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SEROLOGICAL PREVALENCE OF BOVINE BABESIOSIS IN MALI

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SUMMARY

A serological survey of cattle in Mali was carried out to determine the prevalence of antibody activity to Babesia bovis *and* B. bigemina. *It was found that the level of* B. bovis *infection as indicated by antibody activities was too low to be of immediate concern. However, the serological prevalence of* B. bigemina *was high and this may indicate a potential disease problem. It was also found that when zebu and N'Dama cattle grazed together the N'Dama were twice as likely to have positive titres to* Babesia *as were the zebus.*

INTRODUCTION

Babesiosis of cattle caused by *Babesia bovis* and *B. bigemina* is a serious problem in many parts of the world (de Vos, 1979; Corrier, Gonzalez, and Betancourt, 1978; Akafekua, 1976). Ticks of the genus *Boophilus* are the vectors of the parasite and disease incidence is generally limited to the distribution of these ticks (Uilenberg, 1976). There have been limited surveys of ticks and babesiosis in Mali (Morel, 1969, 1973). In order to make more rational decisions concerning the development of cattle production programmes detailed data concerning the distribution and extent of babesiosis are needed.

Mali has two ecological zones with two different types of cattle production. In the humid south the basic agricultural activity is crop farming near permanent villages with cattle used chiefly as a source of traction and organic fertiliser. Tsetse flies are common especially during the rainy season (Goodwin, 1981) and restrict traditional farmers to using trypanotolerant breeds the most common of which is the N'Dama (*Bos taurus*). In the dry north the main agricultural activity is nomadic herding of the drought- and heat-resistant zebu (*Bos indicus*) from dry season to wet season pasture and back again. There is no significant trypanosomiasis problem because the immature tsetse cannot develop in the arid environment (Goodwin, 1981). Pasture and sources of drinking water are sparse, however, especially during the dry season. Millet is planted but is very dependent on rainfall which is often unreliable.

Temperature and humidity are important factors in limiting *Boophilus* spp. distribution in other countries and are likely to be equally important in Mali (de Vos, 1979; Uilenberg, 1976). Additionally when zebu cattle migrate from humid areas to rainy season pasture at the end of the dry season they may carry ticks into areas where, because of the length of the dry season and absence of hosts, they would not normally be found. By this method ticks can become established in these areas during the wet season. Thus an annual reinfestation of ticks may cause babesiosis to occur in climatic situations where, in other countries, the disease has not been noted. A serological survey to assess the distribution and prevalence of antibody activity against *Babesia* was therefore conducted.

