Scand. J. Immunol. **36,** Suppl. 11, 107–109, 1992

Anaplasmosis in Uganda. II. Prevalence of Bovine Anaplasmosis

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Ssenyonga GSZ, Kakoma I, Montenegro-James S, Nyeko PJ, Nanteza A, Buga R. Anaplasmosis in Uganda. II. Prevalence of Bovine Anaplasmosis. Scand J Immunol 1992;36(Suppl. 11):107–9

The prevalence of bovine anaplasmosis was studied in 320 Zebu cattle randomly selected from three regions of Uganda (central, south-western and north-western) using DOT-ELISA, Western immunoblotting, Rapid Card Agglutination Test (RCAT), Capillary Tube Agglutination Test (CAT), Complement Fixation Test (CFT), and parasitological techniques. Dried blood on Whatman filter paper no. 1 was eluated in PBS 0.05% Tween 20 prior to testing at an initial dilution of 1:25.

The incidence of parasitaemia ranged from 25% in the central region to 35% in the north-western region and the serological prevalence was lower in the central region and highest in the north-west. Prevalence rates assayed by DOT-ELISA and Western immunoblotting were 1.5-fold greater than those tested with RCAT and 3-fold greater than in CAT. The overall prevalence rates by DOT-ELISA and Western immunoblotting compared favourably with CFT data.

The present data utilizing dried blood on filter papers indicate that there is a high prevalence of anaplasmosis in those regions of Uganda surveyed and it confirms our observations and those of others that collecting blood on filter papers is a suitable technique for large-scale screening and for seroepidemiological studies.

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Bovine anaplasmosis, a worldwide tick-borne disease affecting cattle, sheep, goats, water buffalo, and some wild ruminants such as deer and buffalo, is caused by *Anaplasma marginale* and transmitted principally by a number of tick species belonging to different genera [1]. The disease is of great economic importance in cattle in the tropics and subtropics, causing losses through mortality, especially in newly introduced, highly susceptible, exotic dairy cattle, where it results in reduced weight gains and milk production. Preventive measures through chemotherapy, vector control, and vaccinations where they are practised are expensive and affect the economics of livestock production [2]. Recovered animals need a prolonged period of convalescence during which they are less productive. Such animals remain carriers for the rest of their life, and they are potentially dangerous to newly introduced susceptible exotic cattle in that they act as reservoirs of infection in the presence of ticks. Knowledge of prevalence rates is an important component in the assessment of levels of enzootic stability to anaplasmosis and other tick-borne diseases on which the design of effective control measures can be based.

Although anaplasmosis is readily recognized and diagnosed throughout Uganda by clinicians and veterinary assistants, little information is available regarding its distribution, prevalence, and economic importance in the country. This situation has been further complicated by protracted socio-political and economic strife in the country for nearly two decades. According to the monthly reports of the Department of Veterinary Services, among the tick-borne diseases the prevalence of anaplasmosis ranks second to that of East Coast fever.

This study is a follow-up of our previous study [3] to determine the seroprevalence and distribution of anaplasmosis in Uganda, especially appropriate considering that the government has

TABLE I. The serological prevalence of *Anaplasma marginale* in the three regions of Uganda sampled

Region	No. of animals	No. of herds	Parasitological results (%)	DOT-ELISA (%)	Western blot (%)	RCAT (%)	CAT (%)	CFT*
Central	105	10	25 (26)†	57 (60)	58 (61)	36 (38)	19 (20)	—
South-western	100	10	28 (28)	62 (62)	62 (62)	40 (40)	22 (22)	—
North-western	115	10	35 (40)	66 (76)	67 (77)	47 (54)	26 (30)	—
Overall prevalence			29 (94)	61.9 (198)	62.5 (200)	41 (132)	22.5 (72)	58

* The CFT was carried out on only 100 randomly selected samples from the three regions: due to the economic and technical constraints associated with the CFT, it was not feasible to apply it on all the samples.

† Number of animals.

embarked on a major herd health improvement and rehabilitation programme involving introduction of valuable, exotic breeds of cattle, particularly Holstein-Friesians which are highly vulnerable to anaplasmosis and other tropical diseases. In this study, we have utilized the filter paper blood sampling technique on a large scale to establish the seroepidemiological profile of anaplasmosis in Uganda.

MATERIALS AND METHODS

Blood samples were obtained from 320 local Zebu cattle ranging from 6 months to about 4 years of age from the central region (105), south-western region (100), and north-western region (115) of Uganda, and 10 herds from each of the three regions were selected randomly for blood sampling. Ten to 11 animals also selected at random were sampled from each herd.

