for the early detection and prevention of future zoonotic disease threats.

Introduction

Brucella species are gram negative bacteria causing brucellosis, a zoonotic disease of global significance[1]. The species most often involved in human infection are *B. melitensis, B. abortus, B. suis, and B. canis*[2]. The most common clinical findings in human brucellosis are fever and a flu-like illness, followed by osteoarticular manifestations[3]. Lymphadenopathy and a broad palette of genitourinary, neurological, pulmonary and gastrointestinal complications are also possible[3,4]. Human brucellosis has an overall mortality rate of 5%, most commonly linked to endocarditis[3]. In livestock, brucellosis causes abortion, infertility and subsequent loss of milk production[5]. In addition to the high disease burden in animals and humans, brucellosis can cause substantial economic impact due to reduced animal production and patient time lost from work and routine activities[6,7]. *B. abortus* and *B. melitensis* have the greatest economic impact[5]. Animal to human transmission

occurs through direct contact with placenta, amniotic fluid or aborted foetuses or during slaughtering of infected animals, as well as through consumption of unpasteurized dairy products from infected animals[4]. In Latin America, *Brucella* spp. have been isolated from humans, domestic animals (cattle, goats, pigs, sheep, dogs and horses) as well as wildlife (buffalos, foxes, grey weasels, capybaras and ferrets)[8]. Most human isolates have come from Argentina, Peru and Mexico; whereas in Colombia, Brazil, Chile and Cuba, *Brucella* spp. was predominantly isolated from cattle[8].

There are limited reports on the seroprevalence of *Brucella* spp. in humans from South America. One study on the population of an urban slum in Brazil found sero-positivity of 13% for *B. abortus* and 4.6% for *B. canis*[9]. A study from Northwest Ecuador found an overall seroprevalence of only 1.9%[10]. Although, the seroprevalence for participants in the high-risk group (occupational exposure to animals) was 4.8% compared to 1.4% in the low-risk group (no occupational exposure to animals)[10].

Serological studies in animals from South America have primarily been conducted in Argentina and Brazil. In Argentina, *Brucella* spp. exposure has been reported in dogs (14.7%)[11], cattle (3.7%)[12], water buffalo (6.4%)[13], foxes (17.8%)[14], minks (9.2%)[15] and armadillos (16.0%)[16]. In Brazil, *Brucella* spp. exposure has been reported in cattle (2.2% - 3.7%), buffaloes (4.8% - 6.8%)[17,18], equines (1.3%)[19] and rams (2.9%)[20,21]. One additional study from Ecuador also reported exposure in cattle (16.7%)[22,23].

In this study we hypothesized to find a high seroprevalence of *Brucella* spp. in the two indigenous communities, the Wiwa and Koguis, residing in the Sierra Nevada de Santa Marta, Colombia. Due to the limited contact these communities have with the outside world, little is known about their healthcare needs and disease burden. It was expected that these communities would have a high burden of infectious disease due to their limited access to medical services, poor socioeconomic status, inadequate access to sanitation and clean drinking water and simple housing (clay huts with unsealed floors)[24,25]. These communities live in close contact with their livestock (cattle, small ruminants, pigs and poultry), indicating a potential susceptibility to zoonotic pathogens including *Brucella* spp.

Materials and Methods

Study population and data collection

This cross-sectional study used a subset of 539 serum samples and demographic data collected within a larger research study called "Colombia-Germany research program on diagnostics, research, treatment and prevention of Chagas Disease (CD) and emerging infectious diseases in vulnerable groups" between 2021 and 2022. Samples were collected from two indigenous communities, the Wiwa and Koguis whose population was reported as 18,202 and 15,820, respectively, in the 2018 census[26]. The samples were randomly selected from the larger study and stratified by village. The Wiwa participants (n = 264) came from five villages: Surimena (n = 52), Ahuyamal (n = 64), Sabannah de Higueron (n = 68), Dungakare (n = 36) and Potrerito (n = 43). The Koguis participants (n = 275) came from four villages: Zarachui (n = 94), Mamangueka (n = 63), San Jose (n = 114), Avingue (n = 4) (Figure 1). Exposure data for common zoonotic disease risks was also collected for 147 of the included Wiwa participants.

