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Seroprevalence of Bluetongue Virus Antibody in Ruminants from Grenada

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Abstract

The aim of the present study was to estimate the seroprevalence of antibodies to bluetongue virus (BTV) among domestic ruminants of Grenada. Sera samples from cattle (133), goat (314) and sheep (481) were tested using competitive Enzyme-linked immunosorbent assay (c-ELISA). Of the total of 928 samples tested, the overall BTV seroprevalence was 78.4% (95% confidence interval (CI ± 2.65). The seropositivity of ovine, caprine and bovine was found to be 71.7% (95% CI, 67.67% to 75.73%), 80.2% (95% CI, 75.79% to 84.61%) and 98.5% (95% CI, 96.43% to 100.57%), respectively. There was statistical significance in the seroprevalence of BTV among bovine, caprine and ovine ($p < 0.05$). It is evident from this study that blue tongue virus is endemic in Grenada.

Keywords

Blue Tongue Virus, c-ELISA, Seroprevalence, Grenada, Ruminants

1. Introduction

Blue tongue is a non-contagious vector transmitted virus of the Orbivirus genus in the reoviridae family [1]. BTV was first described as "Malarial Catarrhal Fever" or "Epizootic Cattarrh of Sheep" in South Africa [2] and was later isolated in California (North America) in the early 1950s from sheep with "sore muzzle" [3]. The pathogenesis of BTV usually involves vasculitis in target tissues resulting in hemorrhages and hyperemia, and may cause reproductive disorders. Clinical signs of bluetongue disease include fever, generalized hyperemia, frothing

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at the mouth, nasal discharge and cyanosis of the tongue. The severity of bluetongue disease depends on a variety of factors including the infecting viral strain, nutritional status, immune status, age, breed and presence of different environmental stressors [4]. Sheep are the most affected species. Most infections in cattle and goats are asymptomatic. Generally, bluetongue disease tends to be mild and self-limiting. However, it has a negative impact on food animal industry resulting in significant economic losses worldwide. These losses are direct (death, abortion, decreased milk yield and meat efficiency) and indirect due to strict export restrictions for animals and their by-products [5].

In South and Central America the virus is widely present [6]. In the Caribbean region data about BTV are limited. The first study of BTV in the Caribbean by Gibbs *et al.* [7] showed an overall prevalence of 70% in cattle, 67% in sheep and 76% in goats. In that survey, seroprevalence of BTV in Grenada was reported higher (cattle 89%, sheep 94%, goats 72%) than that in other countries of the region [7].

There are at least 26 different serotypes of BTV identified worldwide [8]. Through virus isolation or serotype determination serotypes 1, 3, 4, 6, 8, 12, and 17 have been identified in Central America and the Caribbean. It is reported that only serotype 17 is common to both regions and the isolation of this serotype in the Caribbean has been associated with importation of cattle from the U.S.A. [9].

The status of BTV in Grenada has never been evaluated since the work of Gibbs *et al*. [7], more than 30 years ago. The objective of the study was to estimate the prevalence of antibodies to BTV in cattle, sheep and goats in Grenada.

2. Materials and Methods

2.1. Ethical Approval

The project was approved by Institutional Animal Care and Use Committee (IACUC) (reference SRGI 9003 and SRGI 9004).

2.2. Sample Size Determination

To determine sample size, Michael Thrusfield [10] formula was used ($n = 1.96^2P \times (100 - P/d^2)$), where n = sample size; P expected prevalence; d = desired absolute precision. For bovine using 90% prevalence, sample size obtained was 138 and for sheep and goats using 70% prevalence, sample size of 322 was obtained. Sample size for the current research was close to the calculated figures.

2.3. Sample Preparation

Blood samples from the jugular vein were collected from 133 randomly selected cattle, 481 sheep and 314 goats from around the island, between December 2009-2011. All animals included in the study were unvaccinated and had no evident clinical signs of disease. The collected blood samples were centrifuged at 3000 g for 10 minutes, at room temperature (27˚C - 28˚C) and serum was removed and stored at −20˚C until testing.

2.4. Detection of Antibodies

Commercial ELISA kits using VP7 protein from Bluetongue Virus (BTV) from IDEXX Laboratories, Inc. Montpellier, France were used according to the manufacturer's instructions. This test is based on competition between the serum to be tested and a monoclonal antibody, coupled to a peroxidase and directed to the VP7 protein, which is highly conserved among the 24 known BTV serotypes.

2.5. Statistical Analysis

The prevalence was calculated as the number of positive samples, divided by the total number of samples tested.

We used a Chi-square test to assess whether there was a significant difference in the prevalence obtained among bovine, caprine and ovine species. A p-value less than 0.05 was considered statistically significant. The 95% confidence intervals were inserted for prevalence among all the 3 species of animals.

