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Heartwater in Guadeloupe and in the Caribbean

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Une enquête séro-épidémiologique sur la cowdriose dans les Petites Antilles a été organisée en 1992, de la Grenade jusqu'à Saint-Martin. Un échantillon de 1 p. 100 des ruminants a été choisi au hasard et les sérums ont été analysés par ELISA indirect. Le pourcentage de sérums positifs était de 30 p. 100 à la Guadeloupe, 25 p. 100 à Antigua, 2,2 p. 100 à Saint-Martin, 1,3 p. 100 à St.Kitts et Nevis, 3,8 p. 100 à Montserrat, 1,7 p. 100 à Dominique, 1,5 p. 100 à Sainte-Lucie, 1,5 p. 100 à Saint-Vincent, 3,5 p. 100 à la Barbade, 2,9 p. 100 à la Grenade et de 7 p. 100 à la Martinique. On sait que la population de ruminants de la Guadeloupe et d'Antigua est infectée par la cowdriose. Le pourcentage faible de sérums positifs et l'absence de cas cliniques dans les autres îles suggèrent fortement que les sérums positifs dans ces îles sont problablement imputables à des réactions croisées non-spécifiques entre *Cowdria* et d'autres micro-organismes (peut-être *Ehrlichia*), qui restent à être identifiées. Cependant, il convient de porter une attention particulière aux pourcentages relativement élevés de moutons positifs à la Martinique (15 p. 100) et à Montserrat (11 p. 100).

Mots clés : Bovin - Ovin - Cowdriose - Epidémiologie - *Cowdria ruminantium* - Test ELISA - Maladie transmissible par tiques - *Amblyomma variegatum* - Guadeloupe - Caraïbes.

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INTRODUCTION

Thirteen years after the diagnosis of heartwater (12) and 10 years after the beginning of the research program in Guadeloupe, where do we stand ? What is the exact distribution of heartwater in the Lesser Antilles ?

The distribution of heartwater was previously determined using different methods:

- collection of adult *Amblyomma variegatum* on cattle and inoculation of tick supernatant into susceptible goats (1) : the islands of Guadeloupe, Marie-Galante and Antigua appeared to be infected (2, 3). This was confirmed in Guadeloupe and Antigua by post mortem diagnosis on the brain of animals which had died from heartwater ;

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- serological survey using IFAT on Kümm antigen (4) : the islands of St. Lucia, Martinique, St.Kitts & Nevis, and St.Martin were suspected to be infected because at least 5 % of the ruminants were seropositive for *Cowdria.* With an IFAT using the Gardel stock cultivated on endothelial cells as antigen, the only suspected islands with seropositive reactions over 5 % were Martinique and St.Martin (8) ;

- by testing the same sera collected in 1983 using a competitive ELISA (CELISA) followed by confirmation of positive reactions by Western blotting (11), the islands of Dominica, Grenada, Martinique, Montserrat, St.Kitts, St.Lucia, St.Martin and St.Vincent were suspected to be infected. However, the islands of Grenada and St.Vincent were not infested by *A. variegatum* and the question of the specificity of the different tests arose.

The development of an indirect ELISA (9) and of a regional project to eradicate the tick, led to a new sero-epidemiological survey in the Lesser Antilles. The objective of the survey was to determine the distribution of heartwater in the Lesser Antilles and in particular to further investigate whether the disease actually occurs in the suspected islands or not.

MATERIAL AND METHODS

Experimental design

The survey was carried out between February and September 1992 in collaboration with the veterinary services of the Lesser Antilles. Eleven islands were surveyed : Grenada, St.Vincent, Barbados, St.Lucia, Martinique, Dominica, Guadeloupe, Montserrat, Antigua, St.Kitts & Nevis, St.Martin. One percent of the overall ruminant population estimated at 200, 000 heads was sampled. The islands being divided into districts, parishes or municipalities, a cluster sampling technique was applied : 1 % of herds in each area was randomly selected and all animals within selected herds were sampled. In each herd, data were collected on the farming system including tick control methods. The breed, sex, age, presence of ticks were recorded for each sampled animal. In addition, each island was classified according to its status regarding the infestation by the tick : 0 = tick not reported, 1 = tick reported but not established, 2 = tick very limited in distribution and under control, 3 = tick limited in distribution, 4 = tick widespread.

Serological test

All sera were tested for the presence of antibodies to *Cowdria ruminantium* using an indirect ELISA (9).

