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## CLINICAL AND SEROLOGICAL EVIDENCE OF BOVINE BABESIOSIS AND ANAPLASMOSIS IN ST. LUCIA\*

by

R.T. Knowles, M. Montrose, T.M. Craig, G.G. Wagner, R.F. Long

One hundred and fifty-nine Holstein calves were imported into St. Lucia from the U.S.A. An outbreak of babesiosis occurred 17 days post-arrival, and an outbreak of anaplasmosis occurred 5 months after importation. Sera obtained 3, 6 and 12 months post-importation revealed a high prevalence of IFA titres to Babesia bovis and B. bigemina 3 months after arrival and an increase in titres to Anaplasma marginale 6 months after arrival. Sera obtained from native cattle from several places on the island indicated infection rates of 80, 65 and 64% with A. marginale, B. bigemina and B. bovis, respectively. The rapid card test only indicated a 25% prevalence of infection of native cattle by A. marginale. This low prevalence was probably due to deterioration of serological activity during shipment.

#### INTRODUCTION AND CLINICAL OBSERVATIONS

The mountainous island of St. Lucia lies in the Windward islands group of the Caribbean at 14.01 N 61.00 W. The climate is tropical with an annual rainfall which varies from 1016 mm in the dry south to 6350 mm in the central rain forest.

Both Babesia and Anaplasma are present in the native cattle population. Although the tick vector, Boophilus microplus, is widely distributed throughout the island, neither of the infections are much of a problem and clinical cases are rarely seen. Prior to this report no work had been undertaken to determine the level of Babesia infections in native cattle, although it was assumed that

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the infections were widespread. In a survey by the Ministry of Agriculture in 1978 and 1979, 40% of the cattle sera tested were found positive by the Anaplasma card test.

One hundred and fifty-nine Holstein calves were imported into St. Lucia from Miami, FL, U.S.A. In April, 1979 the group consisted of 155 heifers and 4 bulls, 10-14 weeks of age, and with an average weight of 68 kg. The calves had been selected from two locations; 105 from a farm in Florida and the remaining 54 from a farm in Indiana.

All calves were placed in a 2500 m<sup>2</sup> grass paddock, at the center of the (121 ha) Government farm at the southern end of the island. The cattle already on this farm consisted of a dual purpose herd of some 180 cows and followers. These indigenous cattle grazed the surrounding pastures and were separated from the newly imported stock by a single wire fence. The calves were fed on a combination of hay, brewers grains, coconut meal, wheat middlings, chopped sugar cane and molasses.

Seventeen days after importation 63 of the 159 calves showed clinical evidence of disease. The clinical picture presented was one of an acute onset of high fever (range 40.3-41.7°C), anorexia, depression, weakness and cessation of rumination. Respiration and heart rates were increased and the mucous membranes of many of the calves indicated a severe anemia. Five calves were reluctant to rise and one showed evidence of icterus and hemoglobinurea. On the clinical evidence, a diagnosis of babesiosis was made. This diagnosis was not microscopically confirmed. The 63 calves were treated with a 5% quinuronium sulphate solution (Acaprin Ludobal-Bayer, W. Germany) at the rate of 2 ml/100 kg divided into two equal intramuscular doses at a two hour interval. The following day an additional 30 calves showed similar clinical signs. At that time all remaining calves were treated with quinuronium sulphate as above. Three calves died. At postmortem these calves had jaundice, cerebral edema and extensive fibrinous pneumonia. Starting the 25th day after importation, all calves were given 600 mg tetracycline daily for three weeks by intramuscular inoculation.

A week after the initial onset of the illness 22 of the more severely debilitated calves were moved to an adjoining pasture which had a covered area where they could feed with less competition from the stronger recovering calves. For the next six weeks, fibrinous pneumonia became an ever increasing problem particularly amongst those calves which had been more severely affected by the Babesia infection. Individual calves were continually being removed from the main herds for treatment with antibiotics. During this period four calves died or were destroyed. Three of the calves showed signs of incoordination followed by an ascending paralysis.

The calves were sprayed against ticks at monthly intervals between May and September and thereafter at two week intervals.

Three months after the importation (July) blood samples were taken from 25 of the imported calves, 21 adult milking cows on the same farm and from 74 adult cattle scattered through the island.