Community members were invited to participate in the study after being informed about the project. Volunteers aged ≥ 12 years were recruited into the study if they had a positive Chagas rapid diagnostic test (RDT) result, while all volunteers < 12 years old were recruited to avoid an additional blood draw required for the Chagas RDT. Blood samples were collected with a vacutainer (8ml) and kept in a cooler at 4°C (provided by World Courier Frankfurt, Germany) in the field before being transferred daily to the laboratory where they were centrifuged and stored at -20°C[27]. Samples were shipped on dry ice to
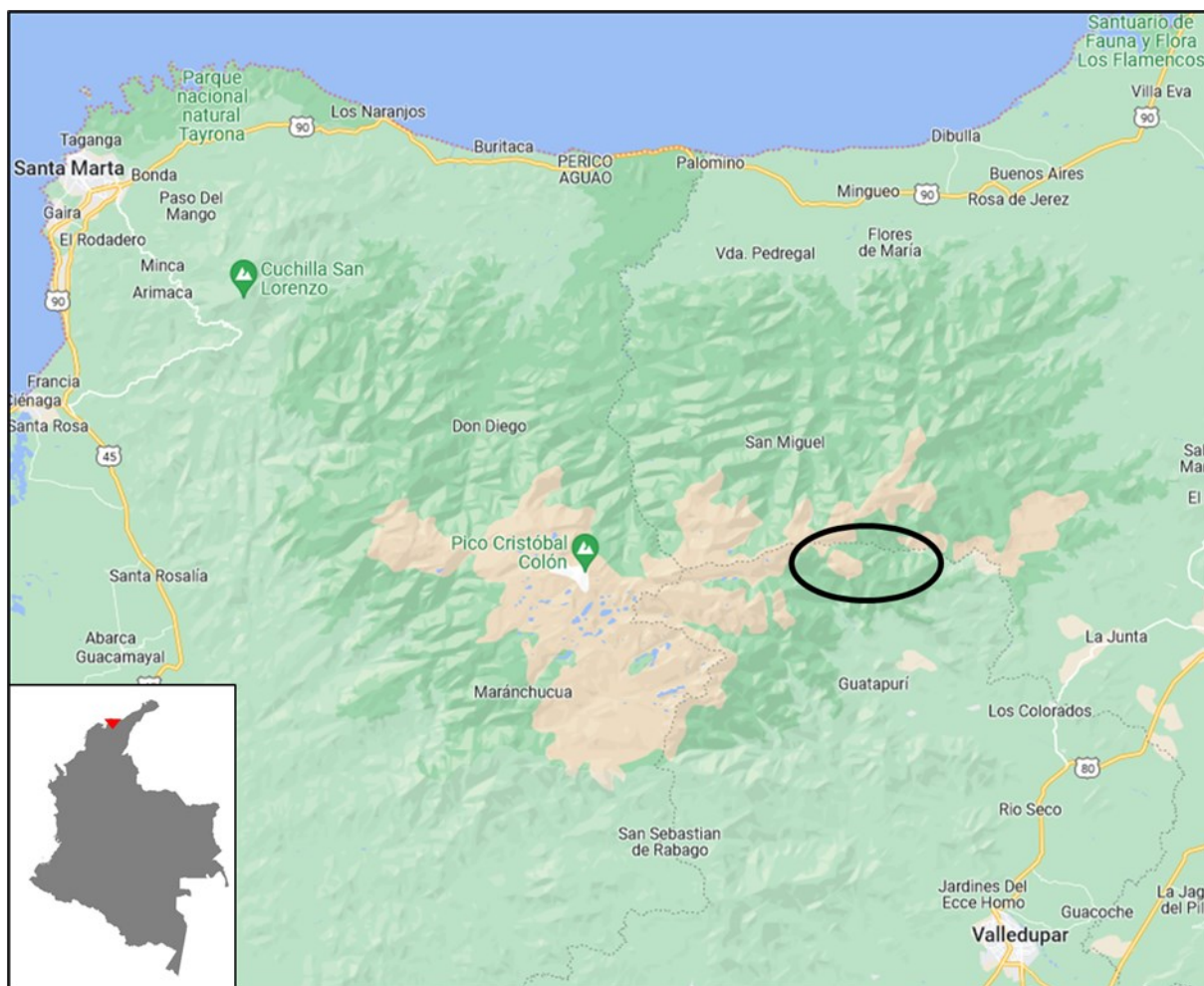
Figure 1. Map of the Sierra Nevada de Santa Marta, Colombia. Included Wiwa and Koguis villages are located within the area marked by the black oval. Insert shows a map of Colombia with the Sierra Nevada de Santa Marta highlighted in red. Adapted from Google Maps and Vemaps[28,29]

