Trop Anim Health Prod (2016) 48:1083–1087
DOI 10.1007/s11250-016-1044-3

SHORT COMMUNICATIONS

First results on small ruminant brucellosis and tuberculosis and caprine arthritis-encephalitis in El Salvador

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Received: 14 October 2015 / Accepted: 8 March 2016 / Published online: 18 March 2016

Abstract This paper reports a first-time study performed in El Salvador on the presence or absence of antibodies to three important animal diseases in small ruminants. The work was conducted in the west and central departments of the country, selecting 42 and 43 cantons with an existing sheep and goat population, respectively. Serum samples were collected from 396 sheep and 335 goats and tested for seropositivity to *Brucella* (*B.*) spp. The specimens from goats were also tested for antibodies to caprine arthritis-encephalitis (CAE) virus. Four (1 %) sheep and none of the goats were seropositive by Rose Bengal test. All animals were negative by indirect ELISA (iELISA) for *B. abortus*. All animals were negative by iELISA for CAE. A total of 383 sheep and 330 goats underwent the single intradermal cervical tuberculin (SICT) test for tuberculosis. Seventy (18 %) sheep and 43 (13 %) goats reacted to the SICT test. Those reactors were subjected to the single intradermal comparative cervical tuberculin (SICCT) test, and one (0.3 %) goat was deemed to be a positive reactor. No mycobacteria were diagnosed in concluding analyses, and further studies are considered necessary to determine the prevalence of the investigated diseases. Additionally, it is recommended that small ruminants should be included in the national eradication program on bovine brucellosis and tuberculosis to prevent potential reservoirs.

Keywords Brucellosis · Caprine arthritis-encephalitis · El Salvador · Small ruminants · Tuberculosis

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Introduction

Brucellosis and tuberculosis are common zoonotic infections worldwide and are a great risk to public health, especially in developing countries where control and eradication programs in cattle and other species have not yet been successful (OIE 2008, 2009; Dürr et al. 2013; Godfroid et al. 2014). The principal route of infection for humans is by consumption of unpasteurized milk and dairy products or close contact with infected animals (Garro et al. 2005; Gumi et al. 2012). Brucellosis in goats and sheep is generally caused by *Brucella melitensis*; however, sporadic infections with *Brucella abortus* in small ruminants have been described in Latin America (Lucero et al. 2008; Martins et al. 2012; Garofolo et al. 2013). Tuberculosis in ruminants is most commonly caused by *M. bovis* and *M. caprae*, two species of bacteria included in the *Mycobacterium* (*M*) *Tuberculosis Complex* (MTC) (Romich 2008; Acevedo et al. 2013). Although small ruminants are susceptible to the strains causing bovine tuberculosis (bTB), infection in sheep is rare (Broughan et al. 2013). In developing countries, small ruminants are often kept together with cattle, and the cross-infection of these species with *B. abortus* as well as bacteria of the MTC promotes the formation of reservoirs which affects the success of eradication programs in bovines (Tschopp et al. 2011; Muñoz Mendoza et al. 2012; Diaz Aparicio 2013). Caprine arthritis-encephalitis (CAE) is a virus infection that causes significant economic loss in goat herds in several

countries all over the world. Caprine arthritis-encephalitis virus (CAEV) induces a lifelong infection and is manifested as chronic arthritis, interstitial pneumonia, mastitis, or encephalomyelitis. Frequently infected animals show no clinical signs but are able to transmit the virus (Gomez-Lucia et al. 2013; Turchetti et al. 2013). The virus is mainly transmitted from dams to their offspring by the ingestion of infected colostrum or milk; however, horizontal transmission by direct contact or via aerosols can also occur (de Souza et al. 2015). High prevalence of seropositive animals has been described in Brazil and Mexico (Martínez et al. 2006; Martins and Lilenbaum 2011).