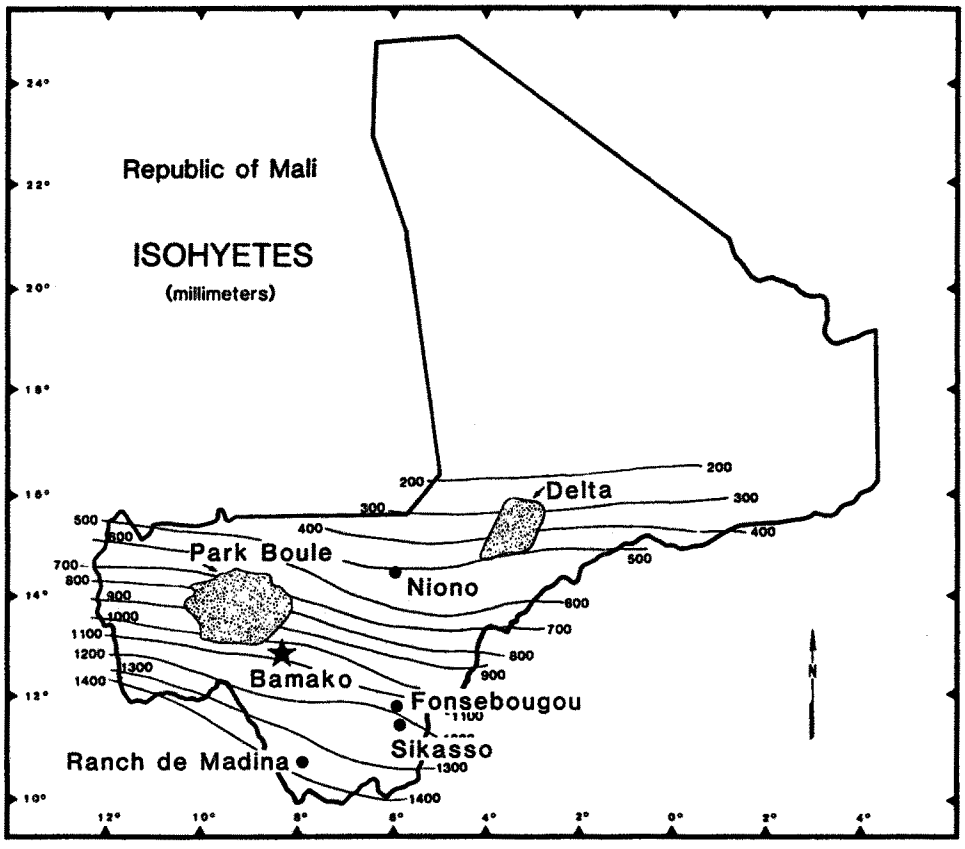

FIG 1. Sampling locations in conjunction with annual rainfall.

MATERIALS AND METHODS

Blood samples for serum were obtained from cattle of all ages in herds from seven villages and towns in different areas of Mali as shown in Fig. 1. Sera were tested for the presence of antibody activity by the indirect fluorescent antibody test (IFAT) (Ross and Lohr, 1968) at dilutions of 1:40, 1:80 and 1:160 in phosphate buffered saline pH 7·2 to 7·4 (PBS). The *B. bovis* and *B. bigemina* antigen slides utilised in the IFAT had been previously prepared.[3] Positive and negative serum controls were included on each slide. The fluorescein conjugated rabbit antibovine immunoglobulin used was from a commercial source.[4] The slides were examined with an epifluorescent system using a mercury vapour light source (Microstar 120).[5] Samples were considered positive if they fluoresced at 1:40 or greater in the same manner as the positive control.

MacDonald's model for malaria (MacDonald, 1950) and Mahoney's adaptation of it for babesiosis (Mahoney, 1977) were used to determine the inoculation rates

[3]IFAT antigen slides and known positive and negative sera were prepared at the Center for Tropical Animal Health, Texas A&M University, and shipped under dry ice to Bamako.
[4]Grand Island Biological Co, Grand Island, NY, USA.
[5]American Optical Co, Rochester, NY, USA.

for *B. bigemina* and *B. bovis* in five different ecological zones. The proportion of each herd infected was determined by IFAT. The inoculation rates for several age groups were averaged to determine the local inoculation rate. Mahoney's definition of an enzootically unstable situation was used to determine the significance of the inoculation rates. These limits for enzootic instability are 0·0005 and 0·005 infective bites/head/day (Mahoney, 1977).

RESULTS

The age prevalence rates for IFAT reactions to *B. bovis* and *B. bigemina* at seven sites representative of both the humid and dry areas of Mali are shown in Tables I and II. The percentage of sera positive for *B. bigemina* in most age groups was several times greater than the percentage positive for *B. bovis*. The percentage of sera positive for *B. bigemina* rarely exceeded 60% at any site while the percentage positive for *B. bovis* was always less than 40%.

The inoculation rates of *B. bovis* and *B. bigemina* infection by ticks at five sites are shown in Table III. The *B. bovis* inoculation rates were below 0·0005 infective bites/head/day while *B. bigemina* inoculation rates were between 0·0005 and 0·0035 infective bites/head/day. The percentage of calves nine months of age with positive IFAT titres from five sites are shown in Table IV. At nine months of age the percentage of calves infected with *B. bovis* was low 16·7% being the maximum at

TABLE I

Age prevalence of positive titres (1:40 or greater) for B. bovis

Site	<1½ years		1½–3 years		Adult	
	%	*N*	%	*N*	%	*N*
Ranch de Madina	33·8	145	12·1	33	10·2	88
Fonsebougou	10·8	120	16·7	30	12·1	33
Niono	15·7	70	10·8	37	10·9	55
Sikasso	17·0	47	30·8	13	15·8	19
Bamako	38·1	139	7·1	14	15·4	26

N = number of sera tested.