Medmissio, Würzburg, Germany (World Courier Frankfurt, Germany) where they were stored at −80 °C[27]

Sample size calculation

To determine the seroprevalence of *Brucella* spp. in the Wiwa and Koguis people from Colombia, a required sample size of 196 participants was calculated using epitools from https://epitools.ausvet.com.au[30]. The sample size was calculated for a precision of 0.05, a confidence of 0.95 and an estimated *Brucella* spp. seroprevalence of 15%. Since no information regarding the seroprevalence of *Brucella* spp. in humans in Colombia was found, this conservative estimate was based on the higher seroprevalence found in the Brazilian study[9].

Ethical considerations

Written informed consent (or witnessed thumb print) was obtained from all participants - or legal guardians of a child where appropriate - prior to sample collection. The studies were performed in accordance with the principles of the Declaration of Helsinki and were approved by the Institutional Ethic Committee for Investigation of Bogota, Colombia (Acta No. 2019-4). Ethical approval and authorization to perform the study was also granted by the Governors of the Wiwa and Kogius

communities with permission to enter their territory.

Serological testing

Serological testing was performed at the Swiss Tropical and Public Health Institute, Basel Switzerland. The serum samples (n=539) were screened by the Serion enzyme-linked immunosorbent assay (ELISA) Brucella IgG *classic* (Serion/Verion Immundiagnostica GmbH, Würzburg Germany) according to the manufactures instructions with the positivity cut-off determined using the manufacturers automated software[31]. The software uses a 4-parameter logistic function to establish a lot-specific standard curve to determine the test result using standard serum included in each run[31]. An additional cut off was set by calculating the mean of the optical density (OD) plus three standard deviations. Serum samples with an OD over this additional cut off were sent to the Institute for Infectious Diseases (IFIK), University of Bern (Bern, Switzerland) for confirmation by the BrucellaCapt test (Vircell, Granada, Spain).

Results

The samples included were from participants aged from 1-90 years, with a median age of 22 years (IQR 11 - 37), with 57% being female. Of the 539 serum samples screened by the Serion ELISA, one sample was ELISA positive and one was equivocal. In the screening phase, thirteen samples (including the positive and equivocal sample from the Serion ELISA) demonstrated an OD greater than the additional cut-off calculated based on the negative results. These 13 samples underwent confirmatory testing by the BrucellaCapt test (performed at the certified laboratory IFIK, Bern, Switzerland). One sample, from a Koguis participant was found positive by the BrucellaCapt test – leading to an overall seroprevalence of 0.2% (95% CI 0.0 – 1.0).

Exposure data in the Wiwa people (n = 147) identified specific practices associated with zoonotic disease transmission: animal slaughtering (84.4%), assisting with birth (23.8%) and milking (18.4%) including cattle, goats and sheep (Table 1). One investigator reported similar practices among the Koguis people. All Wiwas and Koguis questioned reported regular exposure to and handling of livestock and animals (cattle, goats and sheep), thereby limiting the unequivocal identification of risk factors.

Discussion

A very low seroprevalence of <1% was identified for *Brucella* spp. in over 539 sera from two indigenous communities residing in the Sierra Nevada de Santa Marta, Colombia. While studies are limited on brucellosis in Colombia, two case reports on *Brucella* spp. infections in humans describe; a farmer with antibodies to *Brucella* spp. (not speciated) believed to have been infected through contact with cattle[32] and a merchant diagnosed with *B. melitensis* by blood culture, whose infection was suspected to be linked to consumption of unpasteurized milk[33]. A molecular study in the Colombian Caribbean region found 22.2% (6/27) of cheese samples made from unpasteurized cow milk to be positive for *Brucella* spp. DNA[34]. *B. abortus* is the only species previously identified in livestock from Colombia, with herd-level seroprevalence reported between 22.0% – 27.5%[35-39]. The only other species identified in Colombia is *B. canis*, found in humans (9.0%) and dogs (2.0% - 15.0%) in Bogotá and the Antioquia region[40-42]. There is evidence for the absence of *Brucella* spp. exposure in regions of Colombia in two serological studies; one in domestic pigs (n = 350, in 23 farms), and another in collared peccaries (*Pecari tajacu,* n = 58) and feral pigs (n = 15) finding no seropositivity for *Brucella* spp.[43,44].