RESULTS AND DISCUSSION

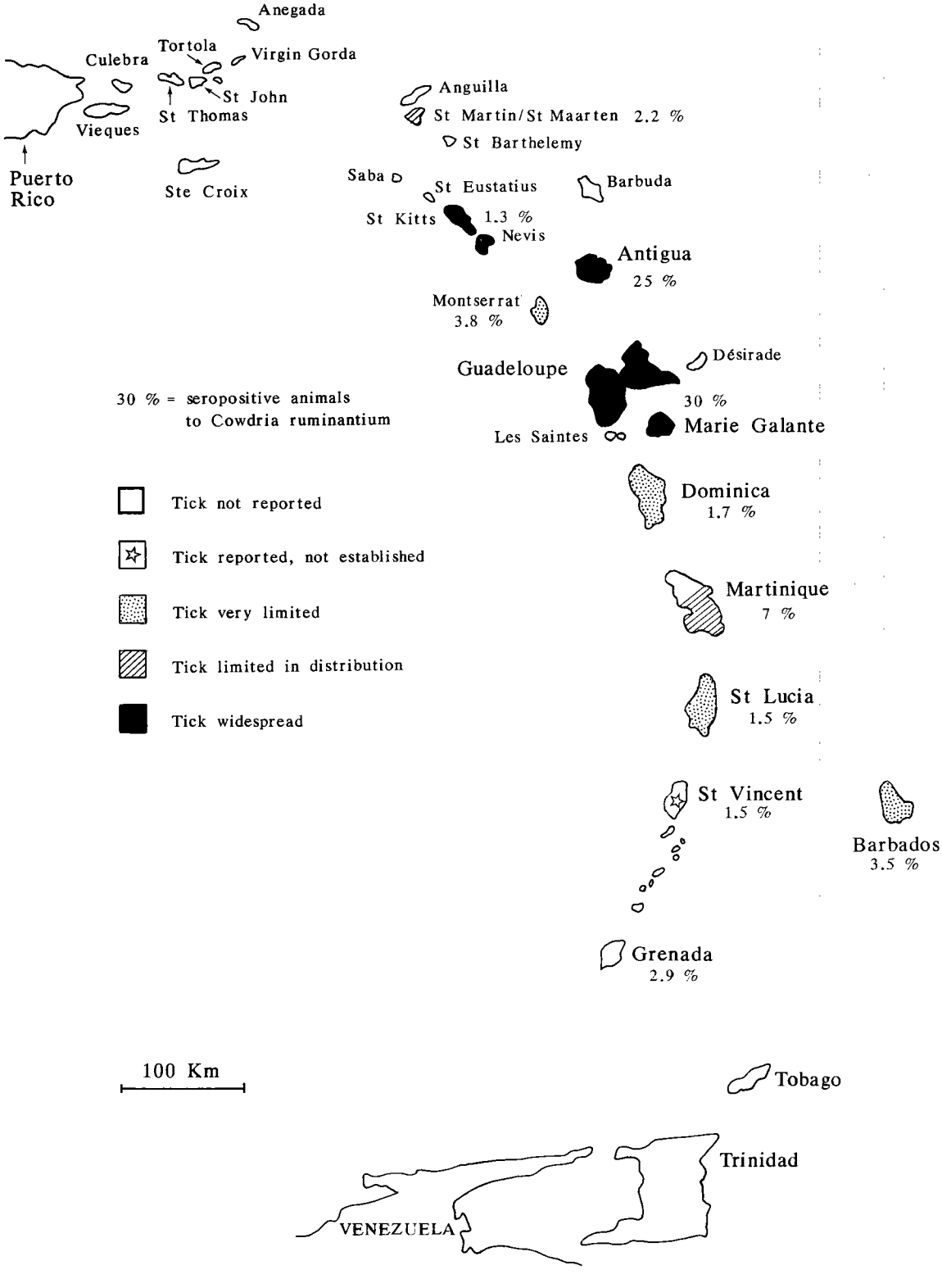
A total of 3,579 sera were collected in 11 different islands and tested for the presence of antibodies to *Cowdria ruminantium.* The results are presented in table I and map 1. Two groups of islands were identified on the basis of serological results :

- Antigua and Guadeloupe were found to have a high percentage of seropositive animals (25 and 30 % respectively). Both Antigua and Guadeloupe have a long history of

TABLE I Percentage of sera from domestic ruminants with antibodies to **C. ruminantium** *detected by indirect ELISA.*

