Short Communication

Seroprevalence of Bluetongue and Associated Risk Factors in Costa Rican Sheep Flocks

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Abstract | Blood samples from a total of 359 sheep from 15 farms were analysed for the presence of antibodies against bluetongue virus (BTV) by commercial enzyme-linked immunosorbent assay (ELISA). Antibodies were detected in 290 sheep from fourteen different flocks, distributed in all analyzed regions (Central, Chorotega, Atlantic Huetar, North Huetar, and Central Pacific) determining regional seropositivity between 63.5% and 100.0%, as well as an overall prevalence of 80.8 %. The within flock seropositivity percentages ranged between 0% and 100.0%. Flocks with the highest seropositivity were found in low altitude regions close to the coast. Risk and protective factors determined in the present study were not in accordance with this insect borne disease. The results of this study indicate that BTV is endemic in sheep herds from Costa Rica, and animals seem not show clinical signs. We recommend carrying out further studies, to determine the presence of BTV in goats and wild ruminants, and to identify serotypes present in the country.

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Introduction

Bluetongue (BT) is a non-contagious viral disease affecting domestic and wild ruminants that is transmitted by insects, particularly biting midges of the *Culicoides* species (OIE, 2011). It is caused by a double stranded RNA orbivirus of the family *Reoviridae*, with more than 25 distinct serotypes distributed worldwide (Boden et al., 1971). The clinical signs of the disease range from a mild febrile illness to edema of lips and face, crusts on lips and muzzle, nasal discharge, conjunctivitis and extensive erosions of the oral mucosa, which can be mistaken for vesicular stomatitis virus, an endemic occurring disease in our country (Rodríguez et al., 1996), and for foot and mouth disease (Buxton & Frazer, 1977), not present in Costa Rica. Various techniques have been used to detect antibodies against bluetongue virus (BTV), however only agar gel immunodiffusion test (AGIDT), and enzyme-linked immunosorbent assay (ELISA) are accepted for international trade according the OIE Manual of Standards for Diagnostic Tests and Vaccines (Breard et al., 2004).

The incidence and geographical distribution of BTV depend on seasonal conditions, the presence of vectors, and the availability of susceptible animals. The midges prefer warm, moist conditions and are in their greatest numbers and most active after rain periods (Animal Health Australia, 2008). More than 40 species of *Culicoides* have been identified as vectors for BTV in the country (Greiner et al., 1990; Tanya et

al., 1992), however *Culicoides insignis* was identified as the primary vector, since it was detected in more than 90.0% of the collections (Greiner et al., 1993; Sáenz and Greiner, 1994).

Prevalence of antibodies against BTV among sheep, goat and cattle had been reported in many tropical and subtropical areas, considered endemic zones, including Central America (Homan et al., 1985a,b; Mo et al., 1994; Mertens et al., 2009; Legisa et al., 2014). In Costa Rica, presence of BTV has been studied in cattle 30 years ago using AGIDT (Homan et al., 1985a,b), and serotypes 1, 3 and 6 were identified (Thompson et al., 1992), indicating the importance of Central America as a possible source of BTV for the rest of the continent (Mertens et al., 2009). However, the serologic evidence of this agent in sheep flocks, an emerging industry in Costa Rica, had not been studied to date. The aim of this study was to determine if antibodies against BTV were present in Costa Rican sheep blood samples, using a commercial competitive ELISA, and to identify risk factors associated to this viral infection.

Materials and Methods

Studied population

Sheep flocks registered at the Costa Rican Association of Sheep Producers were sampled, most of them were used commercially (87.0%), to produce tropical hair breed lambs (100.0%), and these animals were maintained mainly in intensive systems (93.0%). The sampled sheep breeds were Dorper, Pellybuey, Kathadin, Blackbelly, Texel, Suffolk, Santa Ines and their crosses.

