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27th April, 1988

Inspection of Anthrax of the Wild Animals in the South Luangwa National Park in 1987.

SPECIES	NO. EXAMINED	NO. POSITIVE	ISOLATION OF THE ORGANISM
Buffalo	5	5	Positive (culture)
Elephant	4	4	Positive (culture)
Zebra	1	0	
Puku	3	3	
Giraffe	2	1	
Wild dog	3	3	
Kudu	2	1	
Waterbuck	2	2	
Hippo	2	2	
Leopard	1	0	Negative (culture and mice inoculation test)
Unknown	1	1	
Total	26	22	

Except 3 specimens (1 buffalo, 1 elephant and 1 leopard) almost all materials were brought into the laboratory as stained blood smears for diagnosis.

Inspection of Anthrax of the Wild Animals in The South Luangwa National Park

Date of Sampling	Species	Result	Date Exam.
	One Buffalo	Positive	
14, Aug.	One Elephant	Positive	17, Aug.
	One Zebra	Negative	
7, Oct.	One Puku	Positive	22, Oct.
	One Puku	Positive	22, Oct.
5, Oct.	One Buffalo	Positive	22, Oct.
1, Oct.	One Giraffe	Positive	22, Oct.
29, Sept.	One Wild Dog	Positive	22, Oct.
	One Wild Dog	Positive	22, Oct.
31, Aug.	One Kudu	Negative	22, Oct.
23, Sept.	One Waterbuck	Positive	22, Oct.
	One Waterbuck	Positive	22, Oct.
22, Sept.	One Buffalo	Positive	22, Oct.
	One Buffalo	Positive	22, Oct.
22, Oct.	One Wild Dog	Positive	22, Oct.
	One Giraffe	Negative	22, Oct.
	One Elephant	Positive	22, Oct.
27, Aug.	One Elephant	Positive	2, Sept.
27, Aug.	One Elephant	Positive	2, Sept.
27, Aug.	One Kudu	Positive	2, Sept.
1, Sept.	One Puku	Positive	2, Sept.
1, Sept.	One Hippo No.5	Positive	2, Sept.
1, Sept.	One Hippo	Positive	23, July
?	One Buffalo	Positive	14, Aug.
?	Unknown	Positive	22, Oct.
?	One Leopard	Negative	4, Oct.

THE UNIVERSITY OF ZAMBIA

SAMORA MACHEL

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF DISEASE CONTROL

RESULTS OF EXAMINATION OF THE WATER SAMPLES

	Water Samples from	Detection of B. anthracis	
		Cultivation	Mice Inoculation
1.	Chimzombo Lagoon	Negative	Negative
2.	Chimzombo L.R.	"	"
3.	Village well	"	"
4.	Tina Tina	"	"
5.	Vaca Vaca Lagoon	"	"
6.	Luangwa River 1 (upper)	"	"
7.	Luangwa River 2 (middle)	"	"
8.	Goose Lagoon	"	"
9.	Mufue Lagoon	"	"
10.	Luangwa Fura 1	"	"
11.	Luangwa Fura 2	"	"
12.	*Chichela Spring	Positive	Positive

*Water sample was collected near the dead buffalo.

Isolation Experiments of Bacillus anthracis from The Soil

No.of Soil specimens Examined	Results
1. From the points around the dead giraffe	
28 specimens	Negative
2. From the points around the dead elephant	
28 specimens	Negative
3. From the points around the dead buffalo	
28 specimens	Negative

Cause of the death of these animals was not examine bacteriologically

Virology, Rickettsia, Epidemiology

Jpn. J. Med. Sci. Biol., 50, 73 - 77, 1997.

Short Communication

DISTRIBUTION OF RIFT VALLEY FEVER AMONG CATTLE IN ZAMBIA

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SUMMARY: In the present study, 1,421 cattle in 32 herds within nine districts, which are important cattle-producing centers in the nine provinces of Zambia, were tested for Rift Valley fever by the indirect immunofluorescence assay. One hundred and forty-seven cattle (10.5%) in 28 herds (88.9%) in the nine districts tested were positive for Rift Valley fever implying a country-wide distribution. In districts associated with flood plains and/or "dambos" (low lying areas of perpetual flooding), high herd and individual positive rates (100% and >10%, respectively) were found, suggesting a significance of these features in the distribution of the disease.

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Rift Valley fever (RVF) is an acute, febrile, arboviral, zoonotic disease that causes high rates of abortion and neonatal mortality, primarily in sheep, goats and cattle (1). In Zambia the disease was first reported in 1974 when an epizootic involving cattle and sheep occurred in the Chisamba (Central Province) and Mazabuka (Southern Province) districts and in some parts of Copperbelt (2). Since then, several epizootics have occurred in the same areas (2,3). The disease in humans, involving deaths, has also been reported (4). All these reports indicated that the disease may be more or less endemic in the above-mentioned areas and could be contributing to the low livestock productivity and public ill-health in Zambia.

Although seroepidemiological studies have been conducted in the past, these studies have tended to be limited to the areas of repeated RVF occurrences and have, therefore, not indicated how widely the disease is distributed in Zambia. This article reports the results of a seroepidemiological study conducted in various parts of the country to determine the disease's country-wide distribution, factors influencing this distribution as well as to gain an insight on its possible impact on cattle productivity in the affected areas.

Between January 1990 and March 1991, serum samples were collected from commercial and emerging herds in nine districts (Fig. 1) representing important cattle-rearing centers in the nine provinces of Zambia. One district in each province was sampled. Sample herds were selected on the basis of their accessibility, the likely level of cooperation by the owners or managers and the likely availability of animal health and other relevant records. Information regarding the vaccination status of the herd, its reproductive performance and natural surroundings, was obtained from the owners or managers by use of appropriate questionnaires. All vaccinated herds and individual cattle were excluded from the study. The sera were kept at −20 C until required for testing.

The antigens and indirect immunofluorescence assay used in the present study were as described by Morita (5). In brief, Vero-E6 cells infected with ZH548-M12 strain of RVF virus were used as antigen. The sera showing titers higher than 1:16 against the antigen were considered as positive. Table I shows the districts sampled, the numbers of herds and individual cattle within these herds tested in each district and the results of the testing. A total of 1,421 individual cattle in 32 herds were tested for RVF in all the nine sample districts. All the tested herds in six districts and five out of six herds in Choma, three out of six in Chingola, two out three in Chipata districts were positive to RVF. Altogetehr, 147 individual cattle (10.5%) in 27 herds (88.9%) tested were positive for RVF.

Fig. 1. Map of Zambia. Sampling locations are shown by asterisks.

These herds or individual cattle had, reportedly, never been vaccinated against RVF in the past.

Field observations as well as farmer responses indicated that the tested herds in Kabwe, Lusaka, Solwezi, Mongu and Mansa districts were located adjacent to flood plains and/or dambos and grazed in and around these geographic features. (Dambos are shallow streamless depressions that can be seasonally waterlogged and are grass-covered) (5a). All the positive herds had a history of abortion and still-birth although they had annual vaccination programs against bovine brucellosis.

Zambia is composed of nine provinces and the current study included at least one district from each province. These districts are important livestock production centers in these provinces although the productivity is low (6). The results indicate that RVF exits in all the districts studied, implying that the disease may have a country-wide distribution. The results also indicate high positive rates (100% herd rate and greater than 10% individual cattle rates) in the Kabwe,

Table I. Distribution of Rift Valley fever among cattle in Zambia. Results of a seroepidemiological study in nine districts

District	Number of herds tested	Number positive	% herds positive	Number of cattle tested	Number of cattle positive	% cattle positive
Kasama	1	1	100	30	1	3.3
Mansa(d)	1	1	100	198	24	12.1
Chipata	3	2	66.7	162	2	1.2
Chingola	6	3	50	202	11	5.4
Solwezi(d)	2	2	100	181	25	13.8
Kabwe(d)	6	6	100	215	24	11.2
Lusaka(d/fp)	1	1	100	15	3	20.0
Mongu(fp)	6	6	100	206	47	22.8
Choma(d)	6	5	83.3	212	10	4.7
Total	32	27	88.9 av.	1.421	147	10.5 av.

av.: average, d: dambos, fp: flood plain.

Lusaka, Solwezi, Mongu and Mansa districts. These districts are characterized by the presence of either large flood plains and/or dambos and the positive herds were located adjacent to and grazed within and around these features. The results, therefore, suggest that these geographic features may be significant in the distribution and epidemiology of the disease in this country. Previous publications on the status of RVF in the Chisamba, Mazabuka and Lusaka areas expressed the belief that the disease is endemic in these areas (2,5,7,8). The results of the present studies seem to strengthen the previous reports and suggest that RVF could be endemic throughout most of the cattle producing parts of the country. Any future attempt to study the epidemiology and impact of the disease in Zambia should, therefore, involve samples that would represent the whole country.

The objective of this study was to determine the distribution of RVF in Zambia as well as factors influencing the disease distribution. The results indicate that the disease may have a country-wide distribution, that the presence and

proximity of flood plains and/or dambos to cattle-producing areas could be significant to the disease's distribution in those areas, and that the disease may be significantly contributing to the low productivity of cattle in Zambia. Finally, the results suggest that the disease may be endemic in most cattle-producing parts of the country.

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THE DISTRIBUTION OF RIFT VALLEY FEVER AND OTHER HITHERTO UNRECOGNISED VECTOR-BORNE INFECTIONS AMONG CATTLE IN ZAMBIA

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Several sero-epidemiological studies on Rift Valley fever (RVF) in Zambia have been carried out in the past, but they have tended to be limited to the areas of frequent epizootic occurrence and have therefore not indicated how widely the disease is distributed in the country. The current study was aimed at determining the country-wide distribution of the disease, the potential magnitude of its impact and some factors relevant to its distribution. One thousand four hundred and twenty-one individual cattle in 32 herds within 9 districts which are important cattle production areas in the 9 provinces of Zambia, were tested for RVF using an indirect immunofluorescent antibody test. One hundred and forty-seven cattle (10.3%) in 28 herds (84.4%) in all the 9 districts tested positive to RVF implying a country-wide distribution. High herd and individual positive rates (100% and >10% respectively) in districts associated with flood plains and/or dams suggests the significance of these features in the distribution of the disease. The high positive rates could also imply significant impact of the disease in the relevant areas. In addition to RVF, the authors were also interested in the possible presence and distribution of some infections hitherto unrecognised in Zambia, especially Crimean Congo haemorrhagic fever (CCHF), *Coxiella burnetii* and *Rickettsia conorii*. Some sera from the current as well previous RVF studies were also tested for the above infections. Altogether, 189 cattle for CCHF and 377 cattle for *C. burnetii* and *R. conorii*, from 12 herds in Chipata, Kasama, Mongu, Senanga and Choma districts were tested. Only 3 (1.6%) cattle (one each from Kasama, Chipata and Mongu) tested positive for CCHF while 31 (8.2%) cattle in 10 herds (83.3%) were positive for both *C. burnetii* and *R. conorii*. These results suggest the existence but rare occurrence of CCHF in Zambia and widespread and potentially hazardous (in terms of public health) occurrence of the other two infections. Results from similar tests in humans suggest that meat processing plants and farm workers are at higher risk of infection by the above agents.

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Prevalence of Rift Valley Fever in Lusaka and Mazabuka – Zambia

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With 4 tables

(Received for publication October 13, 1987)

Summary

Five out of 53 workers in a cattle abattoir in Lusaka had antibodies against Rift Valley fever (RVF). None of 40 workers in a pig abattoir were seropositive. Transmission of the virus by direct contact with infected cattle is suggested.

In Mazabuka district, 19 out of 167 residents were positive for RVF; 13 out of the 19 had no previous contact with cattle. Hence, the possibility that transmission could occur by mosquito bite can not be excluded in rural areas.

Key words: Rift Valley fever, Zambia

Introduction

Rift Valley fever (RVF) is one of the most important zoonotic diseases in sub-saharan Africa. In Zambia, it was first reported in the Chisamba area of the Central Province in 1974 and later in the Mazabuka district of the Southern province and parts of the Copperbelt (1). The infection is mosquito-borne, and outbreaks of the disease tend to occur during the rainy seasons. Transoval transmission in mosquitoes was reported (2). As the outbreaks of the disease in cattle and sheep are usually followed by those in man (3), the transmission of infection to man is thought to occur through direct contact with infected animals.

In this study, an attempt is made to clarify the mode of transmission of the infection to man in urban and rural areas of Zambia.

Material and Methods

Antigen

ZH 548-M 12 strain of RVF virus was kindly provided by Dr. D. H. L. BISHOP (4).

RVF-virus-infected Vero cells were trypsinized 30 hours after inoculation with the virus. Cell suspensions in Eagle's minimal essential medium supplemented with 10% foetal calf serum was allowed to stand at 60°C for 1 hour to inactivate the virus (Dr. G. VAN DER GROEN, personal communication). The cells were washed 3 times with phosphate buffered saline (PBS) and then

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3—4×10⁴ cells in 10μl volumes were dispensed in each well of a slide (12 wells per slide). Based on indirect immunofluorescence observations, 10—20% of the cells were usually infected.

Indirect immunofluorescence antibody test

10μl of diluted sera were placed on each well and kept for 45 minutes at 37°C. After 3 times washing with PBS, 10μl of fluorescent-isothiocyanate conjugated anti-human Ig or bovine Ig rabbit serum (Cappel Co. Ltd.) at the dilution of 4 staining units, were placed in each well and incubated again for 45 minutes at 37°C. After washing 3 times with PBS, the slide was observed in a fluorescence microscope. If the sample showed specific granular fluorescence in the cytoplasm of the infected cells, it was considered a positive reaction. Antibody titres were expressed as the reciprocal of the dilution of the test serum. Titres of more than 10 were considered as positive.

Sera

Human sera for the study were collected from Lusaka abattoir personnel in May 1987 and from Mazabuka residents in November 1986 and were kindly provided by Prof. S. FALADE and by Mr. T. MIYAZAKI and Miss Y. OZAKI, respectively. Bovine sera from Mazabuka were collected in November 1986 and February 1987 and were made available by Dr. R. KITADA and Dr. F. HASEBE.

Results

1. Prevalence of RVF antibodies in abattoir personnel in Lusaka: As shown in Table 1, 5 out of 53 workers in the abattoir dealing with cattle were seropositive. However, none of the workers in abattoirs dealing with pigs was shown to be positive. Case No. 5 of positives has been working in the abattoir for only 5 months and showed a lower titer of antibodies. All other positives had been working in the abattoir for more than 9 years (Table 2).

Table 1. Prevalence of Rift Valley Fever Antibody in Abattoir Workers in Lusaka

Abattoir	Animal Species	Number tested	Number of positive	%
A	Bovine	53	5	9.4
B	Swine	40	0	0

Table 2. Five positive cases in the abattoir of Lusaka

No.	Section	Sex	Age	Duration of Employment (in years)	Titre of serum
1	Slaughter	M	36	9	80
2	Slaughter	M	38	17	80
3	Slaughter	M	52	27	40
4	Slaughter	M	53	23	80
5	Meat processing	F	27	5/12	20

2. Prevalence of RVF antibodies among Mazabuka residents: The test sera were collected from residents in the rural area of Mazabuka district which is located 120km southwest of Lusaka. As shown in Table 3, positive ratios of males and females were not much different, but positive ratios varied greatly among age groups. Age groups of 25—49, 42—29 and more than 60 years showed distinctively higher positive ratios than other groups. No significant difference in such tendency to seropositivity was observed in age groups of both males and females. The youngest positive case was a 21-years old male (Table 4). In a short questionnaire prepared requesting details of contact with cattle, 13 out of the 19 positives indicated no direct contact with cattle.