Five months after importation (September) there was a sudden marked deterioration in condition, and weight loss in the group of calves now permanently at pasture. Several calves were obviously anemic and two showed evidence of jaundice. Anaplasma bodies were identified in prepared blood smears. All calves in both pastures were treated with 600 mg tetracycline daily for three days and the more severely debilitated ones were given blood transfusions and Vitamin B<sub>12</sub>. Five calves died immediately following the acute period of the disease. Toward the end of September there was a second, less severe outbreak of anaplasmosis and all calves were again treated with tetracycline daily for three days. No losses occurred during this second outbreak and there was a rapid response to treatment. Six months after importation (October), blood samples were collected from the 20 calves previously tested in July. A further sampling was done in April of 1980, one year after importation.

#### MATERIALS AND METHODS

Sera collected from cattle as described above were sent to the Center for Tropical Animal Health of Texas A&M University where they were serologically tested by the indirect fluorescent antibody (IFA) test for Anaplasma marginale, Babesia bigemina, and B. bovis. A modification of the IFA method of Todorovic and Long (1976) as described by Applewhaite et al. (1981) was used. Positive IFA reactions were recorded at dilutions of 1:160 or greater. The Anaplasma card test (Brewer diagnostic Kit for Anaplasmosis, Hynson, Wescott Dunning Laboratory, Baltimore, MD, U.S.A.) was also used to evaluate the sera collected in July 1979.

TABLE 1: Serological prevalence of bovine anaplasmosis and babesiosis in native cattle in St. Lucia

Locality	A. margi	nale	B. bigemina	B. bovis
	IFA	CT	IFA	IFA
Livestock farm	21/21* (100)	13/21	8/21	16/21
milking herd		(62)	(38)	(76)
Vanard	28/38	3/38	20/38	21/38
	(74)	(8)	(53)	(55)
Fond Estate	9/11	4/11	11/11	8/11
	(82)	(36)	(100)	(73)
Mamiku	3/6	1/6	6/6	4/6
	(50)	(17)	(100)	(67)
Cedars	7/9	1/9	7/9	5/9
	(78)	(11)	(78)	(56)
Fond Assau	3/4	0/4	4/4	1/4
	(75)	(0)	(100)	(25)
Central	2/2	1/2	2/2	2/2
	(100)	(50)	(100)	(100)
Nois D'Orange	1/1	0/1	0/1	1/1
	(100)	(0)	(0)	(100)
Vieux Fort	3/4	1/4	4/4	3/4
	(75)	(25)	(100)	(75)
Total	77/96	24/96	62/96	61/96
	(80)	(25)	(65)	(64)

<sup>\*</sup>Number positive/number tested and percent positive in parentheses

#### RESULTS

The serological prevalence of infection of native cattle by A. marginale, B. bigemina and B. bovis was found to be 80, 65 and 64%, respectively (Table I). A considerably higher prevalence of infection was determined for Anaplasma utilizing the IFA test (80%) than with the rapid card test (25%). No differences in the prevalence of infection by any of the organisms were detected in samples obtained from cattle in different geographical areas.

The prevalence of infection of imported calves is given in Table II.

All calves' sera had B. bovis titres by 3 months after importation. At the same time, 88 and 16% were positive for B. bigemina and A. marginale, respectively.

Six months after importation, sera from all calves had positive titres for both Babesia antigens, and 70% were positive for A. marginale. By 12 months, all sera were positive against each antigen.

TABLE II: Serological prevalence (IFA) of anaplasmosis and babesiosis in calves imported into St. Lucia in April 1979.

Date	A. marginale	B. bigemina	B. bovia
July 1979	4/25*	22/25	25/25
•	(16)	(88)	(100)
October 1979	14/20	20/20	20/20
	(70)	(100)	(100)
April 1980	20/20	20/20	20/20
-	(100)	(100)	(100)

<sup>\*</sup>Number positive/number tested and percent positive in parentheses

#### DISCUSSION

The clinical findings described, along with the serological evaluation of both native and imported cattle, point out the danger of importing cattle into

Babesia-endemic areas. Large numbers are not required to instigate an outbreak, especially where B. bovis is concerned. The prevalence of infection of native cattle by A. marginale (80%), B. bigemina (65%) and B. bovis (64%) is probably representative for an endemic region where clinical disease is seen only on rare occasions. The high titres against the Babesia antigens reflect infections that were acquired early by the imported calves. They remained high due to a high reinfection rate occurring via the progeny of ticks feeding on acutely ill animals.