For El Salvador, there is no data available on the status of the abovementioned diseases (personal communication with the Salvadoran Ministry of Agriculture and OIRSA) (Moreno 2002). The purpose of this study was to determine the occurrence of antibodies for brucellosis and tuberculin reactors in small ruminants and the seropositivity for CAEV in goats. This information should contribute to the understanding of the current situation of the infections in sheep and goats and supply a basis for further studies.

Material and methods

For administrative purposes, El Salvador is divided into four regions containing 2.310 cantons (MAG 2014). The study was conducted in Regions I–III from May 2013 to July 2014 (Fig. 1). A two-stage cluster sampling technique was used for each species. Due to the absence of detailed official data on sheep and goat population and their distribution, the cantons were selected by clusters. The number of cantons to be sampled was calculated according to the table by Cannon (2001) with an estimated prevalence of 5 % and a confidence level of 95 %. Forty-three cantons with goats and 42 cantons with sheep were randomly selected using MS Excel. The number of animals to be sampled was estimated according to the present population of the canton (Table 1). Female and male animals older than 6 months were tested. The study animals were managed mainly under a traditional production system, mixed with other species such as cattle, pigs, and poultry. Mean goat herd size was two animals per household while mean sheep herd size was up to six animals per proprietor. A total of 335 goats and 396 sheep were serum sampled for the detection of *Brucella* antibodies, and 330 goats and 383 sheep were skin tested for tuberculosis. The 335 goats were also tested for CAE seropositivity.

Blood samples were collected from the jugular vein and within 24 h transported to the Central Laboratory of Veterinary Diagnostics, Ministry of Agriculture, in San Salvador for analysis. All animals were screened for tuberculosis by the single intradermal cervical tuberculin (SICT) test, and positive as well as inconclusive reactors were submitted to the single intradermal comparative cervical tuberculin (SICCT) test a minimum of 42 days after the SICT test. As specific guidance for testing and interpretation of results for small ruminants is not published, the guidance given for cattle in the OIE Terrestrial Manual (2009) was used. We worked with a caliper (Hauptner-Heberholz, Germany), 3000 IU bovine purified protein derivative (PPD) and 2500 IU avian PPD (Prionics, Switzerland). Due to the small cervical region of goats and sheep, both sides of the mid-neck were inoculated for the SICCT (bovine PPD on the left side and avian PPD on the right side).

The serum samples were screened for *Brucella* spp. applying the Rose Bengal test (RBT) according to the methods described by the OIE for small ruminants, using one part of RBT antigen (SENASA, Honduras) and three parts of serum. In parallel, all serum samples were tested for *B. abortus* using an indirect ELISA (iELISA) commercial kit (Brucellosis

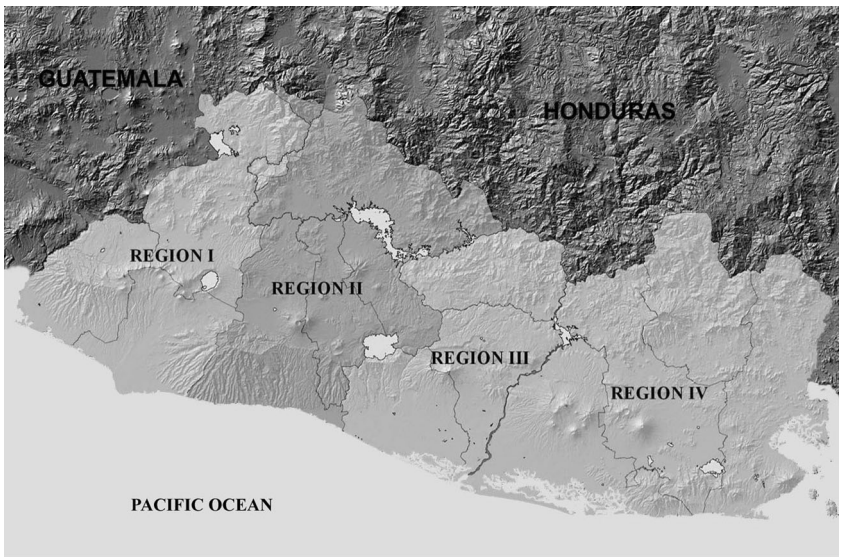

Fig. 1 Administrative division of El Salvador by the Salvadorian Ministry of Agriculture. The study was conducted in Regions I–III. Source: modified map from the Salvadorian Ministry of Agriculture