3. Results

The serological survey of 928 serum samples (314 goat, 481 sheep, and 133 cattle) from different parishes in

Grenada are presented in **Table 1**. The overall BTV seroprevalance was 78.4% (95% confidence interval (CI ± 2.65). The Seroprevalence rates were 80.2 for goat (95% CI, 75.79% to 84.61%), 71.7 for sheep (95% CI, 67.67% to 75.73%), and 98.9 for cattle (95% CI, 96.43% to 100.57%). There was statistical significance in the seroprevalence of BTV among bovine, caprine and ovine ($p < 0.05$).

4. Discussion

There are various techniques described for detecting BTV antibody; these include haemagglutination-inhibition, complement fixation, Agar gel immune-diffusion (AGID) and competitive ELISA. Although all of these techniques successfully detect BTV antibody, only the AGID and ELISA are prescribed for international trade in the OIE Manual of Standards for Diagnostic Tests and Vaccines [11]. Of these two techniques, the c-ELISA is reported to be the most sensitive and specific diagnostic test for the detection of BTV antibody [1]. c-ELISA was used during this investigation.

The results in the present study are the most recently found BTV seroprevalence rates in sheep, goat, and cattle from Grenada. The overall seroprevalence of BTV antibodies was 78.4% (728/928), slightly lower than the previously described seroprevalence rate of 88% in Grenada [7]. Of the three species, cattle had the highest seroprevalence at 98% followed by 80.2% in goats while sheep had the lowest seroprevalence of the three species at 71.7% ($p < 0.05$, χ^2). Variation in seroprevalence of BTV antibodies in ruminants has been described from various countries of the world. In cattle low seroprevalence has been reported (1.5% to 11.0%) in Illinois and Western Indiana [12]; 18.5% in Turkey [5]; 2.69% in Central Iran [13] and 18.9% in Albanian cattle [14]. A medium prevalence rate (43.3%) was reported by Suzan *et al*. [15] in Mexican cattle. Similarly a medium to high seroprevalence has been found in cattle (44.8%) sheep (54.1%) and goats (53.3% in different districts of Soudi Arabia [16]; Hafsa *et al.* [17] found medium Seroprevalence of BTV antibodies in cattle (29%), sheep (14%) and goats (21%) in Algeria. Medium seroprevalence was reported by Mohammad and Seyed [18] in sheep (34.93%) in Iran; Aktar *et al.* [19] found 48.8% BTV antibodies in Sheep in Pakistan. Ravishankar *et al.* [20] found low seroprevalence of BTV in sheep (8.3%) and goats (5.3%) in Kerala state, India.

The distribution of BTV, identified in most countries in the tropics and subtropics, highly depends on the presence of its vector the *Culicoides* midge [21]. The presence of *Culicoides insignis* one of the most frequent species of midges in the Caribbean region may explain the higher prevalence of BTV in the region. The climatic conditions, which do not favor the population growth of *Culicoides* midge, in various countries of the world may be a contributing factor to the comparatively lower seroprevalence compared to the Caribbean region.

Our results, on the other hand, are consistent with the percentages previously described in Grenada and other Caribbean islands. In the late 1970s, it was reported that Puerto Rico and the Virgin islands had a BTV seroprevalence of almost 80% [22]. Years later in the 1980s, Gibbs *et al*. [7] described high prevalence rates of BTV antibody in Barbados (61%), Antigua (76%), Jamaica (77%), St. Lucia (82%) and Trinidad and Tobago (79%), in Caribbean countries. Additionally, Gibbs *et al.* [7] with the use of Agar gel immuno-diffusion (AGID) further described the overall seroprevalence in cattle, goat and sheep as 70%, 76%, and 67% respectively within the Caribbean region.

Clinical disease in native ruminants has not, to our knowledge, been reported in endemic tropical/subtropical regions [23]. In Grenada there is no BTV vaccination program, so positive serum samples indicates exposure to BTV among the tested animal species.

5. Conclusion

The results of this study demonstrate high level of BTV antibodies circulating in the native large and small

Table 1. Seroprevalence of blue tongue virus in sheep, goats and cattle in Grenada, West Indies.

Species	Sample tested	Positive sample	Positive sample (%) & CI
Caprine	314	252	80.2% [75.8%; 92.0%]
Ovine	481	345	71.7% [67.7%; 75.7%]
Bovine	133	131	98.9% [96.43 - 100.5%]
Total	928	728	78.4% [75.7%; 81.0%]

ruminants in Grenada in form of asymptomatic infection. Clinical observation, coupled with routine surveillance of the BTV and virus isolation and identification will assist in better understanding the epidemiology of this disease in Grenada and the Caribbean region.

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