



Research Article

Serological Detection of Caseous Lymphadenitis in Sheep and Goats Using a Commercial ELISA in Grenada, West Indies

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Abstract

Caseous lymphadenitis (CLA) in small ruminants has been diagnosed by culture several times in the state of Grenada. In order to understand the prevalence of CLA, serum samples from 541 sheep and 338 goats from Grenada and Carriacou islands were tested for antibodies against the phospholipase antigen of *Corynebacterium pseudotuberculosis*, the causative agent of caseous lymphadenitis (CLA) using a commercial ELISA kit: "ELITEST CLA". The percentage of positive samples among sheep was 7.9, and for goats it was 31.3%, with a significant difference between these two species of animals. Of the six parishes of Grenada, and one parish of Carriacou, significantly more positive samples originated from St. George parish. There were no significant differences between age groups. The results of this study show that CLA is endemic and widely distributed throughout the islands of Grenada and Carriacou.

Key words: Caseous lymphadenitis, sheep, goats, ELISA, Grenada.

Introduction

Sheep and goats are important production animals in the state of Grenada, a tri-island

nation in the Caribbean, due to their manageable size and feed requirements plus their ability to utilize sloped terrain and limited pasture areas. The rearing of sheep and goats for both meat and milk is

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increasing in Grenada (Annual Report, 2009). Grenada has an estimated small ruminant population of 3,000 sheep and 2,500 goats.

Caseous lymphadenitis (CLA) is a contagious and chronic disease of sheep and goats, caused by *Corynebacterium pseudotuberculosis*, a facultative intracellular bacterium. Abscesses, generally, involving subcutaneous lymph nodes are a common feature. Internal organs, especially lungs and internal lymph nodes can be affected. In both sheep and goats, production losses in weight gain, milk production, reproductive efficiency and carcass quality can occur (Powell, 2014). In a recent study in Canada on 152 goats, 3.9% of mortalities were attributed to CLA. Sporadic cases, including mastitis and occasional outbreaks in cattle, due to this organism have been reported (Shpigel *et al.*, 1993). Nogradi and co-workers (2012) reported musculoskeletal *C. pseudotuberculosis* infection in 35 horses. *C. pseudotuberculosis* has zoonotic potential as well, and several cases of occupational infection, characterized by lymphadenitis, necessitating surgery, and antibiotic therapy, have been reported in humans handling infected animals, particularly, from Australia (Peel *et al.*, 1997, Funke & Bernard, 2011). In sheep and goats, CLA has been diagnosed by cultural methods many times in Grenada. Monitoring of disease status by culturing clinical samples will not help in control of this disease because of the fact that animals may be infected without showing obvious clinical signs. Treatment is generally ineffective, although one study showed that intralesional or systemic administration of tulathromycin may be an acceptable alternative to opening, draining, and flushing of CLA lesions (Washburn *et al.*, 2009). Vaccines can be used to reduce the number of cases and severity of the disease (Windsor, 2014). However, control and eradication can only be achieved by identification of infected animals and removal from the herd. For this purpose, serological tests, including ELISA have been used (Sutherland *et al.*, 1987). There are no published records of this

disease in the Caribbean islands or West Indies, although it has been reported to occur in USA, Canada, Argentina, and Brazil (Williamson, 2001; Arsenault *et al.*, 2003; Estevo Belchior *et al.*, 2007; Guimaraes *et al.*, 2011). Recently, an ELISA based on recombinant phospholipase D (PLD), a secreted protein from *C. pseudotuberculosis* has been developed, and marketed under the trade name "ELITEST CLA". As PLD is not known to be produced by any other sheep pathogenic bacteria, this ELISA is a very specific test (Hyphen Biomed com., 2014). This kit has been successfully used in Norway and Scotland for detection of CLA in small ruminants (Harkiss, 2014). The objective of this study was to generate data on prevalence of CLA in goats and sheep in Grenada by serological testing using "ELITEST CLA", so that control measures can be implemented.

