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REGULAR ARTICLES

The unexpected discovery of *Brucella abortus* Buck 19 vaccine in goats from Ecuador underlines the importance of biosecurity measures

Jorge Ron-Román[1,2,3,4] · Dirk Berkvens[2] · Daniela Barzallo-Rivadeneira[1] · Alexandra Angulo-Cruz[1] · Pablo González-Andrade[1] · Elizabeth Minda-Aluisa[1] · Washington Benítez-Ortíz[1,5] · Jef Brandt[2] · Richar Rodríguez-Hidalgo[1] · Claude Saegerman[3]

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Abstract Very few, mostly old, and only preliminary serological studies of brucellosis in goats exist in Ecuador. In order to assess the current epidemiological situation, we performed a cross-sectional serological study in the goat populations of Carchi ($n = 160$ animals), Pichincha ($n = 224$ animals), and Loja provinces ($n = 2024$ animals). Only two positive serological results (RB negative and SAT-EDTA ≥400 IU/ml) were obtained in lactating goats from the same farm in Quito (Pichincha province). Additionally, milk was sampled from 220 animals in Pichincha province. The present study indicates a low apparent prevalence in Pichincha province and absence in Carchi and Loja provinces. A total of 25 positive milk ring tests (MRT) were obtained in Pichincha province yielding a prevalence of MRT of 11.16%. Subsequent culture was performed on the positive MRT samples. All results were negative, apart from a single sample, obtained from a serologically positive goat in Quito, that was positive for *Brucella abortus* strain 19 (B19). Several hypotheses are forwarded concerning this unexpected result. The most likely hypothesis is the possible accidental use of a needle, previously used for vaccination of cattle with the said vaccine, for the administration of drug treatment to the goat. This hypothesis underlines the necessity of biosecurity measures to prevent this type of accidents.

Keywords Brucellosis · Goats · Ecuador · Vaccine · Biosecurity

✉ Claude Saegerman
claude.saegerman@ulg.ac.be

[1] Instituto de Investigación en Salud Pública y Zoonosis (CIZ), Universidad Central del Ecuador, Quito, Ecuador

[2] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

[3] Research Unit of Epidemiology and Risk analysis applied to Veterinary Sciences (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, B42, Boulevard de Colonster 20, B-4000 Liège, Belgium

[4] Carrera de Ingeniería Agropecuaria, Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas (ESPE), Sangolquí, Ecuador

[5] Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador

Introduction

Brucellosis is a worldwide disease with health and economic impacts (Castro et al. 2005). It is widely distributed in humans and animals, especially in developing countries. Its occurrence is related to the existence of animal reservoirs and high infection rates in livestock, especially in goats and sheep (Corbel 2006).

The main cause of caprine brucellosis is *Brucella melitensis* (biovars 1, 2, and 3) (Godfroid et al. 2010) but some sporadic cases caused by *Brucella abortus* are documented (e.g., Leal-Klevezas et al. 2000). One or more of the following typically characterize the clinical form of the disease: abortion, retained placenta, orchitis, epididymitis, and, more rarely, arthritis together with excretion of the organisms in uterine discharges and milk (OIE 2016a).

Surveillance in goats by indirect diagnostic methods is not a common practice in most countries of South America (PANAFTOSA 2000), where goat breeding is constrained in its development, because of conditions of overcrowding, poor or non-existent disease control measures, and lack of technical assistance,

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which, together with rudimentary empirical management, permit the transmission of brucellosis (Ortega-Sánchez et al. 2009).

Caprine brucellosis due to *B. melitensis* is present in Mexico, Peru, Argentina, Paraguay, and Bolivia (Aznar et al. 2014; PANAFTOSA 2000). Until now, there are no reports in Ecuador of isolation and characterization of *B. melitensis* in bovines or goats, only molecular findings that demonstrate its presence in samples of lymphatic nodes from goats at the slaughterhouse of Quito (Luna et al. 2016). The total number of goats is estimated between 178,000 (INEC and SICA 2002) and 191,000 (OIE 2016b) of which approximately 43% (78,000) are found in the canton of Zapotillo in Loja province.