TABLE II

Age prevalence of positive titres (1:40 or greater) for B. bigemina

Site	<1½ years		1½–3 years		Adult	
	%	*N*	%	*N*	%	*N*
Ranch de Madina	44·0	157	24·2	33	27·0	89
Fonsebougou	57·5	51	66·7	30	57·6	33
Niono	53·9	78	53·2	47	57·1	77
Park Baoule	42·3	52	20·0	10	10·3	29
Sikasso	37·5	38	53·9	13	52·6	19
Delta	52·5	40	72·7	22	66·7	42
Bamako	54·4	139	78·6	14	53·1	32

N = number tested.

TABLE III

Inoculation rates (infective bites/head/day) for B. bovis *and* B. bigemina

Site	*B. bovis*	*B. bigemina*
Fonsebougou	0·0003	0·0019
Niono	0·0003	0·0022
Park Baoule	ND	0·0005
Delta	0·0003	0·0035
Ranch de Madina	0·0005	0·0007

ND = not done.

TABLE IV

Percentage of nine-month-old calves with Babesia *titres of 1:40 or greater*

	B. bovis		*B. bigemina*	
Site	%	*N*	%	*N*
Ranch de Madina	5·8	52	28·3	53
Fonsebougou	11·1	27	63·0	27
Niono	16·7	36	58·2	55
Park Baoule	ND	ND	22·2	18
Delta	10·3	29	69·0	29

N = number tested.
ND = not done.

TABLE V

Prevalence of positive titres (1:40 or greater) for B. bovis *and* B. bigemina *for N'Dama and zebu cattle at Bamako*

	B. bovis		*B. bigemina*	
Breed	%	*N*	%	*N*
Zebu	19·2	26	33·6	125
N'Dama	36·1	147	61·2	147

N = number tested.

any site. On the other hand *B. bigemina* infection was much higher ranging from 22·2% to 69%.

The comparison of antibody activity for each organism in zebu vs. N'Dama cattle is shown in Table V. The N'Dama cattle had a percentage of positive titres almost twice that of zebu cattle from the same area.

DISCUSSION

Annual rainfall at the sites (Fig. 1) ranges from 1,300 to 1,400 mm at Ranch de Madina to less than 550 mm at Niono. Fonsebougou and Sikasso are in the 1,100 to

1,200 mm range. Bamako has between 1,000 and 1,100 mm of annual precipitation and Park Baoule has 750 to 1,000 mm of annual rainfall.

The inland delta of the Niger is a special case because the amount of water available depends on flooding of the Niger rather than local rainfall. During the flood pastures are submerged and cattle are trekked to the Sahelian wet season pasture. Nowhere in Mali do temperatures become low enough to restrict *Boophilus* distribution but at some seasons in some areas the temperatures may be high enough to interfere with development of the tick stages of *Babesia* (Dalgliesh and Stewart, 1979). The main factor affecting *Boophilus* distribution is probably the humidity.

It is obvious from the results that antibody activity to *Babesia* is widespread in Mali. To determine if any threat of disease exists it is necessary to calculate the inoculation rate of the organism in question at a given locality. The inoculation rate determines how likely it is that a particular individual will become infected in a certain period of time (specifically before nine months of age when it loses its age resistance) and varies according to the number of ticks feeding on a single host and the percentage of the tick population that can transmit the infection.

Both MacDonald (1950) and Mahoney (1977) used blood smear examination to determine the proportion of the herd infected. When using a serological test to gain the same information it should be kept in mind that antibody activity may be detected for several years after the parasitaemia has fallen below detectable levels (Kuttler, Adams, and Todorovic, 1977). Thus determination of the percentage of serologically positive individuals at the time of decreasing age resistance gives a direct measure of the proportion of the herd still at risk. Mahoney and Ross (1972) determined that *Babesia* infection prevalence rates between 12 and 75% in nine-month-old calves were the limits of enzootic instability because there were both sufficient susceptibles at risk and a large enough reservoir to infect them. Below 12% the reservoir is too small and above 75% the pool of susceptibles is too small to support a serious outbreak.

It should be noted that when calculating the inoculation rate it was assumed that recovery as measured by IFAT (i.e. reversion to negative antibody activity status) was less than the inoculation rate. This assumption should be valid for at least the first two years after infection since the IFAT remains positive for that period. This assumption is not valid when thin blood smears are used for diagnosis since parasitaemia drops below detectable levels long before IFAT antibody activity falls. If the measured recovery rate is zero recovery must be less than the inoculation rate.