Sample size

The sample size was calculated with an estimated population of 25,000 animals distributed in 138 sheep herds in Costa Rica (20.0% overall expected prevalence, 95.0% confidence level and 5.0% expected error), yielding a total of 244 samples to analyze; however, to enhance the power of the study, a total of 359 sheep were sampled. Within each herd, the number of animals to be sampled was calculated to determine presence or absence of antibodies against BTV, with 95.0% confidence, assuming a sensitivity and specificity of the ELISA of 99.0% (Vandenbussche et al., 2008; Niedbalski, 2011) using the formula described by Cannon and Roe (1982). Since most of the sheep farms presented similar management conditions and the distribution of the agent was unknown, the same chance of infection on each farm was assumed, thus, all animals within each farm had an equal chance of being infected. A random selection of farms within regions was performed in order to get a representative sample population in each region and inside each flock. The study was conducted in 15 Costa Rican sheep herds. According proportional allocation, the farms were distributed as follows: seven in the Central region (46.0%), two in the Chorotega region (13.5%), two in the Central Pacific region (13.5%), two in the North Huetar region (13.5%) and two in the Atlantic Huetar region (13.5%). The Brunca region was not analyzed, since it was not possible to find farms willing to participate in this study. However, less than 10.0% of animals were registered in this region.

Sample collection and survey

Blood was collected between August 2012 and February 2013. Tubes were transported using coolers for keeping a temperature between 4°C to 7°C. Afterwards in the laboratory the samples were centrifuged for 5 minutes at 10,000 g, sera was separated, and frozen at -20°C until processed by ELISA. A questionnaire applied during a Maedi Visna research (Villagra-Blanco et al., 2015) was analyzed again to obtain information in order to determine risk factors associated with BT disease such as housing, animal movement between herds, lamb husbandry, reproductive management and presence of compatible clinical signs of BT in each farm.

Enzyme-linked immunosorbent assay (ELISA)

The IDScreen® Bluetongue Competition Multispecies ELISA (Montpellier, France) was used. This assay reported a sensitivity and specificity of 99.0% (Vandenbussche et al., 2008). The methodology recommended by the manufacturer was used.

Statistical analysis

Frequencies of the general characteristics and management practices of the sheep flocks were calculated. To assess the relationship between BTV and the management practices, the odds ratio (OR) was calculated using a mixed effects logistic regression, being the sheep flock the random variable. The data was analyzed using SAS/STAT ver. 9.2 (SAS Institute Inc.).

Results and Discussion

Seropositive animals were detected in 14 (93.3%)

Table 1: *Number and percentage of animals tested in15 sheep flocks and distribution of seropositive individuals according to flocks and regions*

Farm	Region	Total animals in flock	Animals tested	Positive animals (%)	Breed	Flocks analysed	Regional positivity
7	Central	80	25	25 (100.0)	D,K	7	63.5 %
8	Central	103	25	11 (44.0)	D,K		
9	Central	136	26	16 (61.5)	K,B,P		
12	Central	100	25	21 (84.0)	Om		
13	Central	220	26	20 (76.9)	Om		
14	Central	300	28	8 (28.6)	Om		
15	Central	4	4	0	D,K		
	Subtotal	**943**	**159**	**101 (63.5)**			
5	Central Pacific	500	27	23 (85.2)	Om	2	92.7%
10	Central Pacific	200	28	28 (100.0)	Om		
	Subtotal	700	55	51 (92.7)			
2	Chorotega	115	25	25 (100.0)	D,K,P	2	100.0%
3	Chorotega	140	26	26 (100.0)	Om		
	Subtotal	**255**	**51**	**51 (100.0)**			
4	Atlantic Huetar	30	20	20 (100.0)	K,P	2	100.0%
11	Atlantic Huetar	350	27	27 (100.0)	D,K,S		
	Subtotal	**380**	**47**	**47 (100.0)**			
1	North Huetar	200	21	15 (71.4)	D,K,P	2	85.1%
6	North Huetar	131	26	25 (96.2)	D,K,T		
	Subtotal	**331**	**47**	**40 (85.1)**			
	TOTAL	2609	359	290 (80.8)		15	