The most surprising observation in this study was the acute outbreak of anaplasmosis in young cattle (approximately 8 months of age at the time of the outbreak). A few of the calves had demonstrable serum titres 3 months after arrival which were probably too high to be of colostral origin, but may have been as a result of infection acquired in the United States or soon after arrival in St. Lucia. The serious nature of the outbreak was due either to an especially virulent strain of the organism or to the weakened condition of the calves after episodes of babesiosis and fibrinous pneumonia.

Differences between results obtained by the IFA test and the card test are not surprising. However, the magnitude of the differences was unexpected. The shipment of sera from St. Lucia to Texas encountered several delays and many of the individual sera were rather odoriferous on arrival. The activity of this sera when used in the IFA test points to its value as a serological test under less than optimal conditions.

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## BOVINE HAEMOPARASITIC DISEASES IN JAMAICA

Ъy

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Babesiosis and anaplasmosis are common diseases of cattle in the tropics. A Pan-American Health Organisation survey carried out in 1978 indicated that these diseases were considered a major source of economic loss by all the Caribbean Countries with developed livestock industries. Despite the importance of the diseases particularly to the dairy industry their epidemiology within the region is poorly understood. The present short communication presents the results of a serological survey conducted in Jamaica as a pilot for a larger epidemiological study.

Fifty sera were collected from age-matched (three to 33 months) Jamaica Hope dairy cattle farms in St. Catherine on the southern coastal plain and Trelawny on the northern. The sera were transported to Texas for sero-analysis. Card agglutination (CT) activity for Anaplasma marginale was determined using a kit modification (Hynson, Westcott and Dunning, Baltimore, Maryland) of the method of Amerault and Roby (1968). All other assays used measured activity against A. marginale, Babesia bovis and B. bigemina antigens. Complement fixation (CF) assays were modified from a previously reported technique (Todorovic and Long, 1976). Indirect Fluorescent Antibody (IFA) tests were performed essentially as described in the same article except that epifluorescent illumination was employed. The enzyme-linked immunosorbent assay (ELISA) was slightly modified from the technique described from other workers (Purnell, Hendry, Bidwell and Turp, 1976). A titre of 1:10 was considered positive for the CF test and a titre of 1:160 for

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the IFA test. ELISA absorbance values (OD<sub>492 nm</sub>) greater than 0.4 with A. marginale and B. bovis antigens and 0.5 with B. bigemina antigen were considered positive. The presence of macro-agglutination was CT positive. The results are shown in Table Ia.

There is a significant difference between the results of the IFA and CF tests (Table Ia), the latter indicating fewer positives for all three pathogens. Comparison with previous results (Table Ib) is instructive: the Jamaican and St. Lucian IFA results are similar as are the Jamaican and Guyanese CF results for Babesia infections. Previous experience has shown that the IFA test will detect antibody activity as long as two years after exposure while the CF test will produce reliable results for only four to seven months after exposure. We have insufficient experience with the ELISA test to judge the duration of the antibody response; however, the results suggest that the ELISA is comparable to the IFA in that regard.

The results of the IFA and ELISA tests indicate a high prevalence of haemoparasitic infection with no significant difference between the northern and southern habitats. The prevalence of all three pathogens is comparable with that of St. Lucia and with that of Guyana for A. marginale (Table Ib). This is the first record based on serological evidence of B. bovis in Jamaica although it has been reported from Guyana, St. Lucia and Puerto Rico. The majority of infections in native cattle are latent but the transmission potential of these asymptomatic carriers is indicated by the serious outbreaks of overt disease which occur in recently imported susceptible cattle.

TABLE I: Serological surveys of bovine haemoparasitic disease in the Caribbean Region.

		Location of	ter de la companya d	% Positive	sera	No. of sera
	Serological test	survey	B. bovis	B. bigemina	A. marginale	tested
a	IFA	N. Jamaica	72	76	92	25
		S. Jamaica	64	88	88	25
		National	68	82	90	50
	CF	N. Jamaica	12	28	76	25
		S. Jamaica	24	52	68	25
		National	18	40	72	50
	ELISA	N. Jamaica	76	84	72	25
		S. Jamaica	80	80	92	25
		National	78	82	82	50
	CT	N. Jamaica	_	-	80	25
		S. Jamaica	-	-	88	25
		National			84	50
<b>b</b> .	IFA	St. Lucia	64	65	80	96
	CF	Guyana	11	31	-	770
	CT	Guyana	-	_	85	788

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Amerault, T.E. & Roby, T.O. (1968). Journal of the American Veterinary Medical Association, 153, 1828-1834.