Table 1 Number of animals tested proportionally to the present population

Population of the canton: animals >6 months	Number of animals to be tested
1–10	All animals were tested
11–20	18
21–40	24
41–60	27
60 or more	28

Serum X2 Ab Test, IDEXX Laboratories, USA) following the procedures described by the OIE. Sera from all the goats were tested for CAEV antibodies utilizing a commercial iELISA (CAEV/MVV Total Ab Test, IDEXX Laboratories, USA) according to the methodology described by the OIE (OIE 2008, 2009). The one goat that reacted to the SICCT test was sacrificed and examined for granulomatous lesions. Samples (retropharyngeal, bronchial, and intestinal lymph nodes with and without visible lesions, lung, liver, spleen, kidney, and colon) were taken (OIE 2008, 2009) and sent to the Laboratory of Veterinary Diagnostics and Investigation, Panamanian Ministry of Agriculture, Panama, for microscopic examination of acid-fast organisms by Ziehl-Neelsen and an attempt to isolate mycobacteria. The same specimens were also analyzed using PCR-restriction fragment length polymorphism (PCR-RFLP) of the *hsp65* gene (restriction enzymes: HAEIII and BstEII) by the Institute of Scientific Research and High Technology Services INDICASAT AIP, Panama.

Results

The results of the serological tests, SICT, and SICCT are summarized in Table 2. A total of 396 sheep and 335 goat serum samples were tested for *Brucella* spp. by RBT. Four (1 %) sheep were found to be seropositive, while all the samples from goats were negative. Both sheep and goats were found seronegative by iELISA for *B. abortus*. In the SICT, out of 383 sheep and 330 goats, 9 (2 %) and 11 (3 %) were positive reactors while 61 (16 %) and 32 (10 %) were inconclusive reactors, respectively. The reactors (70 (18 %) sheep and 43 (13 %) goats) were subjected to the SICCT. All the sheep were negative reactors, and only one (0.3 %) goat was a positive reactor. In the case of CAE, all the samples (335) tested by the iELISA were seronegative. The one goat positive to the SICCT was slaughtered and examined for gross tuberculosis lesions. Lesions were detected in retropharyngeal and mandibular lymph nodes that showed a yellowish caseous material upon incision. Disseminated calcifications were found in the wall of the colon and one calcification in the caudal lung lobe. A small induration was detected in the liver. Samples for microscopic examination and cultivation were taken from the organs with lesions and from further organs with no visible alterations. The histopathological examination found granulomas with structures of trematodes in the samples with lesions from the lymph nodes and the colon. The lesion in the lung represented an osseous metaplasia. No acid-fast organisms were observed by histopathology; furthermore, no mycobacteria were isolated from the samples and the PCR results were negative.

Discussion

The present study is a preliminary investigation of two important zoonoses and an important production disease in sheep and goats in El Salvador. It has shown that the prevalence of brucellosis and tuberculosis is negligibly low and that no reactors to CAEV were detected. All the examined animals were seronegative to *B. abortus*, the most isolated species in Latin America and the only isolated species in El Salvador (Lucero et al. 2008; Tique et al. 2010). Most brucellosis cases in small ruminants are caused by *B. melitensis* (Diaz Aparicio 2013; Pérez-Sancho et al. 2014); however, it was not possible at the time of the present study, to extend investigations in El Salvador to include *B. melitensis* or *Brucella ovis*. This important gap in knowledge will need to be addressed by further investigations. Considering the small number of reacting animals to the RBT, the results could be due to cross-reactions of the *Brucella* antigen with antibodies from other bacteria such as *Yersinia enterocolitica*, *Francisella tularensis*, *Escherichia coli*, and *Salmonella* spp. (Megersa et al. 2012; Boukary et al. 2013; Roman et al. 2013). The infection with *B. ovis* is not probable since the RBT does not detect "rough" *Brucella* antigens (Godfroid et al. 2010). Authors describe prevalence below 4 % or only sporadic occurrences of *B. melitensis* and *B. abortus* in Latin American goats and sheep (Lilenbaum