The marketing of goat milk in different parts of the Metropolitan District of Quito (two million inhabitants) has become a common activity and forms the basic income of several families engaged in this business. Ecuadorian law prohibits peddling unpasteurized milk, and although vendors work without government regulation, they try as much as possible to maintain minimum health standards, such as collecting animal droppings, washing the udder, and selling milk in new and clean bottles (El Comercio 2012).

The very few serological studies of brucellosis in goats conducted in Ecuador are old and incomplete or preliminary (e.g., Poulsen et al. 2014). In order to determine the seroprevalence of *Brucella* spp. in goats in three selected areas of Ecuador, as well as isolate the causative agent, we conducted a cross-sectional study (serum and milk samples) in Carchi, Pichincha, and Loja provinces.

Materials and methods

Selected areas

The selection of three areas for this study is based on the potential risks: Bolivar and Mira cantons of Carchi province (presence of bovine brucellosis in cattle and existence of mixed farms) (Ron-Román et al. unpublished data), the urban and peri-urban Metropolitan District of Quito in Pichincha province (business of milk goats in Quito city and high density of inhabitants) and Zapotillo canton of Loja (high density of goats) provinces (Fig. 1).

Sampling design

A survey with census sampling at farm level ($n = 86$) and convenience sampling at animal levels ($n = 2{,}408$) was performed in the three selected areas. In Carchi and Pichincha provinces (small herds), all herds and all animals present in a herd were sampled. In Zapotillo canton of Loja province (large herds), all herds were included and a random selection of 25% of animals present in a herd was sampled.

In Carchi, blood was sampled between December 2012 and February 2013 ($n = 160$ goats in 12 herds). In urban and peri-urban Quito (Pichincha province), blood and milk were sampled between December 2009 and April 2010 ($n = 224$ and 220 goats in 12 herds for blood and milk samples, respectively). In Zapotillo canton of Loja province, blood were sampled in July 2011 ($n = 2{,}024$ goats in 62 herds). The milk samples were collected only in Quito, area with positive results to serology, to perform the isolation and characterization of the pathogen.

Samples

The goats sampled belonged to native, Nubian, and Anglo-Nubian breeds. Jugular vein blood was sampled in vacutainer tubes (10 ml). Each sample was centrifuged; the serum was identified, analysed, and stored at −20 °C. In addition, 100 ml of milk was collected from each lactating goat sampled in peri-urban Quito. All milk samples were identified, stored in a cool box until analysis at the Instituto de Investigación en Salud Pública y Zoonosis (CIZ, Central University of Ecuador).

Blood and milk analysis

Serum samples were analyzed for the presence of antibodies against *Brucella* spp. using two diagnostic tests: slide agglutination test with Rose Bengal (RB) and the serum agglutination tube test with EDTA (SAT-EDTA). These tests were performed as previously described (Alton et al. 1988; OIE 2016a). The modified MRT test as described by Mancera and Ontiveros (2001) for diagnose of brucellosis in goats, was performed as a complementary test on the milk samples. The modification consisted in the addition of 0.3 ml of a NaCl solution [25%] and 0.1 ml of corn oil to each milk sample (1 ml). Afterwards, the samples were incubated at 37 °C for 2 h.

Isolation and identification of Brucella spp.

Milk samples from SAT-EDTA-positive ($n = 2$) and MRT-positive animals ($n = 23$) were centrifuged at 2000 g for 15 min. The supernatant (cream) and sediment were grown in selective Farrell medium (Columbia Agar Base [Oxoid CM0331] with 5% decomplemented horse serum [GIBCO Ref-16050-130], and *Brucella* selective supplement [OXOID SR0083A]) for the isolation of *Brucella* spp.

Replicated colonies with BASE medium (Columbia Agar Base with 5% decomplemented horse serum) were identified and classified by means of macroscopic and microscopic observation, Gram staining, and oxidase [DIFCO-BBL Ref: 261181], catalase, and urease tests. The procedures were