About 10 ml of blood for sera was obtained from the jugular vein of each animal in clean universal bottles. Whatman filter paper no. 1 was spotted with 200 μl of whole blood, dried and stored in self-sealing plastic bags at 4°C until eluted in PBS 0.05% Tween 20 prior to testing. Eluates and sera were aliquoted into 0.5 ml and stored at −70°C. All serological tests were carried out with a starting dilution of 1:25 in PBS 0.05% Tween 20.

Thick and thin blood smears were prepared at the same time for routine parasitology and were stained with Giemsa for the *Anaplasma marginale* organisms.

All the sera and eluates were tested for reactivity against *Anaplasma marginale* antigens Florida strain prepared in the USA as described by Montenegro-James [4] using the following tests: DOT-Enzyme-linked immunosorbent assay (DOT-ELISA), Western immunoblotting, Rapid Card Agglutination Test (RCAT) [5], and Capillary Agglutination Test (CA) [6]. In addition, randomly selected serum samples were submitted to the United States Department of Agriculture (USDA) Diagnostic Laboratory at Centralia, Illinois, for independent confirmation for seropositivity to anaplasmosis by the Official Complement Fixation Test (CFT) in the USA.

RESULTS

The serological prevalence and the incidence of parasitaemia for bovine anaplasmosis in three regions in Uganda are summarized in Table I.

The incidence of parasitaemia ranged from 25% in the central region to 35% in the north-western region of Uganda. Similarly, the serological prevalence as demonstrated by various serological tests was lower in the central region and highest in the north-west. Prevalence rates assayed by DOT-ELISA and Western blotting were 1.5-fold greater than those tested by RCAT and 3-fold greater than in CAT. However, overall prevalence rates by DOT-ELISA and Western immunoblotting compared favourably with that tested with CFT—the officially accepted test for anaplasmosis in the USA (Table I).

DISCUSSION

We have previously demonstrated that dried blood on filter paper was suitable for serodiagnosis of anaplasmosis in Uganda using a battery of selected tests [3]. The present data utilizing that sampling method have confirmed our observations and those of others [7, 8] and provide useful epidemiological information on anaplasmosis in Uganda.

The three regions studied constitute about half the area of the whole country. In these regions anaplasmosis is widespread and appears to be a threat to the entire cattle population as indicated by the overall seroprevalence of 61.9% in DOT-ELISA, 58% in CFT and 62.5% in Western blotting and an overall incidence of *A. marginale* parasitaemia of 29%.

The proportion of positive sera was lowest (19–26%) using the CAT. The prevalence rates were twofold greater when measured by the RCAT and threefold greater when measured by DOT-ELISA and Western immunoblotting. Many samples which tested negative to CAT were positive to DOT-ELISA and Western immunoblotting. The discrepancy observed between the tests could be due to the fact that all the serum samples were frozen for more than 2 months prior to testing, which could have resulted in a decreased sensitivity of the RCAT and CAT. Similar observations were made by Jongejan *et al.* [9] in Zambia. The higher overall seroprevalence rates based on DOT-ELISA (61.9%), Western immunoblotting (62.5%), and CFT (58%), which is the officially accepted serological test for anaplasmosis in the USA, indicate that the vast majority of the indigenous cattle in those regions of Uganda have been exposed to anaplasmosis and that the disease tends to be mild. This would suggest that there might be a situation of enzootic stability for anaplasmosis in those regions. However, anaplasmosis becomes a major problem when fully susceptible adult exotic cattle (*Bos taurus*) are introduced in those enzootic areas [10]. A good example is the high mortality rate among newly imported exotic dairy cattle in Uganda during the period 1977 and 1979 (Ssenyonga, unpublished results).

The most important tick vector of anaplasmosis in Uganda is *Boophilus decoloratus*. This tick is widely distributed throughout Uganda (Ssenyonga, unpublished results). However, bovine anaplasmosis in Uganda tends to be more prevalent during the rainy season when blood-sucking insects such as *Tabanus* spp. and *Stomoxys calcitrans* are also abundant. There is now sufficient evidence that vectors other than ticks, such as *Tabanus* spp. [10] and *Stomoxys calcitrans* [1], transmit anaplasmosis and therefore may play an important role in the epidemiology of bovine anaplasmosis. This situation may prevail in Uganda in the rainy season.

In conclusion, the above data indicate that there is a high prevalence of anaplasmosis in those regions of Uganda surveyed. Since these regions represent only half of the country, more work covering the entire country is required in order to assess more accurately the overall prevalence of bovine anaplasmosis in Uganda.

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