Table 3. Prevalence of Rift Valley Fever Antibody in Mazabuka Residents

Age Group	Male Number tested	Male Number of positive	Male %	Female Number tested	Female Number of positive	Female %	Total Number tested	Total Number of positive	Total %
less than 20	11	0	0	16	0	0	27	0	0
20–24	18	1	5.6	8	0	0	26	1	3.8
25–29	23	5	21.7	8	2	25.0	31	7	22.6
30–39	22	0	0	10	1	10.0	32	1	3.1
40–49	10	1	10.0	11	3	27.3	20	4	20.0
50–59	8	0	0	6	1	16.0	14	1	7.1
more than 60	10	4	40.0	7	1	14.3	17	5	29.4
Total	102	11	10.8	65	8	12.3	167	19	11.4

Table 4. Nineteen positive cases in Mazabuka residents

No.	Sex	Age	Occupation	Contact with Meat Processing	Contact with Aborted calf	Titre of serum
1	M	21	Farmer	+	+	20
2	M	25	Driver	–	–	40
3	M	26	Villager	–	–	20
4	M	25–30	Farmer	+	+	20
5	M	29	Farmer	+	–	80
6	M	30	Farmer	–	–	80
7	M	47	Farmer	+	+	80
8	M	60	Farmer	+	+	20
9	M	60	Farmer	–	–	160
10	M	66	Farmer	–	–	320
11	M	75	Farmer	+	+	80
12	F	28	Housewife	–	–	40
13	F	28	Housewife	–	–	20
14	F	30	Student	–	–	80
15	F	40	Housewife	–	–	80
16	F	46	Housewife	–	–	80
17	F	48	Housewife	–	–	80
18	F	55	Housewife	–	–	80
19	F	65	Housewife	–	–	20

3. Prevalence of RVF antibodies in cattle in the Mazabuka area: On testing cattles sera obtained from Mazabuka, none of 133, 73 and 6 cattle of age groups of 1–6, 7–9 and 10–12 years, respectively, were positive for RVF.

Discussion

From the presently obtained data, transmission of the RVF virus to abattoir personnel might have occurred by direct contact with infected cattle. Evidence to support this, came from the observation that none of the personnel working in the abattoir dealing with pigs was found positive for RVF. Other means of transmission such as by mosquitoes could not be ruled out, since in the present study more than half of the RVF positive persons in Mazabuka area denied any direct contact with cattle. In addition, no significant difference

was noticed in the percentages of positives among females and males, bearing in mind that cattle rearing in Zambia is a job mainly restricted to men. However, such arthropod transmission of RVF to man has not been suggested by WATTS et al. (5) who noted that the prevalence of antibodies to RVF is higher in people dealing with cattle and their products such as butchers, herdsmen and kitchen workers.

Rift Valley fever was reported to recur in the African continent every 5—15 years (6). This is supported, in the present study by the great variation of positivity to RVF among different age groups (Table 3). These variations might have some degree of correlation with epizootics of RVF. According to HUSSEIN et al. (1), RVF was reported in Chisamba area, parts of the Copperbelt and Mazabuka area during 1974 and recurrence of the disease was reported in Chisamba area in 1976. However, in the present study, higher positivity occurred in persons who had been working in abattoirs for more than nine years. Again, residents in Mazabuka area who showed positivity to RVF were those aged 25—29 and 40—49 years (of age). This later observation might suggest an earlier outbreak of RVF in addition to the 1974 epizootic.

Interestingly, traditional cattle included in the study and aged more than 10 years were all negative. This could possibly be attributed to tolerance of these animals to infection with RVF since the disease was reported in the area about 10 years ago. Similar observations were made by HUSSEIN et al. (1) in their retrospective study of cattle in the traditional sector in the Chisamba area only 3—5 years after on outbreak of RVF in the same area.

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Zusammenfassung

Auftreten von Rift Valley Fever in Lusaka und Mazabuka, Zambia

Fünf von 53 Arbeitern aus einem Rinderschlachthof in Lusaka hatten Antikörper gegen Rift Valley Fever (RVF)-Virus. Keiner von 40 Arbeitern eines Schweineschlachthofes war seropositiv. Dies läßt darauf schließen, daß eine Übertragung des RVF-Virus durch unmittelbaren Kontakt mit infizierten Rindern möglich ist.

Im Mazabuka-Gebiet waren 19 von 167 Einwohnern seropositiv auf RVF-Virus: 13 von diesen Personen hatten keinen früheren Kontakt zu Rindern. Es ist deshalb möglich, daß die Krankheit in ländlichen Gebieten auch durch Moskitostiche übertragen wird.

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Short Communication

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Seroepidemiological Survey on Rift Valley Fever in Zambia

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With 2 tables

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Summary

This study was carried out to define the role of cattle as an amplifier of Rift Valley fever. Three areas of different density of cattle population were surveyed. Cattle do not seem to play a significant role as an amplifier of the virus in human beings.

Key words: Rift valley fever, Zambia, epidemiology

Rift valley fever (RVF) is one of the most important zoonotic diseases in sub-suharan Africa, and the transmission of the infection to human is thought to occur through direct contact with infected animals. In a previous study of RVF in Mazabuka area of Zambia, the infection due to mosquito bites was made to be important (1). In this study, an attempt to clarifiy the role of cattle as an amplifier in the spread of the virus to human has been made.

Human serum samples were collected from residents aged between 5 to 80 years in Luanga, Kabwe and Namwala districts which had different density of cattle population (2). A questionnaire survey was also included to determine the extent and mode of contact with cattle in Kabwe and Namwala areas.

Serum samples were examined by indirect fluorescent antibody technique (1). Prevalence rates of RVF antibodies in residents are shown in Table 1 and individual data on the nine positive cases are shown in Table 2. The youngest case was that of 17-year-old female in Namwala area. Thirty-three people aged below 16 years from three different areas were all negative for RVF. Two out of 6 positive cases from Kabwe and Namwala areas had no direct contact with cattle (Table 2). There was no significance in the prevalence rates of RVF antibodies in residents in these three areas having different density of cattle population. Therefore, it seems unlikely that cattle play a significant role as amplifier of the RVF virus. It would appear that the outbreak might have occurred more than 10 years ago

Table 1. Prevalence of Rift Valley Fever Antibody in Residuents and Density of Cattle in Luanga, Kabwe and Namwala District

District	No. of tested	No. of positive	%	Density of Cattle (per 10 km²)
Luanga	141	3	2.1	Less than 1,000
Kabwe	119	3	2.5	2,000
Namwala	84	3	3.6	3,000

Table 2. Individual Data on the Nine Positive Cases

No.	District	Sex	Age	Occupation	*Contact with* meat processing	Aborted fetus	Titer of serum
1	Luangwa	F	20	Gardener	?	?	20
2	Luangwa	F	40	Gardener	?	?	40
3	Luangwa	M	50	Office orderly	?	?	320
4	Kabwe	F	A*	House wife	+	–	20
5	Kabwe	F	35	House wife	+	–	80
6	Kabwe	F	A	House wife	+	–	20
7	Namwala	M	32	Office orderly	+	+	320
8	Namwala	F	17	Student	–	–	40
9	Namwala	F	30	House wife	–	–	80

* Adult

judging from the age distribution. The previous survey of RVF in residents in Mazabuka in 1987 (1) showed the prevalence rate of 11.4 % which seems to be remarkably high in comparison with the present survey. Mazabuka area differs as a vast sugarcane field and its irrigation system when compared to other areas. Although the correlation between the satellite-derived green vegetation index and ecological pareameters associated with RVF was revealed in Kenya (3), the relationship between sugarcane plantation and mosquito population in this study remains obscure. More intensive studies is to be done to clarify the different prevalence of antibodies against RVF between Mazabuka and other 3 areas.

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Zusammenfassung

Eine seroepidemiologische Untersuchung von Rift Valley Fever in Zambia

Diese Untersuchung wurde ausgeführt, um die Rolle der Rinder als Träger von RVF festzustellen. Drei Gebiete mit unterschiedlichen Rinderbevölkerungen wurden untersucht. Es scheint, daß Rinder keine bedeutende Rolle bei der Verbreitung des Virus unter Menschen spielen.

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Short Communication

DISTRIBUTION OF RIFT VALLEY FEVER AMONG CATTLE IN ZAMBIA

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SUMMARY: In the present study, 1,421 cattle in 32 herds within nine districts, which are important cattle-producing centers in the nine provinces of Zambia, were tested for Rift Valley fever by the indirect immunofluorescence assay. One hundred and forty-seven cattle (10.5%) in 28 herds (88.9%) in the nine districts tested were positive for Rift Valley fever implying a country-wide distribution. In districts associated with flood plains and/or "dambos" (low lying areas of perpetual flooding), high herd and individual positive rates (100% and >10%, respectively) were found, suggesting a significance of these features in the distribution of the disease.

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Rift Valley fever (RVF) is an acute, febrile, arboviral, zoonotic disease that causes high rates of abortion and neonatal mortality, primarily in sheep, goats and cattle (1). In Zambia the disease was first reported in 1974 when an epizootic involving cattle and sheep occurred in the Chisamba (Central Province) and Mazabuka (Southern Province) districts and in some parts of Copperbelt (2). Since then, several epizootics have occurred in the same areas (2,3). The disease in humans, involving deaths, has also been reported (4). All these reports indicated that the disease may be more or less endemic in the above-mentioned areas and could be contributing to the low livestock productivity and public ill-health in Zambia.

Although seroepidemiological studies have been conducted in the past, these studies have tended to be limited to the areas of repeated RVF occurrences and have, therefore, not indicated how widely the disease is distributed in Zambia. This article reports the results of a seroepidemiological study conducted in various parts of the country to determine the disease's country-wide distribution, factors influencing this distribution as well as to gain an insight on its possible impact on cattle productivity in the affected areas.

Between January 1990 and March 1991, serum samples were collected from commercial and emerging herds in nine districts (Fig. 1) representing important cattle-rearing centers in the nine provinces of Zambia. One district in each province was sampled. Sample herds were selected on the basis of their accessibility, the likely level of cooperation by the owners or managers and the likely availability of animal health and other relevant records. Information regarding the vaccination status of the herd, its reproductive performance and natural surroundings, was obtained from the owners or managers by use of appropriate questionnaires. All vaccinated herds and individual cattle were excluded from the study. The sera were kept at −20 C until required for testing.

The antigens and indirect immunofluorescence assay used in the present study were as described by Morita (5). In brief, Vero-E6 cells infected with ZH548-M12 strain of RVF virus were used as antigen. The sera showing titers higher than 1:16 against the antigen were considered as positive. Table I shows the districts sampled, the numbers of herds and individual cattle within these herds tested in each district and the results of the testing. A total of 1,421 individual cattle in 32 herds were tested for RVF in all the nine sample districts. All the tested herds in six districts and five out of six herds in Choma, three out of six in Chingola, two out three in Chipata districts were positive to RVF. Altogetehr, 147 individual cattle (10.5%) in 27 herds (88.9%) tested were positive for RVF.

Fig. 1. Map of Zambia. Sampling locations are shown by asterisks.

These herds or individual cattle had, reportedly, never been vaccinated against RVF in the past.

Field observations as well as farmer responses indicated that the tested herds in Kabwe, Lusaka, Solwezi, Mongu and Mansa districts were located adjacent to flood plains and/or dambos and grazed in and around these geographic features. (Dambos are shallow streamless depressions that can be seasonally waterlogged and are grass-covered) (5a). All the positive herds had a history of abortion and still-birth although they had annual vaccination programs against bovine brucellosis.

Zambia is composed of nine provinces and the current study included at least one district from each province. These districts are important livestock production centers in these provinces although the productivity is low (6). The results indicate that RVF exits in all the districts studied, implying that the disease may have a country-wide distribution. The results also indicate high positive rates (100% herd rate and greater than 10% individual cattle rates) in the Kabwe,

Table I. Distribution of Rift Valley fever among cattle in Zambia. Results of a seroepidemiological study in nine districts

District	Number of herds tested	Number positive	% herds positive	Number of cattle tested	Number of cattle positive	% cattle positive
Kasama	1	1	100	30	1	3.3
Mansa(d)	1	1	100	198	24	12.1
Chipata	3	2	66.7	162	2	1.2
Chingola	6	3	50	202	11	5.4
Solwezi(d)	2	2	100	181	25	13.8
Kabwe(d)	6	6	100	215	24	11.2
Lusaka(d/fp)	1	1	100	15	3	20.0
Mongu(fp)	6	6	100	206	47	22.8
Choma(d)	6	5	83.3	212	10	4.7
Total	32	27	88.9 av.	1.421	147	10.5 av.

av.: average, d: dambos, fp: flood plain.

Lusaka, Solwezi, Mongu and Mansa districts. These districts are characterized by the presence of either large flood plains and/or dambos and the positive herds were located adjacent to and grazed within and around these features. The results, therefore, suggest that these geographic features may be significant in the distribution and epidemiology of the disease in this country. Previous publications on the status of RVF in the Chisamba, Mazabuka and Lusaka areas expressed the belief that the disease is endemic in these areas (2,5,7,8). The results of the present studies seem to strengthen the previous reports and suggest that RVF could be endemic throughout most of the cattle producing parts of the country. Any future attempt to study the epidemiology and impact of the disease in Zambia should, therefore, involve samples that would represent the whole country.

The objective of this study was to determine the distribution of RVF in Zambia as well as factors influencing the disease distribution. The results indicate that the disease may have a country-wide distribution, that the presence and

proximity of flood plains and/or dambos to cattle-producing areas could be significant to the disease's distribution in those areas, and that the disease may be significantly contributing to the low productivity of cattle in Zambia. Finally, the results suggest that the disease may be endemic in most cattle-producing parts of the country.

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AFRICAN SWINE FEVER IN ZAMBIA: POTENTIAL FINANCIAL AND PRODUCTION CONSEQUENCES FOR THE COMMERCIAL SECTOR

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ABSTRACT

The first officially recorded outbreak of African swine fever (ASF) in Zambia was in Eastern province in 1965. The disease now covers almost the whole province and is endemic in the indigenous breeds. In 1989, an outbreak of ASF occurred on a commercial property in central Zambia for the first time and was eradicated by depopulation. In order to examine the justification of the drastic control measures and the continued ban on the export of pigs and their products, the impact of the outbreak on the affected property as well as the potential consequences on the commercial pig sector in the district was assessed in the present study. The affected property lost 421,238 Zambian Kwacha (ZK) (US$39,965) as a result of the outbreak and control measures. However, the cost to the district could have been at least ZK14,917,500 (US$1,415,323) if the measures had not been effected. Furthermore, not taking such measures would have increased the risk to the entire commercial pig sector along the line of rail in urban centers.

Key Words: African swine fever, porkers, litters, down time, depopulation, Zambian Kwacha.