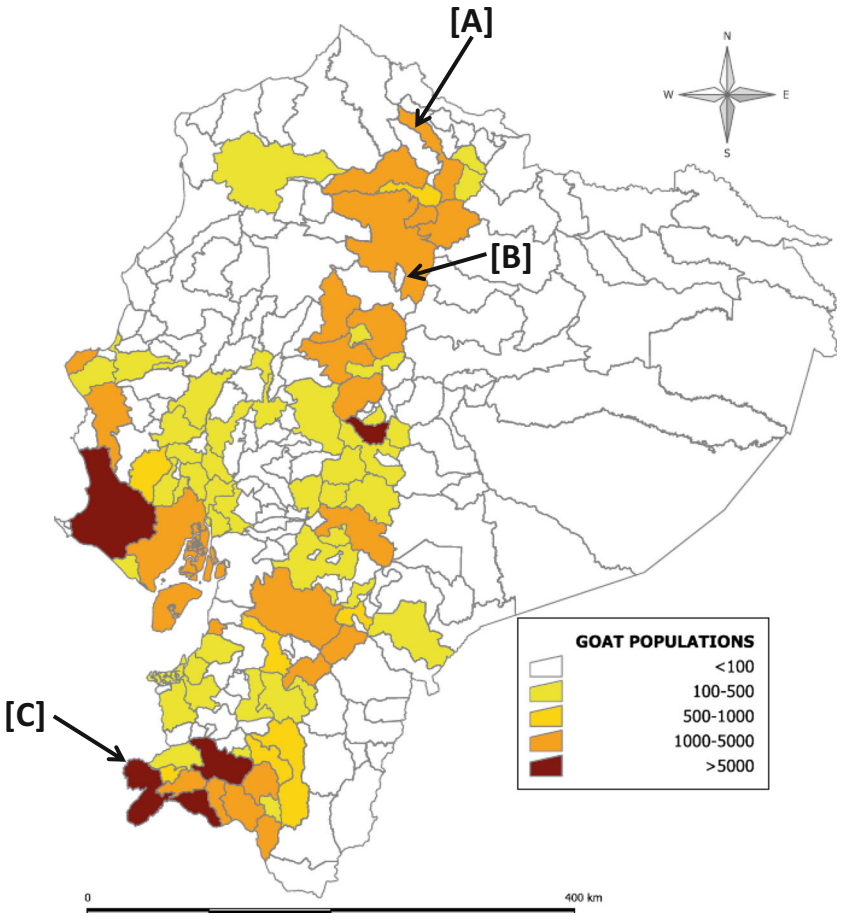

Fig. 1 Goat population per canton and localization of the study areas (INEC and SICA 2002). *[A]* Bolivar and Mira cantons of Carchi province (presence of bovine brucellosis in cattle and existence of mixed farms); *[B]* urban and peri-urban Metropolitan District of Quito in Pichincha province (business of milk goats in Quito city and high density of inhabitants); *[C]* Zapotillo canton of Loja province (high density of goats)

performed as previously described (Alton et al. 1988; Godfroid and Boelaert 1995).

Identification and molecular characterization of *Brucella* spp.

Once identified by biochemical tests, the *Brucella* colonies were analyzed molecularly by three different PCR tests: the IS6501 PCR or PCR-IS711 (primers: IS6501 3 ′: 5′-gat-aga-agg-ctt-gaa-gct-tgc-gga-c-3 ′/IS6501 5′: 5′-acg-ccg-gtg-tat-ggg-aaa-ggc-ttt-t-3′) for genus identification, AMOS PCR (primers: *B. abortus*-specific: gac-gaa-cgg-aat-ttt-tcc-aat-ccc; *B. melitensis*-specific: aaa-tcg-cgt-cct-tgc-tgg-tct-ga; *B. ovis*-specific: cgg-gtt-ctg-gca-cca-tcg-tcg; *B. suis*-specific: gcg-cgg-ttt-tct-gaa-ggt-tca-gg; IS711-specific: tgc-cga-tca-ctt-aag-ggc-ctt-cat) (Bricker and Halling 1994) for species determination, and modified AMOS PCR (Primers: RB51/2308: ccc-cgg-aag-ata-tgc-ttc-gat-cc; eri primer 1: gcg-ccg-cga-aga-act-tat-caa; eri primer 2: cgc-cat-gtt-agc-ggc-ggt-ga) (Bricker and Halling 1995) for the differentiation between vaccine strains and field strains.

Statistical analysis

The seroprevalence was estimated with a binomial exact distribution and computed in Stata/MP 14.1 (StataCorp 2015).

Results

No serological RB test showed the presence of antibodies in any of the animals tested but some animals originating from Pichincha province (see below) tested positive for the SAT-EDTA.