Table 2 Results of serological and intradermic tests from the sampled animals

	RBT	iELISA (BR)	iELISA (CAE)	SICT+[a]	SICT +/–[b]	SICCT+
Sheep	4/396	0/396	–	9/383	61/383	0/70
Goats	0/335	0/335	0/335	11/330	32/330	1/43

[a] Positive reactors

[b] Inconclusive reactors

et al. 2007; Tique et al. 2010; Martins et al. 2012). These reports and the results from this study lead to the assumption that the prevalence for small ruminant brucellosis in Latin America seems to be low in general.

In this study, tuberculosis was not diagnosed in small ruminants. The one goat positive to the SICCT showed visible lesions at necropsy, but they were not caused by bacteria of the MTC nor by *Mycobacteria* spp. Animals can become positive reactors due to non-specific reactions by mycobacteria from the *Mycobacterium Avium Complex* (MAC), non-pathogenic environmental mycobacteria, or other bacteria such as *Nocardia* spp. and *Corynebacterium pseudotuberculosis* (de la Rua-Domenech et al. 2006; Good and Duignan 2011; Bezos et al. 2012). More than 10 % of the tested sheep and goats were positive or inconclusive reactors by the SICT possibly as a result of non-specific sensitization either caused by bacteria of the MAC or environmental mycobacteria. From the literature, it is known that sensitization is frequently caused through contact with domestic or wild birds (Proano-Perez et al. 2006; Álvarez et al. 2008; Buddle et al. 2015). Almost all animal owners in this study kept poultry ranging freely among small ruminants, inferring that the goats and sheep were probably sensitized.

None of the 335 sampled goats (99 goats from the additional group) were seropositive for CAEV. Although not detected in this study, the CAEV has been described in Argentina, Mexico, and Brazil (Martínez et al. 2006; Trezeguet et al. 2010; Rodrigues de Sousa et al. 2014). In these countries, CAE infections seem higher in regions with intense dairy production, and the origin of CAEV in Latin America is traced back to dairy goats imported from the USA or Europe (Peterhans et al. 2004; Assis Bandeira et al. 2009; Ramírez et al. 2010). In El Salvador, a specialization in milk production is practically non-existent, concluding that the seronegativity of goats in this study is due to the fact that all animals were local crossbreds and none of the sampled herds included imported dairy goats.

Finally, the estimated prevalence of this study was set at 5 % with a confidence level of 95 %, leading to the conclusion that the possible existence of the investigated diseases is below a prevalence of 5 %. It is recommended that further studies implying bigger sample sizes are continued, to determine the grade of infection in small ruminants. Although the occurrence of seropositive goats and sheep may be very low, bovine brucellosis and tuberculosis are existent in Salvadorian cattle and present a public health threat as well as a great economic loss. For a successful eradication program in cattle, small ruminants should be included in the official sampling plan to prevent them from forming possible reservoirs.

Acknowledgments This work was financially and technically supported by OIRSA. The authors would like to thank the technical staff and the Central Laboratory of Veterinary Diagnostics, Salvadorian Ministry of Agriculture, for the assistance of the field work and diagnostics. They thank also the Laboratory of Veterinary Diagnostics and Investigation, Panamanian Ministry of Agriculture, and the Institute of Scientific Research and High Technology Services INDICASAT-Panama AIP for their contribution in further diagnostics. Thanks are also expressed to C. Zepeda and E. Bravo for help with statistics.

Compliance with ethical standards

Ethical approval For this type of study, a formal consent is not required. All applicable international and national guidelines for the care and use of animals where followed. Persons gave their informed consent prior to inclusion in the study.

Conflict of interest None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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