INTRODUCTION

The first officially recorded outbreak of African swine fever (ASF) in Zambia was in Chipata and Katete districts of Eastern province in 1965 in "exotic" pigs[1] (Fig. 1). Reports by Wilkinson[5,6], Wilkinson et al.[7] and Mwanaumo et al.[3] have indicated that

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ASF is now endemic in indigenous pig breeds almost throughout the province (Fig. 1). Approximately 300,000 pigs, divided into the commercial (about 36,000) and traditional (about 264,000) sectors[2], are raised in Zambia. The former comprises mainly the Large white and Landrace breeds and their crosses, and is located mainly around urban centers, while the later is mostly made up of indigenous breeds. The Eastern province supports over 66% of the traditional pig population.

The presence of ASF in Eastern province and its devastating effect on commercial pig breeds has led to a long standing ban (since 1965) on the export of pigs and their products from the province to the rest of the country, thus drastically reducing the source and quantities of these products especially for the urban communities. The Luangwa river, where there is a permanent check point, and the Muchinga escarpment serve as natural barriers on the Southern, Western and North-western borders of the province, respectively. To offset this shortfall, livestock development policy has been directed, among other things, towards developing the commercial pig sector. The validity of this continuous quarantine has, however, been frequently questioned. Political leaders have openly condemned the continued existence of the ban and used it in their campaigns.

In May, 1989, the first outbreak of ASF outside the endemic province was

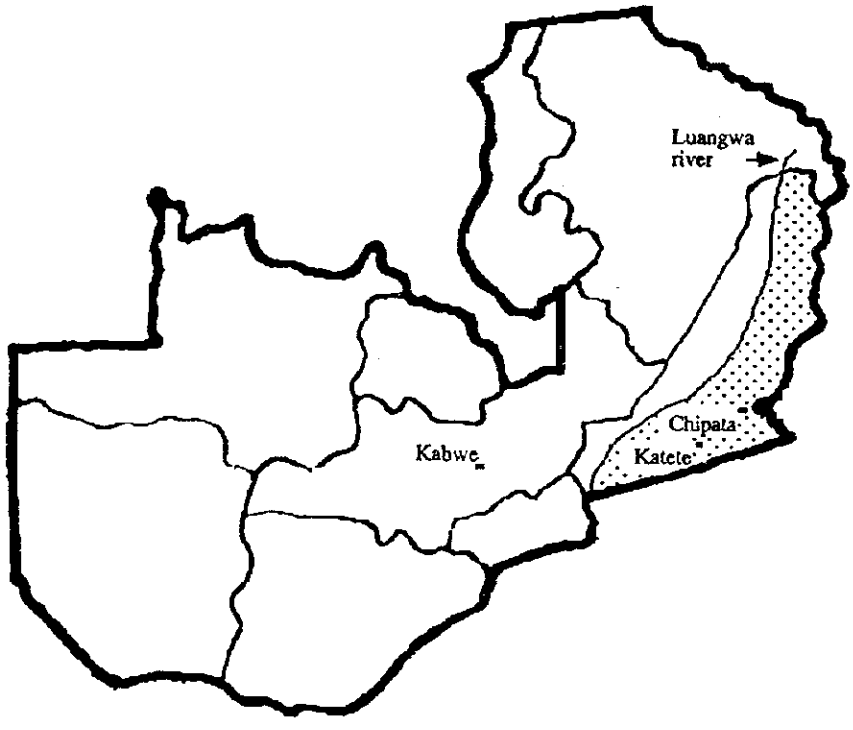

Fig. 1. Map of Zambia showing Kabwe where the ASF outbreak occurred. The shaded area shows part of Eastern province where ASF is endemic.

recorded on a commercial property in Kabwe urban district of Central province[4]. There were initially 164 pigs including 13 that comprised the parent stock on the affected property. The outbreak was eradicated by depopulation of the affected herd, a method which farmers complained about. At the time of the outbreak, there were approximately 10,000 commercial pigs in the district and at risk of infection. This article justifies the control methods used for eradication and subsequently, the continued quarantine of pigs and their products in the Eastern province. This is achieved by assessing the financial impact of the outbreak on the affected property and the potential consequences on the whole commercial pig sector in the district if the measures had not been effected.

Materials and methods

Probably the most appropriate way of justifying the use of these drastic measures is to assess the financial impact of the outbreak on the affected property and subsequently, the potential impact on the whole commercial pig sector in the district and then compare them (i. e. some kind of cost-benefit analysis). The difference could then be regarded as the benefit (if any) of implementing the measures. The financial impact of the outbreak on the affected property was assessed based on the following parameters collected during the course of investigating and controlling the outbreak:

1. The loss of 13 breeding pigs plus the loss of income from potential litters;
2. The loss of 151 porkers;
3. The cost of treatment drugs, disinfectant and diesel used to burn the infected and contaminated carcasses;
4. Loss due to down-time.

All estimates were carried out using 1989 commercial prices and foreign currency exchange rate (US$1 = ZK10.54) and the reported production cost of approximately 25% of the earnings.

The loss of 13 breeding pigs and loss of income from potential litters

At the time of the outbreak, the average weight of the breeding sows and the boar was approximately 120 and 210 kilograms (kg), respectively. Thus 1650 (120 kg x 12 + 210 kg) kg or ZK38,610 (US$3,663.19) was lost due to death of the breeding pigs. In Zambia, the average weight of a piglet at weaning is 20 kg. Since piglets are more likely to be sold after weaning than before, the weight at weaning is used to estimate their monetary value at that age (ZK468). In Zambia a sow is expected to litter on average twice per year with an average live litter size of 9 piglets at weaning. As the sows died in May, it is assumed that one litter was lost to each of the 12 sows, i. e. 108 piglets with a total weight of 2,160 kilograms. Thus ZK50,544 (US$4,795.45) was lost through loss of potential litters.

The loss of 151 porkers

In addition to the parent stock, the property had 151 porkers with a total liveweight of 5,828 kg. These either died naturally of the disease (21 pigs) or were destroyed (130 pigs). This meant a gross loss of ZK136,375.20 (US$12,938.82). When reduced by 25% (the reported approximate production cost), there was a net loss of ZK102,281.40 (US$9,704.12).

The cost of treatment drugs, disinfectant and diesel

At the beginning of the outbreak, swine erysipelas was suspected and hence the breeding pigs (the first to show clinical symptoms) were put on a 3 day penicillin course costing ZK420. After laboratory confirmation of ASF, some 420 liters of diesel costing ZK821 were used to destroy the contaminated carcasses and 40 liters of disinfectant costing ZK3,257 to decontaminate the pens. The total cost of all these activities was ZK4,538 (US$431).

Loss due to down-time

Finally, as part of the eradication measures, the affected property was ordered not to restock for at least one year (June 1989 through May, 1990). Assuming that during this time, another herd of at least 151 porkers could have been raised to market weight (85 kg), the net and gross down-time loss could be ZK300,339 (US$28,495.16) and ZK225,254.25 (US$21,371.37), respectively.

Estimating the Potential Impact of ASF on the Commercial Pig Sector in the District

This was estimated using the 1989 district commercial pig population of approximately 10,000, the average market weight of 85 kg, the price per kg of ZK23.40 (US$2.22) in 1989, and the reported cost of production of 25% (0.75) of the generated income (i. e. 10,000 x 85 x $2.22 x 0.75). The average market weight was used in the estimation due to non-availability of the district herd inventory information. Thus the resultant estimate was very conservative and could be much higher.

RESULTS

The total loss due to death of the parent stock and loss of potential litters was ZK89,154 (US$8,459). ZK102,281 (US$9,704) was lost due to the loss of 151 porkers; ZK4,538 (US$431) due to treatment and decontamination and, ZK225,254 (US$21,371) due to down-time. Thus the innumerable financial impact on the affected property totaled ZK421,227 (US$39,965) (Table 1). Assuming that the destroyed 130 porkers were to die from ASF later, the total loss by the property due to the control measures (items 3 and 4) alone was ZK310,487 (US$29,458).

The net market value of the commercial pig population in the district was estimated at ZK14,917,500 (US$1,415,323) (Table 2). This is also regarded as the potential impact of ASF on the commercial sector in the district if the disease was allowed to establish itself by not implementing the drastic control measures. Thus the benefit to the district, of implementing the drastic measures at a cost of US$39,965 was US$1,375,358 (or approximately US$1.4 million).

Table 1. Estimated financial impact of ASF on the affected property.

Parameter	Number	Pig lost	Estimated total liveweight (Kg)	Estimated value (US$)
Parent stock	13	13	1,650	3,663.19
Potential litters	9	108	2,160	4,795.45
Porkers	151	151	5,828	9,704.12
Drugs & diesel	—	—	—	431.00
Down time	151	151	12,835	21,371.37
Total				39,965.13

Table 2. Potential impact of ASF on the district commercial sector and approximate benefit from implementing depopulation and quarantine measures.

Parameter	Number	Estimated liveweight (Kg)	Estimated net value (US$)
District population	10,000	850,000	1,415,323
Estimated impact on affected property	—	—	39,965
Approximate benefit	—	—	1,375,358

Discussion

For ASF, depopulation and quarantine are currently the only available control measures. Experience in Eastern province[1)] has shown that once established, the disease spreads very rapidly in an area, aided by uncontrolled human movement in and out of the foci of infection to other piggeries as well as the proximity of these piggeries to the foci and each other. It is, therefore, possible that the disease could have quickly spread in the district, as there were several piggeries in the vicinity of the infected property (the nearest being just about 6 km away). The case fatality rates observed during the reported outbreak and in commercial pigs in Eastern province before it[1)] was 100%, suggesting that the approximately 10,000 such pigs in the district could have been wiped out or drastically reduced, causing a net loss of US$1,415,323. The supply of pig products to the nation would have been reduced by at least 30%. The benefit from implementing the measures of some US$1.4 million justifies their use as this amount could be equated to Zambia's budget at that time. Furthermore, once established in Kabwe district, no other measures (on a much larger scale) could have prevented the disease spreading rapidly north and south along the line of rail and throughout the entire commercial pig sector thus negating the current

efforts to develop the sector.

From the foregoing, it is clear that the drastic measures, taken to eradicate this outbreak were justified as the benefit of approximately ZK14.7 million or US$1.4 million was so large, under the Zambian economic standards. Lifting the ban on the inter-provincial exports of pigs and their products from Eastern province would be highly detrimental to the nation's concerted efforts to improve the supply of animal protein and reduce the cost of consuming it. The ban should, not only be maintained but strengthened to prevent the spread of ASF from the endemic province.

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The Health and Productivity of Traditionally Managed Cattle in Lusaka Province, Zambia : Results of a Questionnaire Survey

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ABSTRACT

Several studies have, in the past, been conducted on traditional cattle production systems in Zambia. However, little or no information has been collected recently at the national level. This questionnaire survey was conducted from October, 1995 to February, 1996 to describe the present situation regarding animal health and productivity and general farm management and socio-economic parameters in the traditional sector, in Lusaka Province of Zambia. Four veterinary areas; Kafue, Chipapa, Mutamino and Chinyunyu were studied and the results compared between the study areas and to previous studies. The herd health and productivity parameters investigated included average herd structure which consisted of 5% bulls, 36% cows, 14% heifers, 22% oxen and 23% calves; average herd size which was approximately 15; general and calf mortality rates which were 4% and 6% respectively; general fertility - approximately 63%; crude live birth rate - approximately 23%; offtake - approximately 9% and milk production per cow during the dry season which was approximately 1 litre per day on average. Apart from the offtake, all other parameters were quite close in magnitude to those reported in previous studies. The higher offtake in this study could have been due to the severe drought between 1993 and 1995 which resulted in unusually larger cattle sales in the Province. Between the study areas, little or no variation was observed except for the average herd size.

Key words : cattle, questionnaire survey, traditional sector.

INTRODUCTION

Livestock production accounts for about 35% of the total agricultural production in Zambia [12]. There are two types of livestock production systems : the traditional sector and the commercial sector. Eighty percent of the total cattle population in the country belongs to the traditional sector [12]. In terms of volume, approximately half of the total amount of meat consumed in the country is produced by the traditional sector [11]. However, no official figure is available in Kwacha value concerning their productivity. Previous studies have concluded that the traditional sector has a high potential for increasing cattle production and productivity in Zambia [12, 13].

The traditional cattle are mainly of Sanga and Zebu breeds. Offtake rates are low, estimated at 6% [6, 12] to 7% [13]. This may be because cattle have other commercial and social values in the traditional Zambian society. They provide a flow

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of essential food products, sustain the employment and income of rural areas, contribute to drought oxen, ceremonial slaughter, dowry cattle, manure for crop production and the food and cash security to rural populations [12, 13, 14]. Zambia has the potential of increasing its cattle population two to five times as much as in neighbouring countries [8], since a large part of the country has not yet been used for cattle grazing.

Several studies have been conducted on the traditional cattle production systems in Zambia [5, 10, 13]. However, except for the studies on Barotse cattle in Western Province [6, 7], little information has been collected recently elsewhere in the country. This study, therefore, was conducted to describe the present situation in Lusaka Province in terms of animal health, productivity, management and general farm economics.

MATERIALS AND METHODS

Study area and general agricultural activities

Lusaka Province was selected as the study area based on the short travel distance (one day trip) from the University of Zambia. The Province itself consists of a variety of geographically distinct areas. Under such conditions, traditional farmers practice various types of farming. The Province is located in the southern central part of the country. Lusaka city, the provincial headquarters, which is also the national capital, is situated in the western part of the Province. The Province is divided into four districts: Kafue, Chongwe, Lusaka and Luangwa. Traditional cattle are mainly kept in two districts, Kafue and Chongwe with populations of approximately 6,000 and 30,000 respectively [3]. According to the 1990 agriculture census, the total number of agricultural holders (i.e. farmers) has been estimated at 13,305 who grew maize, sorghum, groundnut, sunflower and cotton; 94% (of 13,305) were small scale farmers who had less than 12 acres of agricultural land; 29% were cattle raisers (80% of which have less than 20 cattle); 87%, 24% and 9% reared chicken, goats and pigs respectively [2].

Questionnaire design

The following subject categories were investigated using a questionnaire.

Cattle herd inventory
Other types of livestock
Characteristics of farm owners
Management procedures and general farm economics
Disease outbreaks and control methods
Health and productivity parameters
Use of oxen

The questionnaire was designed, based on the study objectives and on previous authors' experiences with such questionnaire studies in Zambia [4, 6, 13] and Kenya [9]. Mortality, offtake, intake and calving rates were estimated only for the immediate preceding year (i.e. one cow year).

Farmers' selection, survey timing and data analysis

There are five veterinary areas in Chongwe district and two in Kafue district. Two areas in Chongwe (Mutamino and Chinyunyu) were randomly selected while both areas (Kafue and Chipapa) in Kafue district were included as study areas. Lists of cattle farmers in each of the selected veterinary areas were created by updating existing records or through inquiries with local chiefs and village headmen, after which approximately one quarter of farmers in each area were randomly selected for interviewing. The questionnaire survey commenced in October 1995 and was completed in February 1996. A computer software STATISTIX [1] was used to summarise the data.

RESULTS

During the survey a total of 147 farms were visited. This included 35 farms (out of 173) in Kafue, 39 (out of 193) in Chipapa, 32 (out of 128) in Mutamino and 41 (out of 205) in Chinyunyu veterinary areas. These figures represented one fourth to one fifth of all farmers listed in each veterinary area. The collaboration of the farmers was very good and no farmer refused to provide information for the questionnaire survey. Approximately 60% of the interviewees were the actual farm owners. When the owner was absent at the time of visit, information was collected from their wives or children.