The study demonstrated the absence of antibodies to *Brucella* spp. in Bolivar and Mira cantons of Carchi province (Number of animals tested (Nt) = 160; seroprevalence of 0% with 95% confidence interval (CI)0–1.85%) and Zapotillo canton of Loja province (Nt = 2,024; seroprevalence of 0% with 95% CI = 0–0.15%). The seroprevalence of brucellosis in the district of Quito in Pichincha province was quite low (Nt = 224; seroprevalence of 0.89% with 95% CI = 0.11–3.19%).

Table 1 Serology, culture, and polymerase chain reaction (PCR) results of two SAT-EDTA-positive goats

Sample *N*°	Herd code	Province	Canton	Method of diagnostic						
				RB	SAT-EDTA	MRT	Isolation	PCR IS711	AMOS PCR	mAMOS PCR
178	Tiw 3	Pichincha	Quito	−	400 IUA	+	−	−	−	−
184	Tiw 3	Pichincha	Quito	−	3200 IUA	+	+	+	+	+

RB Rose Bengal test; *SAT-EDTA* serum agglutination test with EDTA; *MRT* milk ring test; *IUA* international units of agglutination; *PCR-IS711* polymerase chain reaction with insertion 711; *AMOS PCR* Abortus, Melitensis, Ovis, and Suis; *mAMOS PCR* AMOS modified (PCR for the differentiation of vaccine strains from field strains)

Of the 220 MRT that were performed in Pichincha province, 25 were positive (milk prevalence of 11.16% with 95% CI = 7.35–16.03%). Only two goats (out of 47 originating from the same farm in the Tiwinsa sector, urban Quito) were positive in SAT-EDTA (high antibody titres) and in MRT (Table 1). From the two seropositive and lactating goats from Quito urban area, one *Brucella* was isolated in milk. This strain was future characterized and identified as *B. abortus* strain 19. The results of the microbiological characterization are in Table 2. A fragment of 498 bp, specific for *B. abortus* biotypes 1, 2, or 4, according to Bricker and Halling (1994), is shown in Fig. 2. In Fig. 3, the absence of the 364 bp fragment (tandem *IS*711) and the *eri* fragment of 178 bp, demonstrate that the strain found in the goat is the *Brucella abortus* strain 19 (B19) vaccine strain (Bricker and Halling 1995). A further 23 lactating goats that were positive in MRT were negative in culture.

Discussion

Brucellosis is a contagious infectious disease, caused by bacteria of the genus *Brucella* spp., which affects both human and several animal species. Caprine brucellosis is mainly due to *B. melitensis* (Godfroid et al. 2010) and some cases of *B. abortus* was previously published (e.g., Leal-Klevezas et al. 2000). The pathogenicity in humans for these two species of *Brucella* is high (Godfroid et al. 2010; Saegerman et al. 2010).

The use of SAT-EDTA, RB, and MRT was previously evaluated for the diagnosis of caprine brucellosis (Falade 1978). There was a good correlation between SAT-EDTA and RB when both tests were negative but RB failed to detect 80% of sera above 50 IU/ml in SAT-EDTA. Also, owing to the relatively poor milking potential of the goat and the false-positive results with MRT, it was concluded that the SAT-EDTA offers a better serological diagnostic tool for caprine brucellosis. This study is in line with this previous information. Unfortunately, studies reporting serological test results in goats should be interpreted with caution, as most of the data have been obtained without isolation of *Brucella* (Mancera and Ontiveros 2001).

Several preliminary results are available in some Faculties of Veterinary Medicine in Ecuador. In Guayas province (west central part of Ecuador), 33% of 800 individual milk samples were positive to MRT in 1970 but with no isolation of *Brucella* (Albornoz 1970). Three

Table 2 Characterization of the caprine *Brucella* spp. isolate

						Growth on colorants				Agglutination with serum	
Bacteriological sample code	Catalase	Oxidase	Urease activity	CO_2 requirement	H_2S production	Thionin 20 μg	Thionin 10 μg	Basic Fuschin 20 μg	Safranin 100 μg	anti A	anti M
Ec-CIZ-Cap-1	+	+++	+ (48 h)	− (48 h)	+++ (24 h)	−	−	+	+	+	−
B2[a]	+	+	+	+	+	−	−	−	−	+	−
B9[b]	+	+	+	−	+	+	+	+	+	−	+
B1[c]	+	+	+	+[d]	+	−	−	+	+	+	−