D: *Dorper;* **K**: *Katahdin;* **P**: *Pelibuey;* **S**: *Suffolk;* **T**: *Texel;* **B**: *Blackbelly;* **Om**: *Other mixed breeds*

Table 2: *Risk factors associated with BTV seropositivity in sheep flocks in Costa Rica*

Variable	Animals		OR	CI (95 %)	
	Positive	Negative		LL	UL
Open flocks	212	147	2.54	1.49	4.35
No quarantine areas	253	106	6.47	3.68	11.4
Partial stabling	333	26	1.10	1.06	1.14

OR: *Odds Ratio;* **UL**: *Upper limit;* **LL**: *Lower limit;* **CI**: *Confidence Interval*

flocks; however, in the seronegative flock only four animals were tested. From a total of 359 serum samples analysed, 290 sheep (80.8%) showed antibodies against BTV, the seropositivity in the regions analysed ranged between 63.5% and 100.0%. Meanwhile, the flock seropositivities determined ranged between 0% and 100.0% (Table 1). This study was the first attempt to detect BTV antibodies in sheep flocks in Costa Rica, an emerging and fast growing industry in the country. Previously, higher seroprevalences (from 15.0% to 75.0%) were reported in cattle by Homan et al. (1985 a, b, 1990, 1992).

Flocks with the highest seropositivity were found in low altitude regions close to the Atlantic and Pacific coast. Furthermore, only one small flock localized in a mountainous area (over 1,500 meters) with just four animals, all born inside this flock, was seronegative (Figure 1). According to Homan et al. (1985a, b) Costa Rica presented an inverse association between antibody prevalence of cattle and altitude of the farm, observation that coincides with the results obtained in our research.

Two management practices were determined as risk factors for BTV seropositivity: buying animals from other farms without any sanitary control (59.1% of the participating farms, OR= 2.54; IC= 1.49 to 4.35), and the lack of quarantine areas or separated boxes for sick animals in each flock (70.47% of the studied flocks, OR= 6.47; IC= 3.68 to 11.40). These risk factors have been described in the literature as factors facilitating the infection of sheep and goat flocks with different virus, bacteria and parasites (Vasileiou et al., 2015), including BTV (Mozaffari et al., 2014), especially if the animals are moved into high-humid