Islands	Number seropositive/total tested (%)				Tick	Conclusion
	Cattle	Goats	Sheep	Total		
Antigua	13/102 (13)	27/76 (35)	20/65 (31)	60/243 (25)	4	HW
Barbados	3/51 (6)	1/47 (2)	3/100 (3)	7/198 (3.5)	2	0
Dominica	3/102 (3)	0/109 (0)	2/88 (2)	5/299 (1.7)	2	0
Grenada	1/60 (1.7)	3/43 (7)	1/101 (2)	5/204 (2.9)	0	0
Guadeloupe	112/592 (19)	156/295 (53)	—	268/887 (30)	4	HW
Martinique	9/297 (3)	4/99 (4)	27/176 (15)	40/572 (7)	3	?
Montserrat	0/91 (0)	0/81 (0)	10/89 (11)	10/261 (3.8)	2	?
St. Kitts & Nevis	0/50 (0)	0/47 (0)	2/56 (2)	2/153 (1.3)	4	0
St. Lucia	6/191 (3)	0/87 (0)	0/134 (0)	6/412 (1.5)	2	0
St. Martin	0/27 (0)	0/29 (0)	2/22 (6)	2/89 (2.2)	3	0
St. Vincent	3/159 (2)	0/37 (0)	1/65 (1.5)	4/261 (1.5)	1	0

Tick : 4 widespread ; 3 limited in distribution ; 2 very limited in distribution ; 1 reported, not established ; 0 not reported.



Map 1 : Distribution of A. variegatum *ticks in the Lesser Antilles and percentage of seropositive animals to* C. ruminantium.

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infestation with the tick and both have experienced clinical cases of heartwater (2, 12) ;

- in the other 9 islands except Martinique, less than 4 % of the sera were positive. This correlates well also with an apparent absence of clinical cases of cowdriosis. However, 11 and 15 % of sheep sera were found to be positive in Montserrat and Martinique respectively, raising the question of the presence of undetected cowdriosis or serological crossreactivity between *Cowdria* and other micro-organisms such as *Ehrlichia* (5, 7). The hypothesis of the presence of an *Ehrlichia* species was already explored in Martinique. In previous surveys (4), 10 % of the sera collected on ruminants in this island were positive for *Cowdria*. However, when a pool of 300 adult *Amblyomma variegatum* collected on these animals were ground and inoculated into a splenectomized Friesian heifer and an intact sheep, no fever, parasitemia or seroconversion to *Cowdria* were observed, indicating that positive sera were not due to *Cowdria* and if an *Ehrlichia* species occurs, it is not transmitted by *A. variegatum*. In the 9 above mentioned islands, the percentage of positive sera was significantly higher in sheep (3.3 %) and in cattle (2.2 %) than in goats (0.8 %). This could be explained by the presence of *Ehrlichia ovina* in sheep and *E. bovis* in cattle. The presence of E. *bovis* has been reported in Brazil (10) but *E. ovina* has never been isolated in the region. Thus the presence of these parasites in the Caribbean needs to be confirmed. In particular, in Montserrat and Martinique, isolation might be attempted by inoculating fresh blood collected from seropositive animals into susceptible ones followed by the isolation of the rickettsiae *in vitro*.

In addition to serological and clinical evidence that cowdriosis is absent from the surveyed islands except Antigua and Guadeloupe, it appears that in several islands, the populations of ticks on livestock are not high enough to allow the transmission of heartwater. Thus, no *A. variegatum* were reported in Grenada. In St.Vincent, only two male *A. variegatum* were reported in 1988 but no more since that time. St. Lucia, Dominica, Montserrat, Barbados have limited foci which are being controlled. During this survey, no *A. variegatum* were found in Dominica and Barbados, one engorged female was found in St.Lucia and one bovine infested by males was observed in Montserrat. Only St. Martin, St. Kitts & Nevis had abundant tick populations on domestic ruminants. However, seroprevalences were very low in these last islands.

The results of this survey were somewhat different from those of previous surveys. The comparison of the results between the different surveys are presented in table II. The different serological tests used (IFAT on Kümm antigen or infected endothelial cells, competitive or indirect ELISA) and the different periods of blood collecting (1982-84, 1992) may account for these differences. A recent comparison of 5 serological tests (6) showed the good sensitivity of all the tests used and the extensive cross-reactions with putative *Ehrlichia* species revealed with all 5 tests. This indicates that the influence of the serological method used was probably not essential to explain the differences between serological results. In contrast, in several islands the situation regarding the infestation by *A. variegatum* has changed between the 2 surveys :

- in Barbados, *A. variegatum* ticks were not present

TABLE II ***Comparison between results of different seroepidemiological surveys on heartwater in the Lesser Antilles.***