EC-CIZ-Cap-1 is the caprine *Brucella* isolate

[a] Control *Brucella abortus* biovar 2

[b] Control *Brucella abortus* biovar 9

[c] Control *Brucella abortus* biovar 1

[d] Positive for most strains

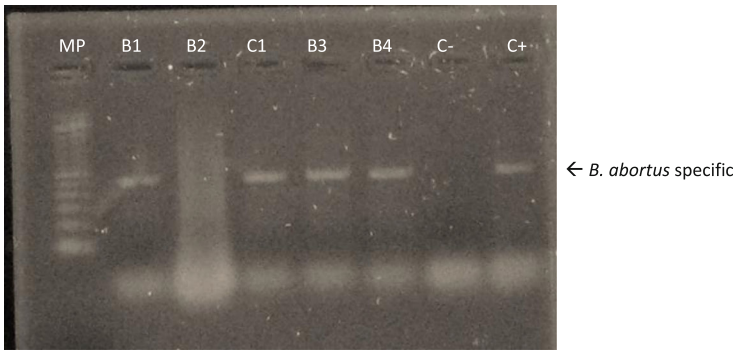

Fig. 2 PCR amplification products from *Brucella* strains tested by the conventional AMOS assay. *MP*: molecular weight marker; *B1*, *B2*, *B3*, and *B4*: samples of *Brucella* strains by bovines; *C1*: samples of *Brucella* strains by caprine (amplification of IS711 which is specific for *B. abortus* biovars 1, 2, or 4 [498 bp]); *C−*: negative control; *C+*: positive control of *B. abortus* biovar 1

Legend: MP: Molecular weight marker; B1, B2, B3 and B4: Samples of *Brucella* strains by bovines; C1: Samples of *Brucella* strains by caprine (amplification of IS711 which is specific for *B. abortus* biovars 1, 2 or 4 [498 bp]); C-: negative control; C+: positive control of *B. abortus* biovar 1.

other serological studies with Huddleson agglutination test in Macará (Granda 1972), Loja (Tapia 1998) and Azuay (Sánchez 1997) provinces indicated a zero or very low seroprevalence.

The present study indicates a low prevalence in Pichincha province and absence in Carchi and Loja provinces.

The discovery of the B19 in milk from a goat with a positive serology result (SAW-EDTA: 3,200 IU/ml; high IgM level) was unexpected. Several hypotheses can be postulated. The first hypothesis is the improper use of brucellosis B19 vaccine in goats in addition to its advised use in cattle. The brucellosis vaccine of choice for goats is Rev 1 and, as recommended, B19 is only mandatory in cattle in Ecuador and common in Pichincha province. The second hypothesis is the use of a needle, which was previously used for B19 vaccination in cattle, for the administration of a drug to goats.

Goats and other species present in a herd are commonly treated by drug injection with the same needle. The second serologically positive goat comes from the same herd, which may form an indication of possible serial use of the same needle. The third hypothesis is the consumption of milk by goats originating from B19-vaccinated cattle. Positive microbiological cultures were obtained during a period of 3 years from the milk of cows vaccinated with B19 (Meyer and Nelson 1969), as well as in colostrum (Corner and Alton 1981). Seropositive titers were observed for a period of 1 year after B19 vaccination of cows (Manthei 1952). A study of oral vaccination with B19 showed the need of a large dose (500 billion cells) and all serological tests were negative in heifers 82 days after vaccination (Nicoletti and Milward 1983). Despite the fact that it cannot be excluded, this hypothesis is deemed unrealistic. The fourth hypothesis is the excretion of B19 in the environment by vaccinated bovines and the use of the same pasture by goats. The intermittent excretion of B19 strain was detected by PCR until 9 years in vaccinated cattle mainly in urine and also in milk samples, which confirmed its multiplication and persistence (Pacheco et al. 2012). However, in this study, cultures were always negative. For identical reasons (large dose needed and short period of positivity in serological tests), this hypothesis also appears improbable. In conclusion, the second hypothesis is retained as the most likely.