Using inoculation rates and the nine month age prevalence rate of positive titres determined from our studies leads to the conclusion that the prevalence of *B. bovis* infection is too low to pose a problem. At Ranch de Madina the inoculation rate was considered a borderline situation (0·0005 infective bites/head/day) and at Niono the age prevalence rate was slightly higher than 12% (16·7%). All the other sites fell outside the danger zone in both inoculation rate and age prevalence rate. Infection with *B. bigemina* presents a different situation. Both the calculated inoculation rates and the percentage of positive yearlings suggest that the situation is unstable. Although outbreaks of disease seem likely to occur the relative virulence of *B. bigemina* in Mali remains to be determined. Clinical babesiosis was not seen and farmers did not mention haematuria as a symptom of clinically ill cattle. Anaemia was a common problem from trypanosomiasis and poor nutrition. *B. bigemina* is not considered pathogenic in Australia (Mahoney, 1977) but reports

from other countries suggest it can cause problems (de Vos, 1979; Corrier *et al*, 1978; Uilenberg, 1976).

The large difference between the prevalence of *B. bovis* and *B. bigemina* titres is interesting. Mahoney (1969) did not find as great a difference in two Australian herds being monitored for *B. bovis* and *B. bigemina* infection by blood smear examination. However, in areas of the Republic of South Africa where only *B. decoloratus* exists *B. bigemina* is found much more fequently than *B. bovis*. In areas where *B. decoloratus* and *B. microplus* coexist the prevalence of *B. bovis* more nearly approaches that of *B. bigemina* (de Vos, 1979).

The two most common species of *Boophilus* in Mali are *B. geigyi* and *B. decoloratus* (Matthysse, 1980). *B. decoloratus* is shown by the South African workers to be a more efficient vector of *B. bigemina* than it is of *B. bovis* (de Vos, 1979). *Boophilus geigyi* is a newly described species difficult to differentiate from *B. decoloratus* (Aeschlimann and Morel, 1965). Recent evidence suggests that it is presently the most common *Boophilus* species in Mali (Matthysse, 1980).

Akinboade and Dipeolu (1981) studied infection rates of *B. geigyi* by *B. bovis* and reported a high percentage of infected eggs, haemolymph and larvae (almost 100%) on the basis of finding vermicules in smears. This result suggests that *B. geigyi* is much more efficient vector of *B. bovis* than the present serological data seem to indicate. The authors did not report transmission experiments.

Along the edge of the tsetse region N'Dama and zebu cattle are often grazed together. These herds allow a direct comparison to be made between the two species of cattle for their relative resistance to babesiosis. The results suggest that zebu are less likely to be infected than N'Dama. This finding is in agreement with observations that *B. indicus* breeds are more tick resistant than *B. taurus* breeds and thus are less likely to be inoculated (O'Kelly and Spiers, 1976). If these results can be confirmed a long range breeding programme may be justified in which crossbreds of N'Dama and zebu could be selected for both resistance to ticks and resistance to trypanosomiasis.

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FREQUENCE SEROLOGIQUE DE LA BABESIOSE BOVINE AU MALI

Résumé—Une enquête sérologique sur le bétail au Mali a été effectuée pour déterminer la fréquence de l'activité des anticorps à *Babesia bovis* et *B. bigemina*. On a trouvé que le degré d'infection à *B. bovis* tel qu'indiqué par les réactions anticorps était trop faible pour constituer un souci immédiat. Cependant la fréquence sérologique de *B. bigemina* était élevée, ce qui peut indiquer un problème pathologique potentiel. On a aussi noté que lorsque les zébus et les N'Dama broûtaient ensemble, les N'Dama avaient deux fois plus de chance d'avoir des titres positifs à *Babesia* que ne l'avaient les zébus.

PREVALENCIA SEROLOGICA DE BABESIOSIS BOVINA EN MALI

Resumen—Se llevó a cabo un análisis serológico en Malí, para detectar la prevalencia de *Babesia bovis* y *Babesia bigemina*. Se encontró, que la prevalencia serológica de *B. bovis* es baja para considerar la enfermedad una amenaza inmediata. Sinembargo, la prevalencia de *B. bigemina* fué alta, un problema potencial. Tambien se encontró, que cuando el ganado Cebu y N'Dama pastorea junto, el N'Dama presenta el doble de títulos serológicos de *B. bigemina*.

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