Materials and Methods

Prior to the commencement of serological testing, the records of the Veterinary Diagnostic Bacteriology Laboratory, St. George's University, Grenada, were examined for reports of cases of CLA in sheep and goats from this country. In all cases, abscess contents were plated routinely on blood agar and MacConkey agar, and isolates suspected to be *Corynebacterium pseudotuberculosis* as described by Quinn *et al.* (1994) were confirmed by using Analytical Profile Index (API) Coryne strips. The growth on primary plating media was classified as "light", "moderate", and "heavy", and "pure" or "mixed". Three or less colonies of any morphological type were considered "insignificant". *Corynebacterium pseudotuberculosis* was isolated from 11 clinical cases of small ruminants in Grenada in the past. Of the 11 consecutive culture-positive samples, 10 were from goats, and one from sheep. Six positive samples were from females, and 5 from males. Abscesses were mostly (6/11) found to occur on neck or mandibular area. Internal abscesses were found in one animal, a sheep (Table 1).

Table 1: Culture-positive Abscess content samples for *Corynebacterium pseudotuberculosis* from 11 small ruminants in Grenada (2004-2012)^a

Year sample received	Species and number	Sex, Age	Lesion/location of abscess	Culture characteristics
2012	Caprine (1)	M, "Adult"	Abscess (2.5" firm), lower neck	Heavy, pure growth
2012	Caprine (1)	F, 3+ years	Abscess in caudal gluteal muscles	Heavy, pure growth
2010	Caprine (2)	F, "Adults"	Caseous abscesses (NI) ^b	Heavy, pure growth from both animals
2010	Ovine (1)	F, 5 years	Abscesses in liver, pancreatic lymphnode	Moderate, pure growth from both samples
2010	Caprine (1)	M, 5 years	Subcutaneous abscess in mandibular region	Moderate, pure growth
2006	Caprine (1)	M, "Adult"	Abscess on left mandible	Moderate, pure growth
2006	Ovine (1)	M, "Adult"	Abscess (NI)	Moderate, pure growth
2004	Goat (1)	F, 2-3 years	Neck abscess	Moderate, pure growth
2004	Goat (1)	F, 2-3 years	Neck abscess (6 cm in diameter) with purulent exudate	Moderate, pure growth
2004	Goat (1)	M, 2-3 years	Neck abscess	Moderate, pure growth

^a Unpublished information from the Veterinary Diagnostic Laboratory, St. George's University, Grenada

^b NI: no information on location

Serum samples from 541 sheep, and 338 goats (16% of the total small ruminant population) from the islands of Grenada and Carriacou were obtained from clinically normal animals of at least 9 months of age. Animals were selected randomly from all 6 parishes of the island of Grenada, and from the island of Carriacou. The number of animals selected is in proportion to the number of small ruminants available in the particular parish/region.

"ELITEST CLA" (HYPHEN Biomed, neuville sur Oise, France) was used and interpreted according to manufacturer's instructions for serological detection of CLA. The test detected IgG antibodies specific for the

conserved *C. pseudotuberculosis* virulence factor, phospholipase D. The test was performed at a room temperature of 20-25°C as per the recommended assay procedure. The cut-off values were calculated as per manufacturer's instructions.

Results

Of a total of 879 serum samples from sheep and goats tested, 149 (16.9%) were positive for CLA. Compared to sheep, significantly more goats (7.9% and 31.3% respectively) were positive for CLA (Table 2). There were significant differences in positive samples from parishes. St. George parish had the highest (43%), and St. Andrews had the

lowest (4%) (Table 3). Age wise, sheep gave significantly lower positive results than goats

in all age groups (Table 4). Age groups did not show significant differences in positivity.

Table 2: Sheep and goat samples positive for antibodies against CLA^a

	Total tested	Numbers positive	Percentage positive (%)
Sheep	541	43	7.9**
Goats	338	106	31.3
Total	879	149	16.9

Note: ** - statistically significant at the .001 level.

^a There is a statistical significant difference between the proportion of sheep and goats testing positive for antibodies against CLA (chi-square=81.01, p-value=<.001). Proportionately more goats tested positive.

Table 3: Parish/ region wise distribution of sheep and goat samples positive for antibodies against CLA^a

Parish/Region	Total samples of sheep tested	Number of sheep positive	Percentage of positive sheep (%)	Total samples of goat tested	Number of goats positive	Percentage of positive goats (%)
St. Andrews	123	0	0	51	07	13.7
St. George	13	02	15.3	82	39	47.5
St. David	48	05	10.4	43	12	27.9
St. Mark	39	03	7.6	14	0	0
St. John	34	05	14.7	16	06	37.5
St. Patrick	07	0	0	37	05	13.5
Carriacou	277	28	10.1	95	37	38.9
Total	541	43	7.9**	338	106	31.3**

Note: ** - statistically significant at the .001 level.