Female headed cattle farms

The study results suggest that only a few female-headed farms contain cattle. There were no such farmers in Kafue, 5% (2/39) in Chipapa, 3% (1/32) in Mutamino and 7% (3/41) in Chinyunyu.

Responsible persons when the owner is absent

Of all the farmers interviewed, 50% in Kafue and Mutamino and 30% in Chipapa and Chinyunyu answered that wives were responsible for farm management during their absence, while 46% in Chipapa and 30% in the other three areas answered that sons were given the responsibility. Mothers, brothers and nephews of the owners became responsible only in 5-8% of the farms. One farm had a farm manager and three farmers gave responsibility to their daughters.

Educational level of the farm owner

Educational level of the farm owner was categorised into four groups; none, primary (some years in primary school), secondary (some years in secondary school) and tertiary (further training) as summarised in Table 1. In Kafue and Chipapa, 30% of the interviewees had not attended school at all, while the majority had spent a few years in primary school. Only two farm owners in Kafue veterinary area had been to University, although their main income source was not agriculture. In Mutamino and Chinyunyu, 12% of the farmers had not received any formal education, while the rest

had received some years of primary education. In all areas, approximately one quarter had had the opportunity to commence secondary school education.

Table 1 : Educational levels of the farm owners in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Total
None	11(31.5%)	11(28.2%)	4(12.5%)	5(12.2%)	31(21%)
Primary	17(48.5%)	15(38.5%)	19(59.4%)	26(64.4%)	77(52%)
Secondary	5(14%)	13(33.3%)	8(25%)	10(24.4%)	36(25%)
Tertiary	2(6%)	0	1(3.1%)	0	3(2%)
No. of farms	35	39	32	41	147

Family size and non-family labour

The average number of family members living on the farm was estimated at 13 in all the four veterinary areas (Table 2).

Table 2 : The average family sizes and their 95% Confidence Interval (CI) in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Average (Mean)	12.8	13.0	13.9	12.5	13.0
Lower 95% CI	9.4	9.9	11.1	10.0	
Upper 95% CI	16.3	16.2	16.8	15.0	

Twenty percent of the interviewees in Mutamino and 10% in each of the other areas employed workers mainly for tending vegetable gardens. Several farmers hired neighbours on temporary and seasonal basis for weeding maize fields.

Farm size and crops grown

The average farm sizes (in acres) in the four study areas are shown in Table 3. There is no significant difference in the average size between areas. The average size for all the study areas was estimated at approximately 25 acres.

Table 3 : The average farm sizes in acres and their 95% CI in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Average (Mean)	19.7	19.3	25.3	34.4	24.9
Lower 95% CI	5.3	10.1	17.8	15.4	
Upper 95% CI	34.2	28.6	32.9	53.3	

All the farmers interviewed grew maize with groundnut as the second most common crop. One third of the farmers also grew sorghum, sunflower and cotton. Half of the farms in Mutamino and Chipapa and 30% in Kafue and Chinyunyu produced cash crops such as tomatoes, rape and other vegetables that are usually sold at Soweto Market in Lusaka.

Other income sources

Not all the farm owners interviewed were dependent on farm income. Some ran taverns, others were teachers and civil servants. There were 25% such farmers in Mutamino, 18% in Chipapa and 10% in both Kafue and Chinyunyu.

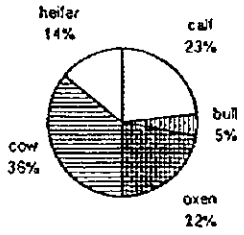
Cattle herd inventory

The herd structure was described by using five categories; bull, cow, heifer, oxen and calf. The total numbers of cattle in each category, by veterinary area, are summarised in Table 4. The average herd structure for the four study areas is depicted in Figure 1.

Table 4 : Total number of cattle and herd structure in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Bull	28(4%)	40(7%)	17(8%)	26(4%)	111(5%)
Cow	267(28%)	193(33%)	78(35%)	242(37%)	780(36%)
Heifer	93(13%)	87(15%)	37(16%)	84(13%)	301(14%)
Oxen	159(22.5%)	141(25%)	48(21%)	135(20%)	483(22%)
Calf	159(22.5%)	114(20%)	46(20%)	167(26%)	486(23%)
Total	706	575	226	654	2161

Figure 1. Average herd structure



Average herd sizes were estimated for each study area and are depicted in Table 5. There was no difference in herd size among three veterinary areas, Kafue, Chipapa and Chinyunyu. However, the average herd size in Mutamino was significantly smaller than in the other three areas. The overall average herd size was approximately 15.

Table 5 : The average herd sizes and their 95% CI in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Average (Mean)	20.2	14.7	7.0	15.9	14.7
Lower 95% CI	11.3	10.6	5.2	9.0	
Upper 95% CI	29.0	18.9	8.9	22.8	

Cattle raising experience in years

The experience of farmers in rearing cattle was also investigated and is depicted in Table 6 as average years per area, of raising cattle. In Kafue, Chipapa and Mutamino these averages were over 20 years. Farmers in Chinyunyu had on average about 14 years of experience, which is lower than in the other three areas. The overall average was estimated at approximately 20 years.

Table 6 : The average years of raising cattle and their 95% CI in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Average (Mean)	20.6	23.0	21.9	13.5	19.5
Lower 95% CI	16.3	17.5	16.3	8.7	
Upper 95% CI	24.9	28.5	27.5	18.2	

Feed/water management and housing

All the interviewed farmers practised communal grazing. Cattle graze within a distance of 1-3 km from the homestead in both the rain and dry seasons. The majority of farmers believed that there was enough forage in their farming areas and could not afford to buy any supplementary feeds for their cattle. Some farmers provided at least salt. Cattle usually go to near-by streams or rivers for water. When there is severe water shortage in the dry season, cattle have to travel for long distances (5-12 km) to reach water sources such as boreholes. The cattle are kept in kraals at night. All farmers have kraals next to or close to their houses. A few farmers own roofed-huts for newly born calves.

Herd health and productivity parameters

Table 7 shows average mortality rates and other farm parameters per area. The general mortality rates ranged from 2 to 6%; the calf mortality rate from 4 to 7 %; general fertility rate from 50 to 74%; crude live birth rate from 20 to 30% and offtake and intake rates from 8 to 10% and 2 to 4% respectively. Several abortion cases were reported. The aborted cases to number of live birth ratio was 1:16 in Kafue, 1:12 in Chipapa, 1:48 in Mutamino and 1:22 in Chinyunyu.

The average milk production per cow in the dry season was approximately one litre per day. Most of the milk produced on a farm is consumed within the household.

Table 7 : The average of herd health and productivity parameters in the four study areas.

Parameters	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
General mortality	3%	6%	2%	5%	4%
Calf mortality	7%	7%	4%	6%	6%
General fertility	49%	66%	74%	70%	63%
Crude live birth	19%	22%	23%	30%	23%
Offtake	9%	8%	10%	8%	9%
Intake	3%	4%	3%	2%	3%

Disease outbreaks and control methods

Nearly 90% of farmers in Mutamino had experienced corridor disease outbreaks. On average, each farmer had lost half of their herds due to these outbreaks between 1992 and 1993. Similar outbreaks had also been experienced in Kafue veterinary area on 74% of the farms, although the losses were not as high as in Mutamino. In Kafue district, blackleg outbreaks were experienced in 1993 and 1994. These were few such outbreaks reported in Chongwe district as well. Trypanosomiasis has been seen only in Chinyunyu veterinary area. Several head of cattle have died of heartwater and babesiosis in all four areas.

None of the public dip tanks in the four veterinary areas were operational at the time of the survey. Three farmers used a diptank belonging to a neighbouring commercial farm while others used sprayers to control ticks. 73% of farmers in Chinyunyu, about 40% in both Kafue and Mutamino and 23% in Chipapa carried out regular tick control. They sprayed/dipped their cattle weekly or twice a month throughout the year. About 25% of the farmers in Kafue and 15% in the other three areas, deworm their cattle once or twice in a year. About 50%, 15%, 7% and 3% of the farmers in Kafue, Mutamino, Chinyunyu and Chipapa respectively keep injectable antibiotics at home for

the treatment of cattle. Farmers do not purchase vaccines for disease prevention. Vaccination was only carried out when blackleg outbreaks were reported.

Use of oxen

Farmers themselves castrate their male cattle when they are 1 to 2 years old. Most oxen are trained as draught animals.

Other types of livestock

Apart from cattle, other types of livestock are also kept on traditional farms. Table 8 shows the number of farms in each veterinary area that rear various types of livestock. It seems that the majority of farms raise poultry, on average about 10 birds per farm. Only one farm in Mutamino raised up to 200 broilers. Goats are second in popularity with 10 animals per farm on average.

Table 8 : Number of farms raising other types of livestock in the four study areas.

	Kafue	Chipapa	Mutamino	Chinyunyu	Overall
Goat	11(31%)	28(72%)	20(63%)	29(71%)	88(60%)
Pig	6(17%)	7(18%)	3(9%)	5(12%)	21(14%)
Poultry	30(86%)	37(95%)	32(100%)	39(95%)	138(94%)
Pigeon	7(20%)	9(23%)	5(16%)	12(29%)	33(22%)
Guinea fowl	6(17%)	16(41%)	6(19%)	12(29%)	40(27%)
Duck	12(34%)	7(18%)	8(25%)	8(20%)	35(24%)
No. of Farm	35	39	32	41	147

Presence of wildlife

Wildlife are seen at half of the farms in Chinyunyu which shares its southern border with the Lower Zambezi National Park. Mutamino has been widely cultivated and only 19% of the farmers have seen wild animals near their farms. In Kafue and Chipapa, 30% of the farmers reported the presence of wildlife such as duiker and wildhog in the vicinity of their farms.

DISCUSSION

This questionnaire survey was conducted as a pilot study to obtain basic background information on which to base and make plans for a future nation-wide research project. The results show a substantial amount of variation between farms within each veterinary area, regarding animal health and productivity parameter, but much less variation between the study areas.

The majority of farmers heavily depended on the agricultural/crop products for their daily lives. They had to grow enough maize to feed their families. In 1995 many farmers sold their cattle for food. Although cattle/livestock production is not the main source of income, it plays an important role as security in case of emergency. Not many farmers have cash incomes and the majority cannot afford to buy expensive supplementary feed and medicines for their livestock. Cattle depend entirely on communal grazing.

Female members of a household are given an equal responsibility in farm management. However, the number of female headed cattle farms was small in the Province. This is due to a traditional custom that when a husband dies, his cattle are given to such close male relatives as sons, brothers or uncles.

The figures in the average herd structure (Fig. 1) are very close to those of Perry et al. [13]. The proportions of bulls, cows, heifers, oxen and calves in the herd in this survey are 5, 36, 14, 22 and 23% respectively compared to 5, 35, 16, 25 and 19% in the survey conducted by Perry et al. [13]. Their figures were averages of seven districts in four Provinces. Therefore, these proportions might be good estimates of the herd structure in the whole country. However, at the farm level, there are various types of herd structures, for example, some farms consisted of only oxen or cows.

The Kafue veterinary area had the largest average herd size of 20 among the four areas (Table 3). The variation in size between farms was also largest in Kafue. One reason may be that there are several Lozi farmers who tend to keep largest sized herds (e.g. more than 100) while practising seasonal grazing in the Kafue flats. Chipapa and Chinyunyu had up to 15 cattle per farm. In both areas, there were a large number of new settlers who had recently moved to these areas. The herd sizes of 7 in Mutamino was the smallest among the four study areas. This may be due to the outbreaks of corridor disease. These average herd size are very close to those in the preliminary farmers lists; 16.4, 14 and 6.7 in Chinyunyu, Chipapa and Mutamino respectively (the value was not available for Kafue veterinary area). This similarity indicates that the sampled farms well represented the reference population.

The average mortality rates in all age groups in the four veterinary areas ranged from 2 to 6%. These rates are close to those of the survey by Perry et al. [13] i.e. 4% in Petauke and Katete, 5% in Mongu and Monze and 7% in Namwala. The calf mortality rate ranged from 4 to 7% in this study while it was 4% in Katete and 8% in Petauke [13]. The study conducted in Western Province reported overall and calf mortality rates of 9% and 21.6% respectively [6]. These rates are much higher than those being reported for Lusaka Province, probably due to the differences in environmental conditions, management, herd size and other factors in the two Provinces.

The general fertility rates ranged from 49 to 74%. These proportions are close to those (44-80%) in the study by Perry et al. [13]. Only 50% of the cows produced calves in the Kafue area. This figure is similar to those reported in Western Province (44% in Perry et al [13] and 55% in Corten [7]. The low calving rate in Kafue could be explained, on a nutritional basis due to the existence of flood plains as Perry et al. [13] did for Western Province. However, more studies are needed for clarification. Relatively high calving rates (approximately 70%) were observed in the other three areas. Abortions are reported in the early (3-4 months) and later stages (8 - 9 months) of gestation. At the time of the survey, the traditional sector in the Province did not use artificial insemination at all and depended on natural breeding. This could lead to increased frequencies of the transmission of some infectious diseases from herd to herd.

Offtake rates in the four areas ranged from 8 to 10%. The commercial offtake rates reported by Perry et al. [13] were 2 to 7% and are a bit lower than those in this study. The main reason may be that farmers in Lusaka Province sold more cattle between 1993 and 1995 in order to buy food for their families. There was severe drought in these areas during this period. It is, thus, necessary to have a long term observation in order to be able to find reasons behind the sale of cattle in the traditional sector.

Government has been charging for veterinary services to farmers since 1988. Farmers now have to pay for all expenses concerning animal health (except when controlling diseases of national importance). It will take some time for them to get used to this new system. However, a small number of farmers in three areas and 50% in Kafue keep injectable antibiotics on the farms and treat their cattle under the supervision of the veterinary assistant. Deworming is not popular probably due to the high price of drugs. When the government stopped organising cattle dipping, a number of diptank committees were created locally wherever a diptank existed. However, after a few years of operation, management and accounting problems stopped further acaricide purchases. Other reasons for the non-functioning of diptanks were physical failures such as cracks in dip tanks and water shortage.

The farming activities of most traditional cattle farmers in Lusaka Province are varied. But less variation was observed between the four study areas. The results indicate that the traditional farmers have difficulties in adopting modern veterinary and livestock rearing practices (e.g. acaricide use in disease control and supply of supplementary feeds).