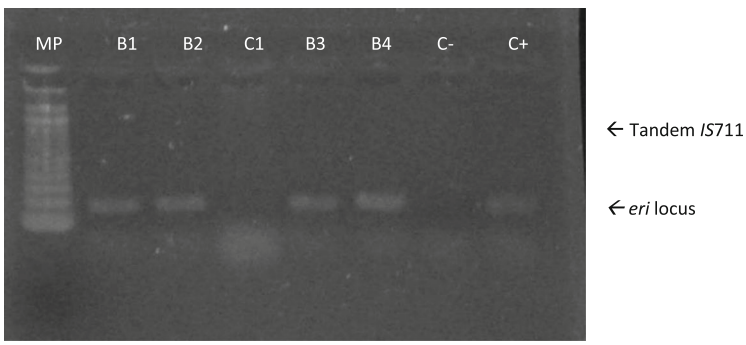

Fig. 3 PCR amplification products from *B. abortus* strains tested by the modified AMOS assay. *MP*: molecular weight marker; *B1*, *B2*, *B3*, and *B4*: samples of *B. abortus* strains by bovines; *C1*: samples of *Brucella* strains by caprine (absence of amplification of tandem *IS711* [364 bp] and *eri* locus [178 bp]); *C−*: negative control; *C+*: positive control of *B. abortus* biovar 1

Legend: MP: Molecular weight marker; B1, B2, B3 and B4: Samples of *B. abortus* strains by bovines; C1: Samples of *Brucella* strains by caprine (absence of amplification of tandem *IS*711 [364 bp] and *eri* locus [178 bp]); C-: negative control; C+: positive control of *B. abortus* biovar 1.

Conclusion

The study demonstrated the absence of antibodies to *Brucella* spp. in Bolivar and Mira cantons of Carchi province and Zapotillo canton of Loja province, the principal goat-producing canton. Isolation of *B. abortus* strain 19 in a goat in Quito district demonstrates the possible cross-infection from vaccinated cattle (B19 vaccination is common here), probably through the accidental use of a needle previously used for vaccination of cattle with B19 vaccine. This finding highlights the necessity of stringent biosecurity measures and quality control of vaccination campaigns.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Albornoz, G., 1970. Diagnóstico de brucelosis por la prueba de "Ring-test" en la provincia del Guayas a nivel de hacienda. Universidad Estatal de Guayaquil.

Alton, G.., Jones, L.., Angus, R.., Verger, J.., 1988. Tecniques for the brucellosis laboratory, 1st Ed. ed. Paris.

Aznar, M.N., Samartino, L.E., Humblet, M.F., Saegerman, C., 2014. Bovine Brucellosis in Argentina and Bordering Countries: Update. Transbound. Emerg. Dis. 61, 121–133. doi:10.1111/tbed.12018

Bricker, B.J., Halling, S.M., 1994. Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR. J. Clin. Microbiol. 32, 2660–6.

Bricker, B.J., Halling, S.M., 1995. Enhancement of the Brucella AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. J. Clin. Microbiol. 33, 1640–2.

Castro, H.A., González, S.R., Prat, M.I., 2005. Brucelosis: una revisión práctica. Acta bioquím. clín. latinoam 39, 203–216.

Corbel, M., 2006. Brucellosis in humans and animals. World Health Organization, Geneva Switzerland.

Corner, L.A., Alton, G.G., 1981. Persistence of *Brucella abortus* strain 19 infection in adult cattle vaccinated with reduced doses. Res. Vet. Sci. 31, 342–4.

El Comercio, 2012. La leche de cabra se vende sin regulaciones [WWW Document]. El Comer. URL http://www.elcomercio.com/actualidad/quito/leche-de-cabra-se-vende.html (accessed 1.1.12).

Falade, S., 1978. A comparison of three serological tests in the diagnosis of caprine brucellosis. Res. Vet. Sci. 24, 376–7.

Godfroid, J., Boelaert, F., 1995. Prescriptions pour le diagnostic sérologique de la brucellose. Belgium: CODA-CERVA (Ed.) 47.

Godfroid, J., Nielsen, K., Saegerman, C., 2010. Diagnosis of brucellosis in livestock and wildlife. Croat. Med. J. 51, 296–305.

Granda, B., 1972. Incidencia de brucelosis caprina en el cantón Macará por el método de Huddleson. Universidad Nacional de Loja.