^a There is a statistically significant difference in the proportion of sheep testing positive for antibodies against CLA by parish (chi-square=31.01, p-value=<.001). The highest parishes testing positive are St. George (15%), St. John (14%), St. David and Carriacou (10% each). There is a statistically significant difference in the proportion of goats testing positive for antibodies against CLA by parish (chi-square=88.50, p-value=<.001). The parishes with the highest proportions are St. George (48%), Carriacou (40%), St. John (38%) and St. David (28%).

Table 4: Age wise distribution of samples positive for antibodies against CLA^a

Age groups	Sheep sample			Goats sample		
	Tested	Positive	Percentage (%)	Tested	Positive	Percentage (%)
9 months-up to 2 yrs	139	11	7.90	80	25	31.25**
2 - up to 3 yrs	176	14	7.95	67	21	31.34**
3 - up to 4 yrs	25	02	8.00	89	28	31.46**
4 - up to 5 yrs	201	16	7.96	102	32	31.37**

Note: ** - indicates statistically significant at the .001 level

^a Overall, sheep have significantly fewer positive than goats at each age group (9 months - 2 years: chi-square= 20.13, p-value<.001; 2-3 years: chi-square= 18.62, p-value<.001; 3-4 years: chi-square=8.89, p-value=.002; and 4-5 years: chi-square= 27.82, p-value <.001). No significant differences were seen between various age groups

Discussion

CLA is a chronic debilitating disease of global importance in sheep and goats, causing considerable economic losses due to culling of infected animals, carcass condemnation and decreased milk, wool production, reproductive efficiency, and mortality as well (Williamson, 2001; Debien *et al.*, 2013). In the Americas, it occurs in Canada and USA (Debien *et al.*, 2013, Powell, 2014). The prevalence of CLA in sheep in Quebec, Canada determined by presence of abscesses and positive culture was 21% (Arsenault *et al.*, 2003). Similar estimates in Australian sheep in 2009, was 12.9% for the province of Victoria (Windsor, 2014). Recent studies in a semi-arid tropical region of India showed a prevalence of 1.3 % and 2.4 %, in sheep and goats, respectively by a specific PCR assay for *C. pseudotuberculosis* (Kumar *et al.* 2012, 2013). Serological tests for CLA have been described and developed further. Sutherland *et al.* (1987) described two enzyme-linked immunosorbent assays to measure antibody to the cell wall antigen and toxin antigen of *C. pseudotuberculosis*. Schreuder *et al.* (1994), used a double-antibody sandwich ELISA for eradication of CLA in two large flocks of sheep in which the disease was endemic. This ELISA was modified for use in sheep and goats, and it had a specificity of $\geq 98\%$ for

both species, and sensitivity of 94% for goats and 79% for sheep (Dercksen *et al.*, 2000). Using this ELISA, 23% of small ruminants, mostly comprising of goats, in two districts of Malaysia, were found to be positive for CLA (Komala *et al.*, 2008). Reboucas *et al.* (2011), described a modified ELISA for the detection of specific immunoglobulins against *C. pseudotuberculosis* secreted antigen. Using this assay, the seroprevalence of CLA in slaughtered sheep in the state of Minas Gerais, Brazil was estimated to be 43.7% (Guimaraes *et al.*, 2011). The present study was possible because of the availability of a specific commercial immunoassay which was found useful in diagnosis of CLA in sheep and goats in Scotland and Norway, respectively (Hyphen biomed, 2014). Among sheep and goats in the present study, proportionately more goats tested positive for CLA, with a statistically significant difference. The reasons for the higher prevalence in goats are unknown. It is notable that 10 of 11 culture-positive samples in our diagnostic laboratory originated from goats (Personal communication, 2014). Among parishes, St. George showed the highest seroprevalence of CLA. The reasons are not understood, and needs further investigation. However, the data should be cautiously interpreted, because of the fact that number of samples collected from parishes varied. Further,

farming is not organized in Grenada, and it is practically impossible to correlate differences in prevalence to animal husbandry conditions and environmental or other factors. It is hoped that the data generated in this study can be of help in designing future investigations. It is obvious that CLA is endemic in Grenada, and possibility of vaccination need to be considered in view of the fact that the herds are small, and culling all positive animals may not be practicable. The current study provides a baseline for continued screening of this disease in small ruminants in Grenada and other Caribbean islands.

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