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SAMPLING STRATEGIES FOR DISEASE STUDIES IN TROPICAL COUNTRIES: EXAMPLES OF MULTI-STAGE CLUSTER SAMPLING AND ITS APPLICATION IN ZAMBIA AND KENYA

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In order to obtain good information on disease parameters, data must be collected at random. Unfortunately, under tropical conditions, the lists of farms and animals required for random sampling are usually not available. It is usually feasible to get lists of larger clusters such as ecological areas and districts, but not smaller elements. In such circumstances, multi-stage cluster sampling is a practical alternative. Essentially, larger aggregates are divided into smaller sampling areas from which the compilation of sampling frames of herds or animals are logistically feasible.
For two-stage cluster sampling, the sample sizes for each stage depend on their relative contributions to the variability of disease occurrence and costs of sampling. However, extensions to multiple stages are more complicated and are often only informally done. Often, the problem can be reduced to a two-stage problem. For example, in the assessment of vaccination coverage in the WHO Expanded Programme of Immunization (EPI), the number of villages to be sampled within an area is estimated and then a fixed number of children per village, from q random starting household are sampled (Lemeshow and Robinson, 1985) . However, in some circumstances, there can be considerable correlation within these chosen clusters, such that a larger number of children per village need to be sampled to estimate rates with a desired precision. There are two potential solutions. One is to first stratify villages into smaller sampling areas to increase the number of household clusters per village and decrease the number of children per cluster (Levy and Lemeshow, 1991). A second method is to randomly sample households. This is not always feasible in larger towns but in our experience is not too difficult for sampling either herds or households in rural settings. The relative number of areas, farms per area and animals per farm in very large herds will then depend on the relative variability and costs of sampling at each level.
In Chongwe District of central Zambia, a two (area and farm) and three (area, farm and animal) stage sampling strategy was used for questionnaire and blood sample collection respectively. Preliminary estimates indicated that farm-to-farm variation was most important (and particularly for small farms), that areas were relatively homogeneous and that the costs of visiting farms once in an area is relatively small. Thus, only 2 of 5 areas but 36 farms per area were sampled. With 3 stage sampling, the price of sampling animals and within herd variability can be incorporated. In general, sampling costs per animal are low so cluster sampling is usually preferred. In Machakos District, Kenya a multi-stage cluster sampling strategy was employed to estimate dog ecology characteristics and collect serum for rabies testing. Again, the small number of dogs per household reduced the problem to a two stage sampling problem. In this case, their were larger area-to-area than farm-to-farm differences so a much higher sampling frequency of areas (60%) relative to farms (>20%) was used.

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PREVALENCE OF ANTIBODIES AGAINST SPOTTED FEVER GROUP RICKETTSIA, MURINE TYPHUS, AND Q FEVER IN ZAMBIA

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La prévalence d'anticorps contre Rickettsia conorii, Rickettsia typhi et Coxiella burnetii a pu être relevée dans plusieurs pays africains. Bien que ces observations confirment la présence de rickettsioses sur le continent africain, aucun rapport sur la situation épidémiologique de cette zoonose en Zambie n'a été publié. La présente étude vise donc à clarifier la prévalence de trois rickettsioses en Zambie chez l'homme (377 échantillons de sérums humains) en utilisant un test indirect d'immunofluorescence. La prévalence des anticorps contre Rickettsia conorii, Rickettsia typhi et Coxiella burnetii a été de 15,9%, 5,0% et 8,2% respectivement. Les taux de séropositivité pour Rickettsia conorii et Coxiella burnetii dans les zones occidentales et orientales du pays ont été plus élevés que dans le nord de la Zambie. Compte-tenu du mode d'élevage plus extensif prévalant dans les zones orientales et occidentales par rapport à la région nord, il est suggéré que ce mode d'élevage constitue un facteur de risque pour l'infestation par Rickettsia conorii et Coxiella burnetii.

INTRODUCTION

The prevalence of antibodies against *Rickettsia conorii, Rickettsia typhi* and *Coxiella burnetii* has been demonstrated in some African countries (1). Although these reports indicate the presence of rickettsioses throughout the African continent, there has been no published report of an epidemiological survey in Zambia. Therefore, this study was designed to clarifythe prevalence of three rickettsioses in Zambia.

MATERIALS AND METHODS

Serum samples were collected from 377 people living innorthern, western, and eastern areas of Zambia. An indirect immunofluorescent antibody test was used for detecting the antibodies against rickettsioses.

RESULTS

The prevalences of antibodies against *R. conorii, R. typhi,* and *C. burnetii* were 15.9%, 5.0% and 8.2%, respectively. The positive rates of antibodies against *R. conorii* in western (23.1%) and eastern (16.8%) areas were significantly higher than that in the northern area (3.0%) of Zambia. The prevalence of antibodies *against C. burnetii* in western (11.8%) and eastern (7.4%) areas was also slightly higher than that in the northern area (3.0%). There was no significant difference among three areas in the distribution of antibodies against *R. typhi.*

DISCUSSION

The results of this study suggest that *R. conorii, R. typhi* and *C. burnetii* are all spread widely in Zambia, although the prevalence of *R. conorii* and *C. burnetii* appears to be especially high in the cattle-breedingareas of Zambia. More intensive studies on the Rickettsioses in Zambia should be pursued.

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Parasitology

Serological Survey on Bovine Anaplasmosis in Zambia

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Bovine anaplasmosis indicates nearly a worldwide distribution except for Northern Europe and NewZealand. In Zambia, it is also one of the important diseases. Diagnosis is usualy done by demonstration of the organisms in the blood smear. As the serological diagnosis, CF test, Agglutination test, FAT and ELISA have been reported. In Zambia, fatal cases have sometimes been observed, but no surveys have been carried out on the extent of prevalence of this disease. Recently, the authors carried out some surveys on prevalence of this disease by using CF test.

Materials and methods

A total of 121 bovine sera were collected from the farm of Natural Resources Development College (NRDC) and Kaleya farm (Commercial farm) in which the clinical disease occured. The other sera were collected from two traditional farms (Wilson Munkonawe's farm and Farm No. 100). Sera were inactivated at 56°C for 30 min and examined by the micro-plate test. The test antigens were obtained from the National Institute of Animal Health of Japan. The positive reactions were decided by the 100% inhibition of hemolysis and the titers of 1:5 and over were regarded as infected.

Result

Positive ratios ranged from 15.8% to 53.3% making an average ratio of 38.0% (46 positive cases out of 121 cattle, Table 1). Titers ranged

from 1:5 to 1:20, mostly 1:10 (Tables 1 and 2). The relations between positive cases and age were not significant (table 2). Though 531 of blood smears from 11 commercial farms were examined, A. marginale was detected only in Kaleya farm and positive ratio in this farm was 18% (9 positive cases of 50). The positive ratio (by CF test) of traditional farms were very high (Table 1). From the above described data it is assumed that the disease is widely distributed among cattle in Zambia.

Further survey for diagnosis of anaplasmosis is needed to examine correlation between the detection of the organisms in blood smears and CF titers, rise and fall, moreover persistance of antibodies by using artificially and naturally infected cattle.

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Table 1. Distribution of CF antibodies to A. marginale by farm

Names of farm	No. of sera tested	Positive (%)
NRDC farm	42	15(35.7)
Kaleya farm	19	3(15.8)
Wilson Mukunawe's farm	30	16(53.3)
Farm No. 100	30	12(40.0)
Total	121	46(38.0)

Table 2. Distribution of CF antibodies of the cows grouped by age

Age	No. of sera tested	CF titer				Positive(%)
		<5	5	10	20	
2	5	3	1	1	0	2(40.0)
3	15	9	0	5	1	6(40.0)
4	17	12	1	4	0	5(29.4)
5	12	9	0	2	1	3(25.0)
6	6	2	1	3	0	4(60.7)
7	9	5	0	4	0	4(44.4)
>7	15	8	0	7	0	7(46.7)
Total	79	48	3	26	2	31(39.2)

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V. Orino and K. Shimizu

SEROLOGICAL SURVEY OF TOXOPLASMOSIS

Eighty sera from workers at the abattoir and 137 pigs were examined with the toxoplasma diagnostic kit. 9 out of 40 human sera at the pig's abattoir were positive, but 5 out 40 at the cattle's abattoir showed positive reactions. In case of the pigs, 18 out of 137 were positive (13.1%).

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13th July, 1988

RESULTS OF TOXOPLASMOSIS IN HUMAN SERA AT THE SLAUGHTER HOUSE

Slaughter house	No. of tested sera	Negative (%)	Positive (%)
Pig's slaughter	40	31(77.5)	9(22.5)
Cattle slaughter	40	35(87.5)	5(12.5)
Total	80	66(82.5)	14(17.5)

RESULTS OF TOXOPLASMOSIS IN PIGS

Farm	No. of tested sera	Negative (%)	Positive (%)
Mkumba farm	31	30(96.8)	1(3.2)
Menaba farm	26	25(96.1)	1(3.2)
Kalangua farm	40	38(95.0)	2(5.0)
Muyeko farm	40	26(65.0)	14(35.0)
Total	137	119(86.9)	18(13.1)

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Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle

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ABSTRACT

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Four populations of *Trypanosoma congolense* and *Trypanosoma brucei brucei* were isolated from cattle under different management practices and environments in Zambia. All four isolates had varied responses to both diminazene aceturate (Berenil®) and isometamidium chloride (Samorin®) as curative drugs in infected mice. Trypanosomes from a traditionally managed herd in a high-tsetse-challenge area had the strains most resistant to Berenil, with maximum curative dose of 45 mg kg^{-1} body weight. Another isolate from a high-tsetse-challenge area was evidently resistant both to Berenil at 40 mg kg^{-1} and to Samorin at 4 mg kg^{-1}. The strains most susceptible to both Berenil and Samorin were from a commercially managed herd of cattle under medium tsetse challenge. They responded to recommended cattle standard doses of 3.5 mg kg^{-1} or 7 mg kg^{-1} Berenil and to as little as 0.25 mg kg^{-1} Samorin.

It is evident that trypanosome strains resistant to Berenil and/or partially resistant to Samorin exist, and that both *T. congolense* and *T. b. brucei* are implicated.

INTRODUCTION

Trypanosomiasis is one of the most serious diseases affecting cattle productivity in Africa. In Zambia, over 60% of the 2.5 million herd in the traditional farming sector, which constitutes about 80% of the total national herd, and a smaller proportion in the commercial farming sector, are at risk of the disease (Mumba and Chizyuka, 1987). Economically important animal infective species, *Trypanosoma (Duttonella) vivax*, *Trypanosoma (Nannomonas) congolense*, and *Trypanosoma (Trypanozoon) brucei brucei*, all co-exist in the field.

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Since 1916, besides control of the vector tsetse fly (*Glossina* spp.), greater emphasis has been put on the need to control the disease in cattle by trypanocides (Mumba and Chizyuka, 1987). Prolonged irregular treatments or indiscriminate use of trypanocides can cause an ideal condition for the development of drug-resistant strains (Fairclough, 1963a,b; MacLennan and Jones-Davies, 1967; MacLennan and Na'isa, 1970; Gray and Roberts, 1971; Wilson et al., 1975; Leach and Roberts, 1981; Tacher, 1982; Rottcher and Schillinger, 1985; Trail et al., 1985; review, Gardiner, 1989). The existence of drug-resistant trypanosomes is also well documented (Fairclough, 1963a,b; Mwambo et al., 1988; Zweygarth and Rottcher, 1989; review, Gardiner, 1989). In Zambia, Joshua (1987) isolated *T. congolense* and *T. b. brucei* strains from cattle which were resistant to recommended cattle therapeutic doses of 3.5 mg kg^{-1} or 7 mg kg^{-1} for diminazene aceturate (Berenil®) and to 0.5 mg kg^{-1} for isometamidium chloride (Samorin®) in mice.

In spite of efforts to control the disease in cattle by trypanocides, not much has been done to assess their efficacy and impact on trypanosomes in cattle under various trypanocide treatment regimens. The advent of a Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) covering about 8000 km^2 in Zambia (review, Jordan, 1985) necessitates extensive field investigations, in order to establish the extent of trypanosomiasis prevalence and the existence of drug-resistant trypanosomes in cattle, as a prerequisite to wide-scale application of trypanocides.

The present study was carried out to determine curative doses for Berenil and Samorin in mice infected with four trypanosome isolates from cattle reared under different management practices and environments in Zambia.

MATERIALS AND METHODS

Trypanosome stocks

Representative trypanosomes were isolated from cattle in herds located at four different areas (Fig. 1). Each herd was screened for trypanosomiasis by examining wet blood film preparations on site using a field microscope and ×400 magnification (Olympus, Japan). Syringes (1 ml) precharged with 0.5 ml phosphate glucose saline (PGS) were used to draw blood from ear veins of all parasitaemic cattle. Aliquots of 0.5 ml pooled blood were intraperitoneally inoculated into 10 inbred albino mice. Infected mice were monitored by tail clip wet blood film preparations under a phase contrast microscope (Olympus, Japan). Trypanosomes were harvested from parasitaemic mice and stored at −190°C in liquid nitrogen according to the method outlined in the ILCA manual (Murray et al., 1983) before being brought to Japan.

The Chipata isolate came from a typical traditionally managed herd in a trypanosomiasis-endemic area with high tsetse challenge located near the

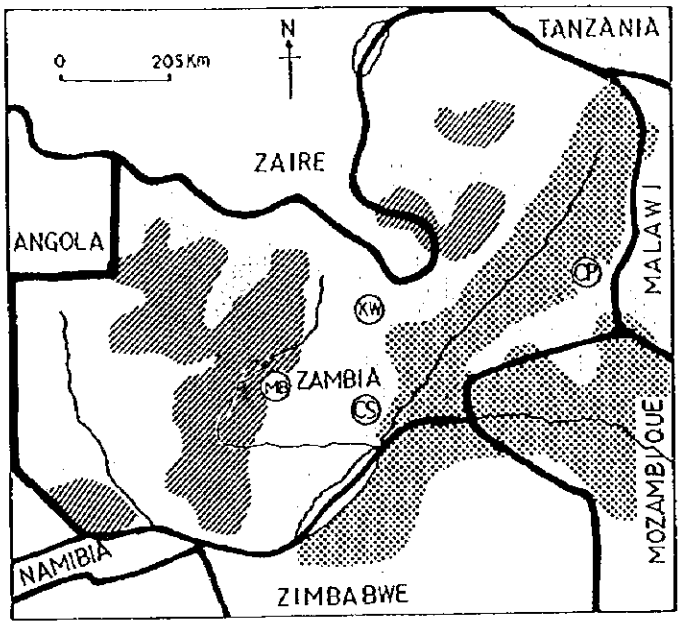

Fig. 1. Map of Zambia, showing areas from which trypanosome stocks were isolated within the Regional Tsetse and Trypanosomiasis Control Programme Area: CP, Chipata; CS, Chisamba; KW, Kabwe; MB, Mumbwa. Thick lines, international boundaries; stippling, common tsetse fly belt; shading, other major tsetse fly belts.

Luangwa river basin tsetse fly belt, Eastern Province. This herd relied entirely on the national trypanosomiasis control programme.

The Chisamba isolate came from a commercially managed herd in Lusaka Province under medium tsetse challenge with high trypanosomiasis prevalence. This herd was on regular prophylactic (Samorin) and therapeutic (Berenil) treatments.

The Kabwe isolate was obtained from a semi-traditionally managed herd located in the Central Province. It had neither a long history of trypanosomiasis nor drug use. Oxen for draft power and trypanocidal drugs have recently been introduced in this herd through a donor-agency-sponsored scheme.

The Mumbwa isolate was obtained in 1987 as a cryostabilate from the Central Veterinary Research Institute (CVRI), Lusaka, Zambia, for teaching purposes. It was originally isolated from Mumbwa district near the Kafue river basin tsetse fly belt, Western Province. No information on the farm type nor history of drug use was obtained for this herd.

Mice

Type Jla:ddY (20–30 g) purchased from the Japan Laboratory Animal Co., Inc. (Nerima, Tokyo, Japan) were used in the investigations. Mice were

housed in a fly-proof isolation building. Water and commercially purchased pellet feed were provided ad libitum.

Drugs

Berenil (Hoechst, Lot 467 U 682) and Samorin (May and Baker, DX 0026/58/49/00-2) were commercially purchased from Zambia. The drugs were stored at 4°C in powder form, and prepared by dissolving the required quantities in sterile distilled water just before use.