INEC, MAG, SICA, 2002. ECUADOR - Agricultural Census 1999/2000 – Main Results [WWW Document]. III Censo Nac. Agropecu. URL http://www.fao.org/fileadmin/templates/ess/ess_test_folder/World_Census_Agriculture/Country_info_2000/Reports_2/ECU_SPA_REP_2000.pdf (accessed 7.20.16).

Leal-Klevezas, D.S., Martínez-Vázquez, I.O., García-Cantú, J., López-Merino, A., Martínez-Soriano, J.P., 2000. Use of polymerase chain reaction to detect *Brucella abortus* biovar 1 in infected goats, Veterinary Microbiology. doi:10.1016/S0378-1135(00)00200-5

Luna, L., Chávez, G., Mejía, L., Barragán, V., Trueba, G., 2016. Molecular Detection of *Brucella* Species in Ecuador. Intern J Appl Res Vet Med 14, 185–189.

Mancera, A., Ontiveros, M., 2001. Prueba de anillo en leche o anillo de Bang para el diagnóstico de brucelosis en bovinos, in: Díaz, E., Hernández, L., Valero, G., Arellano, B. (Eds.), Diagnóstico de Brucelosis Animal. México, pp. 79–83.

Manthei, C.A., 1952. Evaluation of vaccinal methods and doses of *brucella abortus* strain 19. Proc. 56th Annu. Meet. Livest. Sanit. Assoc. 115–125.

Meyer, M.E., Nelson, C.J., 1969. Persistence of *Brucella abortus*, strain 19 infection in immunized cattle., in: Proceedings, Annual Meeting of the United States Animal Health Association. p. 159.

Nicoletti, P., Milward, F.W., 1983. Protection by oral administration of *brucella abortus* strain 19 against an oral challenge exposure with a pathogenic strain of *Brucella*. Am. J. Vet. Res. 44, 1641–3.

OIE, 2016a. CHAPTER 2.1.4 Brucellosis (*Brucella abortus*, *B. mellitensis* and *B. suis*) [WWW Document]. OIE. URL http://www.oie.int/fileadmin/Home/esp/Health_standards/tahm/2.01.04_BRUCELLOSIS.pdf (accessed 7.20.16).

OIE, 2016b. OIE World Animal Health Information System [WWW Document]. WAHIS Interface. URL http://www.oie.int/wahis_2/public/wahidwild.php/Countryinformation/Animalsituation (accessed 7.20.16).

Ortega-Sánchez, J.L., Martínez-Romero, A., García-Luján, C., Rodríguez-Martínez, R., 2009. Seroprevalencia de brucelosis caprina en el municipio de Tlahualilo, Durango. México. REDVET. Rev. Electrónica Vet. 10.

Pacheco, W.A., Genovez, M.E., Pozzi, C.R., Silva, L.M.P., Azevedo, S.S., Did, C.C., Piatti, R.M., Pinheiro, E.S., Castro, V., Miyashiro, S., Gambarini, M.L., 2012. Excretion of *Brucella abortus* vaccine B19 strain during a reproductive cycle in dairy cows. Braz. J. Microbiol. 43, 594–601. doi:10.1590/S1517-83822012000200022

PANAFTOSA, 2000. Brucelosis y Tuberculosis, situación de los programas en las Américas (No. 1). Rio de Janeiro, Brasil.

Poulsen, K.P., Hutchins, F.T., McNulty, C.M., Tremblay, M., Zabala, C., Barragan, V., Lopez, L., Trueba, G., Bethel, J.W., 2014. Brucellosis in dairy cattle and goats in northern Ecuador. Am. J. Trop. Med. Hyg. 90, 712–5. doi:10.4269/ajtmh.13-0362

Saegerman, C., Berkvens, D., Godfroid, J., Walravens, K., 2010. Bovine brucellosis, in: Lefèvre, P., Blancou, J., Chermette, R., Uilenberg, G. (Eds.), Infectious and Parasitic Disease of Livestock. Lovoisier, France, pp. 991–1021.

Sánchez, P., 1997. Diagnóstico de brucelosis caprina, en el Cantón Santa Isabel, mediante el método de aglutinación en placa, año 1996. Universidad de Cuenca.

StataCorp, 2015. Stata: Release 14. Statistical Software. College Station, TX: StataCorp LP.

Tapia, N., 1998. Prevalencia de brucelosis caprina en el área "Centro Laja." Universidad Nacional de Loja.