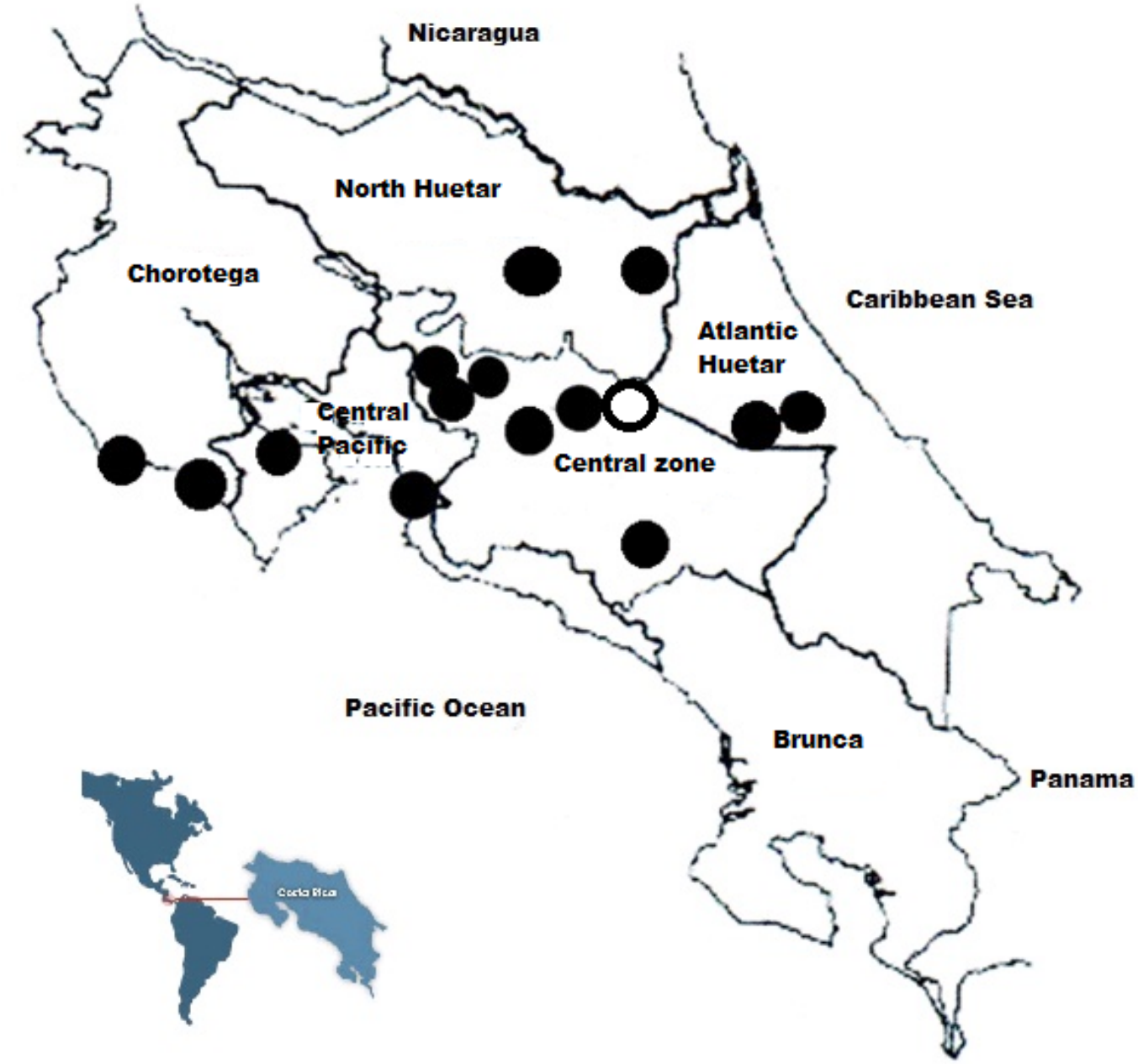

Figure 1: *Location of the participating flocks with Bluetongue virus (BTV) seropositive sheep (black dots) and seronegative animals (white dots) within the five analysed regions of Costa Rica*

endemic coast areas (Homan et al., 1990). Lack of sanitary control and lack of quarantine areas are not considered risk factors for BTV (Bosnić et al., 2015), the only risk factor would be exposure to *Culicoides* (Sáenz and Greiner, 1994), which was not analyzed in the present study.

On the other hand, all seronegative individuals (19.2%) belonged to flocks with partial stabling, indicating this management practice as a protective factor for BTV infection (OR= 1.10; IC= 1.06 to 1.14) (Table 2), since stabled animals are likely exposed to fewer *Culicoides* (Meiswinkel et al., 2000). However, BT disease occurs, when seronegative animals are bitten by *Culicoides*, typically as adults, and controlling exposure to these mosquitoes in Costa Rica might actually lead to clinical disease if infection is delayed (Holbrook, 1996).

Finally, no clinical signs of disease were observed in the analyzed sheep, findings that are in accordance with Mo et al. (1994). Sheep in endemic areas are naturally resistant to BT, and clinical disease is only observed when non-native ruminants, particularly European breeds, are introduced into these areas (OIE, 2011).

Measures for preventing and controlling the disease

in endemic areas are based mainly on sentinel monitoring programs, in combination with surveillance programs of insect vectors (OIE, 2011).

The positive results obtained in this study confirmed the presence of antibodies against BTV in Costa Rican sheep flocks. Risk and protective factors determined in the present study were not in accordance with this insect borne disease, probably due to the type of study (cross-sectional). We recommend carrying out further studies, to determine the presence of BTV in goats and wild ruminants, and to identify serotypes present in the country.

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Conflict of interest

There is no conflict of interest in this study.

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