Experimental procedure

Four groups of 10 mice, one group for each isolate, were intraperitoneally inoculated with 0.2 ml pooled parasitaemic blood in PGS, bled by cardiac puncture from anaesthetized donor mice at high parasitaemia. Infected mice were monitored daily for the onset of parasitaemia and for 2 to 3 days after onset. Mice with 10^4 to 5×10^5 trypanosomes ml^{-1} of blood, estimated by wet film parasite counts and scored using a chart outlined in the ILCA manual (Murray et al., 1983), were weighed and intraperitoneally treated at varying doses. Berenil treatment started with 1.75 mg of diminazene aceturate per kg body weight, calculated on the basis of 44.5% of the active ingredient in Berenil for a dose of 0.5 ml per 30 g of mice and adjusted accordingly. Treated mice were examined and trypanosome counts estimated 24 hours later. Mice with no change or having higher counts were given subsequent treatment double the previous dose, monitored and treated as before, until they became aparasitaemic. In mice with a marked reduction in trypanosome counts, subsequent treatment was withheld for another 24 hours; parasitaemias generally cleared during that period.

Aparasitaemic mice were monitored daily for relapses, and those that succumbed were observed for another 48 hours before being treated with double the previous dose. Mice were declared cured when no more relapse was detected for at least 180 days. From all treated and relapsing mice, trypanosomes were obtained by tail bleeding and subinoculated into 5 mice before each subsequent treatment. Subinoculated mice were treated at onset of parasitaemia with doses corresponding to those they had previously received. Trypanosome strains attaining the highest effective dose (HED) were subinoculated into 40 clean mice which were divided into eight subgroups of 5 mice, and each subgroup was treated with a narrow range of Berenil doses to establish the actual maximum curative dose (MCD).

A similar, parallel investigation was simultaneously carried out to assess the efficacy of Samorin as a curative drug for the same trypanosome isolates used in the Berenil tests. Four groups of 10 mice were inoculated, monitored and treated as for Berenil. The starting dose was 0.5 mg of isometamidium

chloride kg^{-1} body weight calculated for a dose of 0.5 ml per 30 g of mice. All experimental procedures were similar to that for Berenil.

RESULTS

Responses for the relapsing Chipata isolate with the trypanosome strains most tolerant to Berenil are given in Table 1. It had a highest effective dose (HED) of 56 mg kg^{-1} in 40% of the relapsing mice. The actual maximum curative dose (MCD) was 45 mg kg^{-1} in subinoculated mice (Table 2). Average time to Berenil treatment relapse ranged from 12.8 to 29 days in the relapsing group and from 6.5 to 14.0 days for the subinoculated mice. This isolate was, however, more susceptible to Samorin with an HED of 2 mg kg^{-1} in 80% of the treated mice (Table 3).

Results for the Mumbwa, Kabwe and Chisamba isolates are summarized in Table 4. Mumbwa isolate had strains more tolerant to both Berenil and Samorin. It had an HED of 56 mg kg^{-1} in 10% of the relapsing Berenil-treated mice; this corresponded to an MCD of 40 mg kg^{-1} in the subinoculated mice (not in the Table). Average time to relapse ranged from 14.7 to 30 days in Berenil-treated relapsing mice and from 5 to 12 days in subinoculated mice. Samorin-treated mice had an HED of 4 mg kg^{-1}, which was also the MCD in 90% of all the subinoculated mice.

Kabwe isolate responded to Berenil treatments with an HED of 28 mg kg^{-1} in 40% of the relapsing mice. In Samorin-treated mice, there was an HED of 4 mg kg^{-1} in only a single relapsing mouse; this value was also the MCD in all the subinoculated mice. All other Samorin-treated mice were permanently cured at doses of either 1 mg kg^{-1} or 2 mg kg^{-1}.

The strains most susceptible to both Berenil and Samorin treatments were

TABLE 1

Responses of Chipata isolate to different doses of Berenil treatments in relapsing mice

Group	Dose (mg kg^{-1})	No. of mice	Drug efficacy[1]			Avg. no. of days to relapse	Mortality (no. (%))
			NE (no. (%))	TE (no. (%))	PE (no. (%))		
1	1.75	10	10 (100)	0	0	NA[2]	0
2	3.50	10	8 (80)	2 (20)	0	12.8	0
3	7.00	10	3 (30)	6 (60)	1 (10)	15.9	0
4	14.00	9	0	8 (80)	1 (10)	18.7	0
5	28.00	8	0	4 (40)	4 (40)	29.0	0
6	56.00	4	0	0	4 (40)	NA	0

[1]Number and percentage of mice for which Berenil had: NE, no effect; TE, temporary effect; PE, permanent effect.
[2]NA, not applicable.

TABLE 2

Responses of Chipata strain to different doses of Berenil treatments administered to determine the maximum curative dose

Group	Dose (mg kg^{-1})	No. of mice	Drug efficacy[1]			Avg. no. of days to relapse	Mortality (no. (%))
			NE (no. (%))	TE (no. (%))	PE (no. (%))		
1	0.0	5	NA[2]	NA	NA	NA	5 (100)[3]
2	7.0	5	1 (20)	4 (80)	0	6.5	5 (100)
3	25.0	5	1 (20)	3 (60)	1 (20)	9.3	4 (80)
4	30.0	5	2 (40)	1 (20)	2 (40)	10.0	3 (60)
5	35.0	5	1 (20)	1 (20)	3 (60)	10.0	2 (40)
6	40.0	5	0	2 (40)	3 (60)	14.0	2 (40)
7	45.0	5	0	0	5 (100)	NA	0

[1]Number and percentage of mice for which Berenil had: NE, no effect; TE, temporary effect; PE, permanent effect.
[2]Na, not applicable.
[3]Average number of days to 100% mortality was 5.4 days after onset of parasitaemia, but considerably longer in the treated groups, with an average of 19.7 days.

TABLE 3

Responses of Chipata isolate to different doses of Samorin treatments

Group	Dose (cum. dose)[1] (mg kg^{-1})	No. of mice	Drug efficacy[2]			Avg. no. of days to relapse	Mortality (no. (%))
			NE (no. (%))	TE (no. (%))	PE (no. (%))		
1	0.5	10	10 (100)	0	0	NA[3]	0
2	1 (1.5)	10	10 (100)	0	0	NA	2 (20)
3	2 (3.5)	8	0	0	8 (80)	NA	0

[1]Cumulative dose in parentheses.
[2]Number and percentage of mice for which Samorin had: NE, no effect; TE, temporary effect; PE, permanent effect.
[3]NA, not applicable.

from Chisamba isolate. Berenil treatment response had an HED of 14 mg kg^{-1} in 10% of the treated mice. All other mice in this group responded to 3.5 or 7 mg kg^{-1}, with 1.75 mg kg^{-1} giving temporary clearance of parasitaemia in all treated mice (not in the Table). Response in Samorin-treated mice had an HED of 1 mg kg^{-1} in 60% of the treated mice. As little as 0.25 mg kg^{-1} effected permanent cure in 20% of subinoculated mice. Relapse on Samorin treatments occurred on average at 4 to 6 day intervals.

TABLE 4

Summary of therapeutic responses for Mumbwa (MB), Kabwe (KW) and Chisamba (CS) isolates to Samorin (Sam.) and Berenil (Ber.) treatments

Isolate	Mice per group	Drug efficacy				Avg. no. of days to relapse (range) (Ber. / Sam.)	Mortality (%) (Ber. / Sam.)
		Samorin		Berenil			
		LED[1,3] (mg kg^{-1} (%))	HED[2,3] (mg kg^{-1} (%))	LED[1,3] (mg kg^{-1} (%))	HED[2,3] (mg kg^{-1} (%))		
MB	10	NA[4]	4 (100)	3.5 (10)	56 (10)	5.0–30.0 / NA	10 / 20
KW	10	1 (80)	4 (10)	14.0 (30)	28 (40)	7.3–11.5 / 4–9	30 / 0
CS	10	0.25 (20)[5]	1 (60)	3.5 (60)	14 (10)	12.8–22.0 / 4–6	0 / 0

[1]LED, lowest effective dose in mg per kg body weight which permanently cured mice (% cured in parentheses).
[2]HED, highest effective dose in mg per kg body weight which permanently cured mice (% cured in parentheses).
[3]NB: All other mice in each group were cured at doses lower than HED.
[4]NA, not applicable.
[5]Data obtained from subinoculated group of 5 mice.

DISCUSSION

Although clear variations exist in our results, it would be highly debatable to designate a certain strain as drug resistant or susceptible using a mouse model for cattle results, because of differences in pharmacokinetics between mice and cattle. Sones et al. (1988) observed that a partially resistant *T. congolense* which responded to 2 mg kg^{-1} Samorin in cattle required 20 mg kg^{-1} in mice for effective cure. Mwambo et al. (1988) isolated a *T. congolense* strain which was resistant to 14 mg kg^{-1} Berenil and susceptible to 1 mg kg^{-1} Samorin in cattle, but it responded to 56 mg kg^{-1} Berenil and 20 mg kg^{-1} Samorin in mice. Zweygarth and Rottcher (1989) designated a *T. b. brucei* strain responding to 6.3 or 16 mg kg^{-1} Berenil in mice as susceptible and those responding to 50 mg kg^{-1} as resistant. Jennings et al. (1977) used a 40 mg kg^{-1} dose for maximum Berenil effect in mice to avoid toxic side effects. Other trypanosome strains designated as Samorin resistant are reported refractory over a wide range of doses from 3 to 20 mg kg^{-1} (Kaminisky et al., 1989, 1990; Zweygarth and Rottcher, 1989; Nyeko et al., 1989). Nevertheless, there is no doubt that mice models are useful in assessing relative susceptibilities of trypanosomes to drugs.

To avoid relapses due to the central nervous system involvement (Jennings et al., 1977), all treatments in this study were given within the first 7 days. In all re-treated mice, the cumulative drug effect may be expected to induce higher final curative doses, but this was not reflected in the subinoculated mice. The observed average times to relapses were generally low, a strong indication of relapse due to drug tolerance. Jennings et al. (1977) observed relapse due to the nervous system involvement of between 20 and 50 days, whereas Sones et al. (1988) observed an average of 6.0 or 8.8 days for a Samorin-resistant trypanosome strain. Discrepancies in our observations may be discussed with reference to tsetse challenge and managerial practices.

Although Chisamba herd is within an expanding tsetse encroachment zone (Corten et al., 1988) and has been maintained on regular prophylactic and therapeutic treatments over the past 10 years, it had the most sensitive isolate. This may be directly due to low tsetse challenge and to good herd and drug management practices. Chipata herd, which is reared under poor environmental conditions, including high tsetse challenge, free-range communal grazing system, and a high risk to chronic trypanosomiasis, has a problem. Reasons for the high Berenil resistance in this isolate may be varied, but high tsetse challenge, poor record keeping and irregular treatment regimes, exacerbated by lack of logistic support and disease awareness by farmers, seem to be the main contributing factors (Mumba and Chizyuka, 1987). The low tolerance to Samorin may be attributed to its high cost, which makes it a very rarely used drug in the traditional farming sector. The partial resistance observed in Kabwe isolate may also be attributed to increased irregular treat-

ments, poor record keeping, and/or to the introduction of oxen in the area. The persistent resistance to both Samorin and Berenil observed in Mumbwa isolate seems to indicate its prolonged exposure to both drugs before it was isolated. It also supports the idea that a trait for drug resistance is highly stable once developed (Gray and Roberts, 1971; Zweygarth and Rottcher, 1989).

The apparent resistance to both Berenil and Samorin is a matter of great concern. Although occurrence of double resistance to these drugs is considered to be rare (Fairclough, 1963a,b; Jordan, 1986), the phenomenon has been noted in trypanosomes (Rottcher and Schillinger, 1985; Zweygarth and Rottcher, 1989; Kaminsky et al., 1990). It is evident that trypanosomes resistant to Berenil, Samorin or even to both drugs exist in Zambian cattle. This problem is probably more widespread than is at present appreciated, particularly so in commercially managed herds under high tsetse challenge and with a long history of trypanocide use, often in the absence of veterinary supervision. More extensive field investigations are necessary to confirm the extent of the drug resistance problem in cattle and to establish the status of a non-rodent-infective *T. vivax* before large-scale use of trypanocides is adopted.

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Parasitology Research

Trypanosoma congolense: the in vitro akinetoplastic induction sensitivity assay

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Abstract. Incubation of *Trypanosoma congolense* in diminazene aceturate (Berenil) or isometamidium chloride (Samorin) induced akinetoplastic (AK) forms in vitro. The AK values (expressed in percent) obtained were found to be useful for rapid assessment of relative drug sensitivities. In susceptible clones, AK forms were induced at all drug concentrations tested, whereas in resistant clones they were induced only at higher concentrations. The Berenil-resistant clone exhibited AK values of 0.9% ± 0.6%–8.9 ± 2% at concentrations of 1–100 µg/ml at 4–10 h post-inoculation (p.i.), whereas the Berenil-susceptible clone displayed values of 9.3% ± 3%–19.2% ± 5% at 0.1–50 µg/ml. Motile trypanosomes were not seen at 100 µg/ml at 4 h p.i. or at 10 or 50 µg/ml at 10 h p.i. The Samorin-resistant clone showed AK values of 0.5% ± 0.1%–43% ± 3% at concentrations of 0.1–100 µg/ml at 4 and 10 h p.i., whereas the Samorin-susceptible clone exhibited values of 5.3% ± 2%–45% ± 4% at 0.0005–100 µg/ml. These results were supported by the findings obtained using a mouse infectivity test.

The prolonged use of trypanocides in various animal-trypanosomiasis control programmes in Africa over the last five decades (Fairclough 1963a, b; Williamson 1980; Tacher 1982; Trail et al. 1985; Braide 1987) has brought about an increase in the prevalence of drug-resistant trypanosomes in the field (Kupper and Wolters 1983; Pinder and Aunthie 1984; Röttcher and Schillinger 1985; Abebe 1987; Mwambo et al. 1988; Gardiner 1989). Nevertheless, there is a continuing need to improve upon the existing methods of determining drug susceptibility in trypanosomes, particularly the development of more rapid, reliable and relatively simple alternative approaches for possible wide-scale application under field conditions.

A simple infection-treatment approach in mice and other laboratory rodents is commonly used to evaluate the efficacy of drugs in eliminating trypanosomes from the peripheral blood (Bishop 1959; Hawking 1963). Unfortunately, the use of mouse models involves various limitations in both labour and time for wide-scale screening purposes (Jennings et al. 1977a, b, 1979; Sones et al. 1988; Zweygarth and Röttcher 1989). In a previous study, Chitambo et al. (1991) reported that resistant and susceptible *Trypanosoma congolense* and *T. brucei brucei* can easily be differentiated in vivo by akinetoplastic (AK) induction within 10 h following treatment with Berenil or Samorin. The AK forms induced can be clearly distinguished under light microscopy because they exhibit no detectable kinetoplast on Giemsa or Feulgen staining (Werbitzki 1910; Mühlpfordt 1963; Newton and Le Page 1967; Hadjuk 1976; Ono 1977; Rion and Bernard 1980). However, under field conditions, blood sampling at 10 h post-treatment is not always feasible.