The serion ELISA used in our study is coated with native extract from *B. abortus,* containing genus-specific antigens for the detection of antibodies against human pathogenic *Brucella* spp[31]. The manufacturer reported a high sensitivity (>99%) and specificity (99.3%)[31]. An independent evaluation,

Table 1. Exposure among the Wiwa people (n = 147) living in the Sierra Nevada de Santa Marta, Colombia, to potential risk factors for the transmission of zoonotic pathogens.

Exposure	Positive, n (%)
Participate in animal slaughter:	
Chickens	108 (73.5)
Turkeys	5 (3.4)
Pigs	16 (10.9)
Cattle	7 (4.8)
Goats	72 (49.0)
Sheep	4 (2.7)
None	23 (15.6)
Assist in animal birth:	
Pigs	2 (1.4)
Cattle	5 (3.4)
Goats	8 (5.4)
Sheep	2 (1.4)
Other	5 (3.4)
None	112 (76.2)
Milking of animals:	
Cattle	12 (8.2)
Goats	8 (5.4)
Sheep	6 (4.1)
Other	3 (2.0)
None	120 (81.6)

however, found a lower sensitivity of 84%, subsequently there is a possibility of false negatives with the ELISA[45]. Although, we do not believe this to affect the findings of this study as the samples with the highest ELISA OD readings were confirmed negative by the BrucellaCapt test performed at the certified IFIK, Bern, Switzerland. The BrucellaCapt test covers the three smooth lipopolysaccharides (LPS) containing human pathogenic *Brucella* spp. (*B. melitensis*, *B. abortus* and *B. suis*) with a reported 100% sensitivity and specificity[45,46]. *B. canis*, however, has a rough LPS and is often not adequately diagnosed by routine serological tests[47]. The reports of *B. canis* in Colombia may warrant further epidemiological investigations to determine the exposure to dogs and *B. canis* among the indigenous tribes of the Sierra Nevada de Santa Marta.

While we found limited evidence of *Brucella* spp. amongst the Wiwa and Koguis tribes, this should not discount the potential risk of zoonotic diseases of these remote, vulnerable communities, with notably highly limited access to health care facilities.

Zoonotic diseases are responsible for substantial morbidity and mortality, particularly in low resource

communities with high dependence on livestock[48]. Globally, zoonoses account for 60.3% of emerging infectious diseases[48]. The major drivers of their emergence or re-emergence include expansion of cities or farmland with deforestation, increasing human population and urbanization, globalization of food systems, climate change and environmental contamination[48-50]. Remote tribes having been protected through their isolation and with high proportions of immunologically naïve inhabitants may be particularly vulnerable to these drivers and exposure to new pathogens. Given the high level of exposure to animals in the populations of this study, it was unexpected to find such a low seroprevalence for this normally common zoonotic disease. The low rates could be attributed to the isolated raising and maintenance of livestock with small herd numbers, reducing the contact rate and transmission risk[51]. Introduction of infected animals poses a risk to these vulnerable populations and discussions with the community on potential surveillance programs are warranted. One Health surveillance systems provide a means of early detection to prevent outbreaks in both animal and human hosts[52]. These systems are particularly effective for zoonoses that have limited human-to-human transmission risk, as is the case with brucellosis, which can be detected and controlled in the animal host preventing transmission to humans[53,54].

A limitation of this study is that despite testing more than double the calculated sample size, the sample may not reflect adequately the geographical distribution in the Sierra Nevada de Santa Marta, Colombia. The Wiwa and Koguis participants accounted for 1.5% and 1.7% of their entire populations, respectively – and the sampled villages were located to the East of the Sierra Nevada de Santa Marta. We cannot discount the possibility of differential exposure in other Wiwa and Koguis villages. Further, is that we do not have the risk factor data for the Koguis participants, including the *Brucella* spp. positive individual. Consequently, we cannot comment on the possible route by which this individual became exposed to the bacteria.

Conclusion

The evidence of *Brucella* spp. exposure in the Wiwa and Koguis tribes was found to be very small with a seroprevalence of <1% by screening/confirmatory testing. Consultation with these communities for the implementation of systematic One Health investigations for the detection and prevention of potential emerging zoonotic threats are recommended due to their isolation and limited access to healthcare.

Acknowledgements

Conflict of interest

None of the authors reports any conflict of interest.

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