Islands	Inoculation of ticks		Serological surveys (% sero+)					Tick Infestation	Conclusion
	No of ticks	Results	IFAT Kümm	IFAT Gardel	CELISA	W. Blot	ELISA		
Antigua	500	1+/5	4.3	ND	4.9	ND	25.0	4	HW
Barbados	0	ND	0.8	ND	ND	ND	3.5	2	0
Dominica	28	0/2	3.2	ND	24.0	21.3	1.7	2	0
Grenada	0	ND	2.5	ND	5.4	3.6	2.9	0	0
Guadeloupe	2 086	12+/19	24.3	ND	38.6	ND	30.0	4	HW
Martinique	500	0/6	10.7	5.9	6.7	4.2	7.0	3	?
Montserrat	0	ND	1.0	ND	2.6	2.6	3.8	2	?
St. Kitts & Nevis	225	0/4	8.0	3.3	10.6	4.6	1.3	4	0
St. Lucia	212	0/4	4.7	4.2	4.5	ND	1.5	2	0
St. Martin	107	0/6	10.3	7.5	3.2	3.2	2.2	3	0
St. Vincent	0	ND	2.6	ND	9.9	6.1	1.5	1	0
Years of sampling	1982 - 1984		1982 - 1984				1992		

ND : not determined.

during the first survey but are now established at a very low level ;

- in Dominica, ticks appeared in 1983, were present at a relatively low level during the first survey but are now under control ;

- in Martinique, ticks were very abundant in 1982, and are now very rare in spite of their wide distribution ;

- in Montserrat, ticks were absent during the first survey, were very abundant the following years but are now under control ;

- in St.Lucia, ticks were numerous during the first survey but are now under control.

These modifications of tick populations between 1982 and 1992 are likely to be responsible for the differences in the results.

CONCLUSION

The present survey strongly suggest that heartwater is absent from Dominica, Grenada, St.Kitts & Nevis, St.Lucia, St.Martin and St.Vincent. Further research is needed to confirm the absence of heartwater in Montserrat and Martinique.

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A sero-epidemiological survey for heartwater was organized in 1992 in the Lesser Antilles, from Grenada to Saint Martin. Blood from about one percent of the ruminant livestock of the islands was randomly sampled and the sera were tested with an indirect ELISA. The percentage of positive sera was found to be 30 % in Guadeloupe, 25 % in Antigua, 2.2 % in St.Martin, 1.3 % in St.Kitts & Nevis, 3.8 % in Montserrat, 1.7 % in Dominica, 1.5 % in St.Lucia, 1.5 % in St.Vincent, 3.5 % in Barbados, 2.9 % in Grenada and 7 % in Martinique. Ruminants from Guadeloupe and Antigua are known to be infected with heartwater. The low percentage of positive sera and the absence of clinical cases in the other islands strongly suggest that positive sera in these islands are probably due to non-specific cross reactions between *Cowdria* and other micro-organisms (possibly *Ehrlichia*) which remain to be isolated. In particular, the high percentages of positive sheep sera in Martinique (15 %) and Montserrat (11 %) should be further investigated.

Key words : Cattle - Sheep - Cowdriosis - Epidemiology - *Cowdria ruminantium* - ELISA - Tickborne disease - *Amblyomma variegatum* - Guadeloupe - Caribbean.

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En 1992, se organizó un estudio sero epidemiológico para la cowdriosis en las Antillas Menores, desde Grenada hasta San Martín. Se recolectaron al azar muestras sanguíneas de alrededor 1 p. 100 del hato de las islas, las cuales se examinaron mediante el ELISA indirecto. El porcentaje de sueros positivos fue de 30 p. 100 en Guadalupe, 25 p. 100 en Antigua, 2,2 p. 100 en San Martín, 1,3 p. 100 en San Kitts & Nevis, 3,8 p. 100 en Monserrat, 1,7 p. 100 en Dominicana, 1,5 p. 100 en Santa Lucía, 1,5 p. 100 en San Vicente, 3,5 p. 100 en Barbados, 2,9 p. 100 en Grenada, 7 p. 100 en Martinica. Se sabe que los rumiantes de Guadalupe y Antigua están contaminados con cowdriosis. En cuanto a las otras islas, el bajo porcentaje de sueros positivos y la ausencia de casos clínicos sugiere que los sueros positivos encontrados en éstas, podrían deberse a reacciones cruzadas no específicas entre *Cowdria* y otros microrganismos (posiblemente *Ehrlichia*) los cuales aún no han sido aislados. Se recomienda un estudio posterior, principalmente para explicar los porcentajes positivos en Martinica (15 p. 100) y Monserrat (11 p. 100).

Palabras claves : Bovino - Ovino - Cowdriosis - Epidemiología - *Cowdria ruminantium* - ELISA - Enfermedad transmitida por garrapatas - *Amblyomma variegatum* - Guadalupe - Caribe.