The advent of in vitro systems for propagation of the various stages of trypanosomes (Yorke et al. 1929; Bishop 1967; Hirumi et al. 1977; Evans 1978; Gray et al. 1979; Hirumi and Hirumi 1984; Baltz et al. 1985) led to the development of in vitro drug sensitivity assays (Hawking 1963; Borowy et al. 1985a, b; Brun and Kunz 1989; Kaminsky and Zweygarth 1989; Kaminsky et al. 1990). Although the results obtained using these in vitro methods may be more precise, they do not provide appropriate means of distinguishing resistant from susceptible trypanosomes after incubation (Ross and Taylor 1990).

Quantitative methods that have since been applied mainly involve in vitro growth inhibition or radioisotope-based assays (Brun and Kunz 1989; Kaminsky et al. 1989; Ross and Taylor 1990). However, these approaches often require the adaptation of trypanosomes to an in vitro system, and high degrees of technical expertise and logistics are also involved. Kaminsky et al. (1990) developed a simple 24-h drug incubation infectivity test (DIIT) that combined the use of in vitro drug incubation and a mouse infectivity test to differentiate between resistant and susceptible trypanosomes; however, the use of mice represented a major limitation for

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this assay. Recently, Brun and Rab (1991) described a more simplified and rapid radio-labelled hypoxanthine-based sensitivity test for *T. congolense*, but it also requires the use of a liquid scintillation counter to distinguish the sensitivity of trypanosomes after incubation in trypanocides.

The main objective of the present study was to investigate whether *T. congolese* clones exhibiting varying degrees of sensitivity to Berenil or Samorin in mice could be evaluated according to the induction of AK forms after in vitro incubation with diminazene aceturate (Berenil) or isometamidium chloride (Samorin) and to determine whether such AK-induction values could be exploited as a means of distinguishing resistant from susceptible trypanosomes.

Materials and methods

Trypanosomes

All trypanosomes used in this study were originally isolated in 1989 from Zambian cattle that had been reared under different management systems and in different locations (Chitambo and Arakawa 1991). These isolates were stored and transported to Japan as cryostabilates. In Japan, they were subsequently raised, cloned and either cryopreserved or maintained in mice.

The Berenil-resistant (BR) clone was derived from the Chipata strain, which was originally isolated from a typical, traditionally managed herd of cattle that were located in a high tsetse fly (*Glossina morsitans*) and trypanosomiasis challenge area but were maintained on an irregular trypanocidal regimen. This isolate was found to be resistant to Berenil and partially resistant to Samorin in treated mice.

The Berenil-Samorin-susceptible (BSS) clone and the Samorin-resistant (SR) clone were both derived from the Chisamba strain, which was originally isolated from a commercially managed herd of cattle that were located in a low tsetse fly and intermediate trypanosomiasis challenge area but were maintained on a regular trypanocidal regimen. This isolate was sensitive to both Berenil and Samorin. The SR clone was then derived from a sub-population of the BSS clone by the administration of repeated sub-curative doses of Samorin to mice; after 6 months, this isolate became resistant to Samorin.

Designation of derivative clones

For the BR clone, the minimal curative doses (MCD) in mice were 45 mg/kg Berenil and 1 mg/kg Samorin. The MCD values for the SR clone in mice amounted to 16 mg/kg Samorin and 7 mg/kg Berenil. For the BSS clone, the MCD values in mice were 0.5 mg/kg Samorin and 7 mg/kg Berenil. The further designation of this clone was broken down into Berenil- (BS) and Samorin-susceptible (SS) clones, respectively.

Trypanocides

Berenil (Hoechst, Lot 467 U 682) and Samorin (May and Baker, DX 0026/58/49/00-2) stock solutions were prepared by dissolving the required quantities of each drug in sterile distilled water just before their use. These trypanocides were commercially purchased from Zambia and were stored at 4° C.

Medium

Modified Iscove's medium (Flow Laboratories, UK) to which 3.024 mg sodium bicarbonate/ml had been added (pH adjusted to 7) was used as a carrier for the various drug solutions and to dilute parasitaemic blood. Shortly before its use, the medium was supplemented with 2 mM L-glutamine, 0.1 mM hypoxanthine, 0.075 mM adenosine, 2 mM sodium pyruvate, 50 µg gentamicin, 0.2 mM 2-mercaptoethanol and 20% heat-inactivated fetal bovine serum (FBS; Flow Laboratories, UK). This medium was prepared as described elsewhere for long-term cultivation of trypanosomes (Baltz et al. 1985; Kaminsky and Zweygarth 1989).

Animals

JLA:ddY mice weighing 20–30 g were used to raise and to maintain the various trypanosome clones. Mice were intraperitoneally inoculated with the respective clones and were monitored for parasitaemia onset by examination of the peripheral blood. At peak parasitaemia of $\geq 5 \times 10^6$ parasites/ml blood, mice were bled by cardiac puncture following the induction of ethyl-ether anaesthesia. Heparinized blood was used for in vitro drug incubation assays immediately after sample collection.

Experimental procedure

Trypanocide stock solutions were prepared by dissolving the drugs in sterile distilled water (Samorin, 2 mg/ml; Berenil, 4.25 mg/ml). The concentration of Berenil was calculated on the basis of 44% of the active ingredient. These solutions were prepared at double the concentrations required such that their final dilution at 1:1 (v/v) with blood would yield the intended drug concentrations.

The Berenil stock solution was serially diluted 10- or 2-fold with medium to attain final drug concentrations of 100, 50, 10, 5, 1.0, 0.5 or 0.1 µg/ml. Samorin was also serially diluted 10-fold with medium to achieve final drug concentrations of 100, 10, 1.0, 0.1, 0.01 and 0.001 µg/ml, with an additional 0.0005-µg/ml solution being prepared from the 0.001-µg/ml solution.

Three 96-well tissue-culture plates were used (Flow Laboratories, USA). For each concentration of Berenil or Samorin, 100 µl of the respective drug solutions was added to an equal volume of heparinized blood containing the respective trypanosome clones (BR, SR, BS or SS clones). For each clone, three columns on the culture plate were used as replicates. In each row from A to G, each well contained the respective drug concentrations, whereas the last row (H), which contained medium only, served as a control.

The plates were incubated at 37° C under 5.1% CO_2 and 19% O_2 injection in air ($N_2-O_2-CO_2$ incubator BNP-110; Tabai Espec. Corp., Osaka, Japan). The first plate was examined after 4 h; the second, after 10 h; and the third, after 24 h incubation. Five thin blood smears from each sample were prepared from the first and the second plate after 4 and 10 h incubation, respectively. All slides were fixed in methanol and stained with Giemsa for light microscopic examination. At least 200 trypanosomes/slide (magnification, ×1,000) were counted and the AK rates were established as the average of the total AK values obtained from each replicate, expressed in percent.

Samples which exhibited motile parasites after 24 h incubation as judged from the examination of wet blood-films were drawn into 1-ml syringes and intraperitoneally inoculated into groups of five mice. Inoculated mice were then monitored for the onset of parasitaemia by examination of the peripheral blood.

Results

After 4 and 10 h incubation, it became evident that different AK-induction rate occurred in all trypanosome

Fig. 1A, B. Cytomorphological differences between A intact and B akinetoplastic forms of *Trypanosoma congolense* induced by in vitro incubation with trypanocides. Bar = 11 µm

Table 1. Responses of the BR clone of *Trypanosoma congolense* to various Berenil concentrations in vitro

Berenil (µg/ml)	Replicates (*n*)	% AK value[a] 4 h	% AK value[a] 10 h	Parasite survival[b] 24 h	Mouse infectivity (%)[c]
100	3	7.8±4	NPS	NPS	ND
50	3	0.8±0.5	8.9±2	NPS	ND
10	3	0	7.9±4	+ve	0/5
5	3	0	4.9±3	+ve	0/5
1	3	0	0.9±0.3	+ve	3/5 (60)
0.5	3	0	0	+ve	5/5 (100)
0.1	3	0	0	+ve	5/5 (100)
Control	3	0	0	+ve	5/5 (100)

[a] Average AK value ± SD ($n=3$) expressed in percent
[b] +ve, Parasites survived 24 h incubation; NPS, no parasite seen
[c] Number of mice infected/number inoculated (percentage of infection); ND, not done

clones examined. However, AK forms were not observed in any of the control groups, which were incubated in medium containing no trypanocides, and all control samples were infective to mice after 24 h incubation. Both intact *Trypanosoma congolense* and AK forms induced by in vitro incubation are shown in Fig. 1.

Responses of the BR clone to the various Berenil concentrations are given in Table 1. The 4-h AK-induction values were 7.8% ±4% and 0.8% ±0.5% after incubation in Berenil concentrations of 100 and 50 µg/ml, respectively. After 10 h incubation, no parasite was seen at 100 µg/ml; other 10-h AK-induction values ranging from 0.9% ±0.3% to 8.9% ±2% were observed at concentrations of between 1 and 50 µg/ml. No AK forms were detected following 4 h incubation at 0.1–10 µg/ml or after 10 h incubation at 0.1 and 0.5 µg/ml. Trypanosomes that survived 24 h incubation with Berenil at 0.1–1 µg/ml were infective to mice; the infection rates were 100% at 0.1 and 0.5 µg/ml and 60% at 1 µg/ml.

For the BS clone, no parasites were seen after 4 h incubation with Berenil at 100 µg/ml; the other AK-induction values ranged from 9.3% ±3% at 0.1 µg/ml to 18.2% ±4% at 50 µg/ml (Table 2). Again, no parasite was seen after 10 h incubation with Berenil at 10 or 50 µg/ml; the other 10 h AK-induction values ranged from 10% ±3% at 0.1 µg/ml to 19.2% ±5% at 5 µg/ml. Trypanosomes that survived 24 h incubation at 0.1 or 0.5 µg/ml were not infective to mice. The actual distribution and the range of effective concentrations observed

Table 2. Responses of the BS clone of *Trypanosoma congolense* to various Berenil concentrations in vitro

Berenil (µg/ml)	Replicates (*n*)	% AK value[a] 4 h	% AK value[a] 10 h	Parasite survival[b] 24 h	Mouse infectivity (%)[c]
100	3	NPS	NPS	NPS	ND
50	3	18.2±4	NPS	NPS	ND
10	3	17.7±5	NPS	NPS	ND
5	3	13±3	19.2±5	NPS	ND
1	3	11.5±6	17.9±3	NPS	ND
0.5	3	10±5	12±4	+ve	0/5
0.1	3	9.3±3	10±3	+ve	0/5
Control	3	0	0	+ve	5/5 (100)

[a] Average AK value ± SD ($n=3$) expressed in percent
[b] +ve, Parasites survived 24 h incubation; NPS, no parasites seen
[c] Number of mice infected/number inoculated (percentage of infection); ND, not done

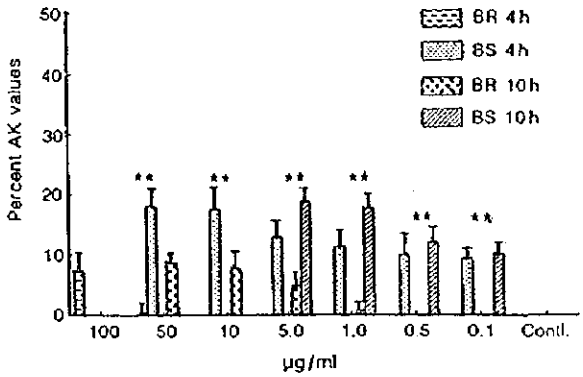

Fig. 2. Direct comparisons of the akinetoplastic (*AK*) induction values obtained for BS and BR clones of *Trypanosoma congolense* after in vitro incubation for 4 or 10 h in the presence of various concentrations of Berenil. Significant differences: ** $P<0.01$. Bars represent mean values ± SD ($n=3$, Student's *t*-test)

in BS and BR clones (Fig. 2) were significantly different ($P<0.01$).

Responses of the SR clone (Table 3) and the SS clone (Table 4) revealed a considerably higher range of AK-induction values as compared with the responses to Berenil. After 4 h incubation at the various Samorin concentrations, AK-induction values in the SR clone ranged from 0.5% ±0.1% at 1 µg/ml to 18.5% +3% at 100 µg/ml. All other drug concentrations between 0.0005 and 0.1 µg/ml had no apparent effect on this clone. Higher AK-induction values were evident in the SR clone after 10 h incubation in Samorin, with the range being from 8% ±2% at 0.1 µg/ml to 43% ±3% at 100 µg/ml. Again, AK forms were not observed at Samorin concentrations of 0.0005–0.01 µg/ml. Trypanosomes that survived 24 h incubation exhibited infectivity rates of 40% after incubation at 0.01 µg/ml and 100% at 0.001 and 0.0005 µg/ml.

Table 3. Responses of the SR clone of *Trypanosoma congolense* to various Samorin concentrations in vitro

Samorin (μg/ml)	Replicates (*n*)	% AK value[a] 4 h	% AK value[a] 10 h	Parasite survival[b] 24 h	Mouse infectivity (%)[c]
100	3	18.5±3	43±3	NPS	ND
10	3	8±5	29±4	NPS	ND
1	3	0.5±0.1	16±3	NPS	ND
0.1	3	0	8±2	+ve	0/5
0.01	3	0	0	+ve	2/5 (40)
0.001	3	0	0	+ve	5/5 (100)
0.0005	3	0	0	+ve	5/5 (100)
Control	3	0	0	+ve	5/5 (100)

[a] Average AK value ± SD ($n=3$) expressed in percent
[b] +ve, Parasites survived 24 h incubation; NPS, no parasites seen
[c] Number of mice infected/number inoculated (percentage of infection); ND, not done

Table 4. Responses of the SS clone of *Trypanosoma congolense* to various Samorin concentrations in vitro

Samorin (μg/ml)	Replicates (*n*)	% AK value[a] 4 h	% AK value[a] 10 h	Parasite survival[b] 24 h	Mouse infectivity (%)[c]
100	3	20.5±3	45±4	NPS	ND
10	3	12.2±5	39.5±3	NPS	ND
1.0	3	9.6±4	36.4±5	NPS	ND
0.1	3	7.1±5	31.1±2	NPS	ND
0.01	3	6.8±4	29.9±3	NPS	ND
0.001	3	6±3	20.5±3	+ve	0/5
0.0005	3	5.3±2	13.6±2	+ve	0/5
Control	3	0	0	+ve	5/5 (100)

[a] Average AK values ± SD ($n=3$) expressed in percent
[b] +ve, Parasites survived 24 h incubation; NPS, no parasites seen
[c] Number of mice infected/number inoculated (percentage of infection); ND, not done

In the SS clone, AK-induction values were observed at all drug concentrations used. After 4 h incubation with Samorin, values ranging from 5.3%±2% at 0.0005 μg/ml to 20.5%±3% at 100 μg/ml were observed. The 10-h AK-induction values ranged from 13.5%±2% to 45%±4% at Samorin concentrations of 0.0005 and 100 μg/ml, respectively. Trypanosomes that survived 24 h incubation were not infective to mice. At a Samorin concentration of 100 μg/ml, no significant difference was found in AK values obtained for the SR and the SS clones following incubation periods of 4 or 10 h. Significantly different AK values ($P<0.05$ or $P<0.01$) were apparent only at lower drug concentrations (Fig. 3).

Prepatent periods in mouse infectivity tests generally ranged from 5±1.5 to 10±3 days post-inoculation. The onset of parasitaemia always occurred earlier in the control samples. Mice that failed to exhibit parasitaemia by at least 4 weeks post-inoculation were considered to be negative.