Purnell, R.E., Hendry, D.J., Bidwell, D.E. & Turp, P. (1976). Veterinary Record, 99, 102.

Todorovic, R.A. & Long, R.F. (1976). Tropenmedizin und Parasitologie, 27, 169-181.

## RABIES

Country	Species	No. of Cases/Quarter	No. Vaccinated/Quarter
Grenada	Canine	-	14
	Feline	-	2
Guyana	Bovine	21	-

## TETANUS

Country	Bovine Q	<u>Caprine</u> Q	<u>Equine</u> Q	Ovine Q	Porcine Q	Total Q
Grenada	-	3	-	3	<b>3</b>	9
Guyana	-	-	-	-	-	-
Jamaica	-	5	3	6	14	28

## **HAEMOPARASITES**

Country	Species .	Anaplasmosis	Piroplasmosis	Dirofil- ariasis	Trypano- somiasis
Grenada	Bovine	2	-	-	-
	Canine	-	-	12	-
Guyana	Canine	-	-	1	-
	Ovine	-	-	-	46
Dominica	Bovine	16	-	-	<u>-</u>
Jamaica	Bovine	6	-	-	-
	Canine	-	-	11	-

## LEPTOSPIROSIS

Outa	rte	×1 17	Tot	010
UMIA	TTE	ירוע	100	818

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Species	No. Tested	No. Positive
Canine	1	1
Human	1	1
Canine	1	1
Bovine Canine Caprine Equine Human Porcine	424 33 36 2 282 3	415 13 24 2 143 0
	Canine Human Canine Bovine Canine Caprine Equine Human	Canine 1  Human 1  Canine 1  Bovine 424 Canine 33 Caprine 36 Equine 2 Human 282

## BRUCELLOSIS

Country	Species	No. Tested	No. Farms	No. Pos.	No. Farms
Jamaica	Bovine	11368	518	44	1

## TUBERCULOSIS

Country	Species	No. Tested/Quarter	No. Farms	No. Pos.	No. Farms
Jamaica	Bovine	5611	432	17/(S)	2

MASTITIS	Country	Bovine	Canine	Caprine Q	Equine	Feline Q	Ovine Q	Porcine Q	Total Q
	Dominica	11	1		1	i	1		14
	Grenada	26	ı		ŧ	<b>.</b>	Ŋ		<b>69</b> .
	Jamaica	846/433*	ı		1	1	ı		915/502*
METRITIS	Dominica	'n	ſ	m	ı	ŧ	-	.1	6
	Grenada	1	•	ı	ı	ŧ	i	S	۲
	Jamaica	23/23*	1	23/23*	í	ı	3/3*	3/3*	52/52*
									13.
MANGE	Dominica	t	1	1	1	. <b>i</b>	25	11	36
	Grenada	ı	17	1	ı	t	ì	4	21
	Jamaica	16/16*	ı	ŧ	1	1	ı	25/25*	41/41

\* No. of Farms

### INFECTIOUS POULTRY DISEASES

### Guyana

	No. of Farms	No. Birds Affected			
Fowl Pox	1	60			
Coryza	4	660			

### BLACKLEG

Species No. Vaccinated No. Farms Country 3447 300 Jamaica Bovine

## SWINE ERYSIPELAS

Country No. Vaccinated No. Farms 108

2718 Jamaica

## FERTILITY EXAMINATIONS & REPRODUCTION

Country	Bovine	Caprine	Canine	<u>Feline</u>	<u>Ovine</u>	Porcine	Total
Grenada	126	46	27	3	43	27	272
Jamaica	6688/59	* -	-	-	-	-	-

\* No. of Farms

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Total	1268	33	187	3634
007	1	ı		
Porcine	331	ı		*608/858
Ovine	268	o,		
Feline	1	ı		
Equine	7	-		4
Caprine	257	1		1649/360*
Canine	100*	16		*
Bovine	305	9		1127/688*
Country	Grenada	Guyana**	Dominica	Jamaica

\*No. of Farms

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