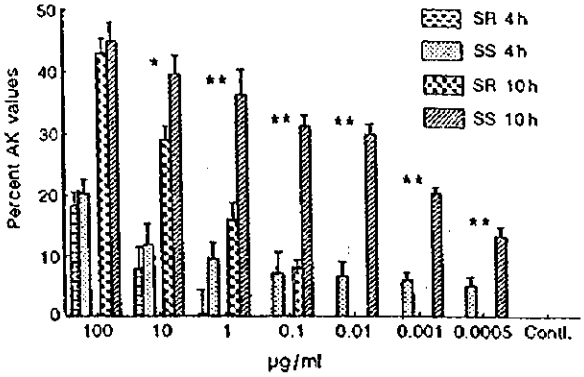

Fig. 3. Direct comparisons of the akinetoplastic (*AK*) induction values obtained for SS and SR clones of *Trypanosoma congolense* after in vitro incubation for 4 or 10 h in the presence of various concentrations of Samorin. Significant differences: * $P<0.05$, ** $P<0.01$. Bars represent mean values ± SD ($n=3$, Student's *t*-test)

Discussion

It is unfortunate that the evaluation of either growth inhibition or incorporation of radio-labelled hypoxanthine currently used in quantitative assays to distinguish resistant from susceptible trypanosomes following in vitro drug incubation (Kaminsky et al. 1989; Ross and Taylor 1990; Brun and Rab 1991) renders these highly promising assays technically inappropriate for wide-scale application or for routine diagnostic use. In most cases, these assays are suited for trypanosomes that have been well adapted to in vitro culture, which may take ≥2 weeks to achieve (Kaminsky et al. 1989). This is a particularly serious limitation for the often poorly equipped field-diagnostic laboratories that are directly involved in trypanosomiasis control in Africa.

Alternatively, the induction of AK forms by Berenil or Samorin in vivo have proved to be useful in distinguishing the relative drug sensitivity of trypanosomes by a cytomorphological approach (Chitambo et al. 1991). It is also known that trypanosomes can easily be kept alive for 2–5 days in liquid medium without the need for their special adaptation to in vitro systems (Yorke et al. 1929; Kaminsky and Zweygarth 1989). This enables the induction of AK forms using a short-term in vitro incubation assay.

In the present study, we observed that freshly cultured bloodstream forms of *Trypanosoma congolense* responded differently to various concentrations of Berenil or Samorin after 4, 10 or 24 h in vitro incubation. Increasing the drug concentration or prolonging the incubation period resulted in a corresponding increase in the ability to differentiate trypanosome sensitivity on the basis of the AK-induction rates or the rate of parasite elimination or by a mouse infectivity test. The AK forms were induced in the susceptible clones over a wide range of Berenil and Samorin concentrations, whereas

they were induced in the resistant clones only at higher drug concentrations.

The observed effective incubation periods and the minimal effective concentrations that were capable of inducing AK forms in susceptible but not in resistant strains were found to be close to those reported by other authors as being capable of differentiating resistant from susceptible trypanosomes in vitro using other detection methods (Kaminsky et al. 1989, 1990; Ross and Taylor 1990; Brun and Rab 1991).

The post-treatment plasma concentrations of Samorin and Berenil previously found in cattle following standard therapeutic doses are also reasonably close to the effective concentrations observed in vitro during the present study. Kratzer et al. (1989) detected Samorin plasma concentrations of about 0.02 µg/ml for up to 100 days after treatment, which dropped to <0.001 µg/ml after 140 days. Plasma concentrations of Berenil were found to be 1–2 µg/ml within 24 h after treatment and about 0.01 µg/ml at 28 days thereafter (Klatt and Hajdu 1976; Kellner et al. 1985).

Although Berenil eliminated trypanosomes more rapidly than did Samorin, especially at high concentrations, this did not correspond to high AK-induction values. There may be various reasons for this, but Berenil and trivalent tryparsamides are known to exhibit strong, direct cytotoxic activity against trypanosomes in vitro (Hawking 1963). Therefore, Berenil seems to eliminate the susceptible trypanosomes rapidly prior to the achievement of AK induction. However, the low AK-induction values that were also evident at lower concentrations seem to suggest that other factors may also be involved. The overall AK values for both Berenil and Samorin were generally lower than those observed in mice (Chitambo et al. 1991). None of the AK values observed in the present study reached 50%, the minimal AK value that corresponds to an effective treatment dose in mice. It is possible that certain unknown innate factors present in vivo help to modify the pharmacokinetics of Berenil and, probably, of Samorin as well. Such host-parasite-drug interactions may contribute to the enhanced AK-induction rates observed in vivo. However, the conditions used in the present study may also exacerbate these discrepancies.

Although we could estimate the relative susceptibility of various trypanosome clones to Berenil or Samorin using AK-induction values, further efforts are needed to optimise this assay so as to achieve a rapid test-tube sensitivity test and to validate this method using a much larger group of different trypanosome species and strains from different sources.

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In vivo assessment of drug sensitivity of African trypanosomes using the akinetoplastic induction test

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Following treatment of mice infected with *Trypanosoma congolense* or *T brucei brucei* with various doses of isometamidium chloride or diminazene aceturate, the induction of akinetoplastic (AK) forms was observed in the trypomastigotes of both species within 10 hours of drug administration. The levels of AK-induction were closely correlated with the levels of resistance to each compound found using a standard in vivo drug assay in mice. In general, ineffective doses of either compound conferred AK-induction rates of less than 30 per cent; relapsing cases had between 30 and 50 per cent while curative doses had AK-induction rates of 50 per cent or more. In vivo determination of AK-induction rates using ordinary light microscopy is thus a potentially feasible alternative indicator to the conventional use of mice infection and treatment methods for assessing drug sensitivity in African trypanosomes.

AFRICAN animal trypanosomiasis affecting livestock productivity in sub-Saharan Africa is mainly caused by *Trypanosoma congolense, T brucei brucei* and *T vivax*. In the absence of an effective vaccine, control depends on the use of chemotherapy and control of the tsetse fly vector (*Glossina* species). However, reports of trypanosome populations with resistance to the currently available trypanocides have increased in recent years (Fairclough 1963a, b, Finelle 1977, Zweygarth and Rottcher 1989, Gardiner 1989, Boid et al 1989, Chitambo and Arakawa 1991). Nevertheless, isometamidium chloride (Samorin; Rhone Merieux), diminazene aceturate (Berenil; Hoechst), suramin sodium (Naganol; Bayer), quinapyramine sulphate (Trypacide S; Rhone Merieux) and homidium bromide (Ethidium; FBC) are still going to be drugs of choice for some time to come (Braide 1987).

Conventional drug sensitivity tests normally involve the use of mice. However, the results from these drug assays may not be highly predictive for cattle (Sones et al 1988); confusion from relapses due to resistance and, or, central nervous system involvement may arise (Jennings et al 1979, Abebe 1987); a prolonged observation period of 30 to 90 days is necessary to confirm permanent cure (Sones et al 1988, Zweygarth and Rottcher 1989) while certain trypanosomes may require a long adaptation period in mice. Also, the mouse model cannot be used for non-rodent infective trypanosomes. Alternative approaches may involve the use of in vitro systems (Borowy et al 1985a, b, c) or the use of both in vitro and in vivo protocols as in the drug incubation infectivity test (DIIT) (Kaminsky et al 1990). Furthermore, Boid et al (1989) considered the use of enzyme electrophoresis for the detection of suramin resistance in *T evansi*. However, none of these alternatives is free of shortcomings.

Bloodstream forms of most African trypanosomes have a self-duplicating organelle, the kinetoplast, which is associated with the mitochondrion and contains genetic material, kDNA (Werbitzki 1910, DuBuy et al 1965, Newton 1967, Newton and Le Page 1967, Ozeki et al 1970, 1971, Delain et al 1971, Ono et al 1971, Riou and Benard 1980). Inoki and Matsushiro (1959) observed that when pararosaniline, a red biological stain known to have a strong trypanocidal effect in susceptible *T b gambiense* species, was inoculated into mice infected with various *T gambiense* strains, akinetoplastic (AK) forms were observed in the peripheral blood within four hours of treatment. Th

rate of this AK induction was shown to be higher in susceptible than in resistant strains following treatment.

The objective of the current study was to examine the effect of isometamidium chloride, diminazene aceturate and pararosaniline on the induction of AK-bloodstream forms in mice infected with trypanosomes originally isolated from Zambian cattle and to determine whether observable differences in AK-induction rates between resistant and susceptible African trypanosomes could be of value in the detection of trypanocide resistance.

Materials and methods

Drugs

Basic pararosaniline (Sigma) was purchased in Japan. Diminazene aceturate and isometamidium chloride were purchased from Zambia. Injection solutions were prepared by dissolving the required quantities of each compound in sterile distilled water; just before use for diminazene aceturate and isometamidium chloride, and three weeks before for pararosaniline because of its low solubility in water.

Mice

Type Jla: ddY mice weighing 20 to 30 g were purchased from the Japan Laboratory Animal Co, Tokyo. They were housed in a fly-proof isolation building with equal light and darkness per day. Water and pelleted feed was provided ad libitum.

Trypanosomes

All trypanosomes used in this study were isolated in 1989 from Zambian cattle which were raised under different management practices at various locations. Details on the general characteristics and the origin of each isolate are given in Table 1 (Chitambo and Arakawa 1991). MB stock, a mixed population of *T congolense* and *T b brucei*, which was obtained as a cryostabilate from the Central Veterinary Research Institute, Lusaka, Zambia, was originally isolated from Mumbwa but no herd history was provided.

A clone of *T congolense* (ChiTat 1·0), resistant to diminazene aceturate (BR-clone) with a minimum curative dose (MCD) in mice of 45 mg kg^{-1}, but susceptible in mice to 2 mg kg^{-1} isometamidium chloride was derived from a parent resistant strain (CP) without drug selection.

Another *T congolense* clone (LusTat 1·0) was derived from a diminazene aceturate and isometamidium chloride susceptible (BSS) original field isolate (CS-strain) and was made resistant to isometamidium chloride as follows; five mice previously infected with the BSS-strain were treated with 0·25 mg kg^{-1} isometamidium chloride at their first parasitaemia peaks. One hour, six hours and 12 hours after treatment, pooled tail-bled blood

TABLE 1: Characteristics and the origin of the various trypanosome isolates

Stock code	Species	Therapeutic responses: Diminazene aceturate LED mg kg^{-1} (%)	Diminazene aceturate MCD mg kg^{-1} (%)	Isometamidium chloride LED mg kg^{-1} (%)	Isometamidium chloride MCD mg kg^{-1} (%)	Herd location (province)	Management practice
CS	*Tc*	1·25 (100)	14·0 (10)	0·25 (20)	1·0 (60)	Chisamba (LP)	Commercial
CP	*Tc*	7·0 (60)	45·0 (40)	NA	2·0 (100)*	Chipata (EP)	Traditional
MB	*Tc/Tb*	7·0 (50)	40·0 (20)	NA	4·0 (100)*	Mumbwa (CP/WP)	Unknown
KW	*Tc/Tb*	3·5 (70)	28·0 (40)	2·00 (90)	4·0 (10)	Kabwe (CP)	Emergent

Tc Trypanosoma congolense, Tb T b brucei
LED Lowest effective dose which resulted in temporary clearance of parasitaemia (number indicates percentage of mice relapsed, n = 10)
MCD Minimum dose reached to give 100 % permanent cure (number indicates percentage of mice cured in the relapsing [or no effect]* groups, n = 10)
NA Not applicable
LP Lusaka province, EP Eastern province, CP Central province, WP Western province and CP/WP Border location

from each of the treated mice was reinoculated into five fresh mice. Subsequently, all mice which became parasitaemic were treated with double the previous dose used. This protocol was then repeated four times, gradually increasing the dosage twofold to a maximum of 4 mg kg^{-1}. Thereafter, all mice with relapsing parasitaemias were repeatedly given 1 to 2 mg kg^{-1} of isometamidium chloride. Following six months of subcurative treatments in relapsing mice, LusTat 1·0 (SR-clone) was selected after it was found to have a MCD value for isometamidium chloride of 16 mg kg^{-1} but remained susceptible to 7 mg kg^{-1} diminazene aceturate.

An uncloned line, also derived from the CS-strain, with MCD values in mice of 7 mg kg^{-1} diminazene aceturate and 0·5 mg kg^{-1} isometamidium chloride, was maintained drug free and was used as a BSS-strain in both the diminazene aceturate and isometamidium chloride treatment groups.

Experimental procedure

All experimental mice were inoculated intraperitoneally with approximately $2·5 \times 10^5$ trypanosomes harvested from donor mice previously infected with the various trypanosome clones or stocks and anaesthetised with ethyl-ether. No irradiation was necessary for any of the trypanosome strains used.

Experiment 1. Two groups of 15 mice were allotted to each of the five diminazene aceturate treatment groups. The first group was infected with the BR clone (ChiTat 1·0), while the second group was infected with the BSS strain (CS stock).

Two groups of 18 mice were assigned to each of the six isometamidium chloride treatment groups. One group was infected with the SR clone (LusTat 1·0) while the other group was infected with the BSS strain.

The last four groups, containing three mice each, were used in the pararosaniline treatments. In each of the BR and SR groups, mice were infected with the respective clones, while the BSS groups were each infected with the BSS strain.

All infected mice were monitored for the onset of parasitaemia by examining the peripheral blood from day 3 after inoculation. Average prepatent periods were 4 ± 1·5 days in the BR and SR strains and 7 ± 2·0 days for the BSS strains. Parasitaemia was expressed in scores based on counts from wet blood films (Murray et al 1983). When the parasitaemia reached 5×10^5 cells or more ml^{-1} of blood, two to three days after the onset of parasitaemia, mice were treated intraperitoneally with various doses of diminazene aceturate or isometamidium chloride. In the BR and BSS groups, five groups of three mice were given diminazene aceturate at 3·5, 7·0, 14·0, 28·0 or 45·0 mg kg^{-1}. In the SR and BSS groups, six groups of three mice were given isometamidium chloride at 0·5, 1·0, 2·0, 4·0, 8·0 or 16·0 mg kg^{-1}. Mice in the pararosaniline treatment groups were all given 10 mg kg^{-1} pararosaniline as previously used in *T gambiense* infected mice (Ono 1977).

Experiment 2. Four populations of the original trypanosome field isolates CP, KW, MB and CS were also assessed for their AK-induction responses. Two sets of four groups of six mice were infected with each of the isolates at a parasitaemia of 5×10^5 cells or more ml^{-1} of blood. Three mice in each group were then treated with 7 mg kg^{-1} diminazene aceturate or 1 mg kg^{-1} isometamidium chloride. These regimens were selected because they represented not only the observed minimum curative doses in mice for a susceptible strain (CS strain) (Chitambo and Arakawa 1991) but are also the recommended cattle therapeutic doses.

Light microscopy

In experiment 1, five thin blood smears were prepared from each treated mouse at zero, four, 10 and 24 hours after treatment, while in experiment 2, slides were prepared at zero and 10 hours after treatment. At each of these time intervals, parasitaemias were also estimated by examining the peripheral blood from each treated mouse and the group average values were established. All thin blood smear slides were fixed in methanol and stained with Giemsa. Stained slides were examined by light microscopy (Nikon, Japan) and photographs taken (Nikon UFX II A system). At least 500 trypanosomes were counted per slide to determine the akinetoplastic rate as a percentage.

Results

AK rates before treatment ranged from nil to 0·5 per cent in all trypanosome populations used.