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Epidemiology of Rhodesian sleeping sickness in the Lambwe Valley, Kenya

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A total of 912 cases of sleeping sickness have been recorded from the Lambwe Valley from 1959 to 1984. After a period of decreasing prevalence in the 1970s an outbreak of disease occurred between 1980 and 1984. The incidence of disease for this five-year period was highest in areas adjoining the Ruma National Park, reaching 54‰ in Area I. Attack rates were highest in the 50+ age group (125) and children had significantly lower attack rates (8‰) in this area of peridomestic transmission. Sex ratios of patients (M/F) were near 1·0 in areas in closest proximity to the thickets in the National Park, while in distant areas the ratios rose to 6·0. The distribution of the number of patients within different households was studied; fewer households than expected had 0 or one patient, and more than expected had three or more patients. No difference in attack rates were found between Nilotic and Bantu groups. Twelve different zymodemes were found in 136 stocks of *Trypanosoma brucei rhodesiense*. Four new zymodemes appeared in 1980 in the latest outbreak and accounted for 73% of the stocks isolated from man during this outbreak. Neutralization tests indicated that each trypanosome zymodeme may also represent a different serodeme.

The Lambwe Valley is a small area contained within relatively defined geographical boundaries which was populated mainly in the last 30 years. The human inhabitants and their domestic animals came into contact with an isolated population of *Glossina pallidipes*, which in recent history at least has not been contiguous with any other *G. pallidipes* fly belt. Due to increased human settlement, agriculture and tsetse control measures, the *G. pallidipes* population has become increasingly restricted to the confines of the Ruma National Park. The park's large resident game-animal population and extensive evergreen thicket, however, provide an ideal environment for the tsetse flies, and for transmission of trypanosomiasis within the National Park and to man and domestic animals in adjacent areas (Karluki *et al.*, 1989). The relatively recent appearance of Rhodesian sleeping sickness in the Lambwe Valley in the late 1950s permitted a description not usually possible in other typical zones of infection where relatively few sporadic cases occur in widely dispersed populations (Molyneux, 1983).

The first section of this paper deals with the descriptive epidemiological aspects of Rhodesian sleeping sickness in the Lambwe Valley from 1959 to 1984. The second section describes the disease in a population that was defined by census and survey in 1981–1982 (Wellde *et al.*,

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1989*d*) and in which continuous surveillance was maintained between 1980 and 1984. The third section concerns the biochemical and serological analyses of trypanosome stocks isolated from man in the Lambwe Valley.

MATERIALS AND METHODS

Patient Status

It was clear from hospital records that some sleeping sickness patients were subsequently readmitted to hospital with the same diagnosis. In order to define the status of these patients we reviewed the individual health records and found that readmission usually occurred after therapy with suramin. Using the patient data collected from 1980 to 1984 as a guide (see Wellde *et al.*, 1989*h*), we classified the patients as follows:

PRIMARY SLEEPING SICKNESS INFECTION: parasitological diagnosis in individuals with no previous history of sleeping sickness.

RELAPSE SLEEPING SICKNESS INFECTION: parasitological or clinical diagnosis of sleeping sickness in patients within three years of treatment of a primary sleeping sickness infection. These cases were predominantly relapses following suramin therapy.

REINFECTION: parasitological diagnosis of sleeping sickness in former patients three years or longer after treatment of a primary infection without intervening therapy.

Epidemiological Methods

The earliest records of sleeping sickness in the Lambwe Valley were contributed by Mr. R. G. Highton, former entomological field officer, Kisumu. These records had been used as a basis for other descriptions of the disease in the Lambwe Valley area (Willett, 1965; Watson, 1972). Other information pertaining to the introduction of Rhodesian sleeping sickness into the Lambwe Valley was obtained from the monthly reports of the Kisii Hospital. Records of 758 sleeping sickness patients (which included age, sex and geographical information) were recovered, dating back to 1963.

Sleeping sickness cases were classified as being residents of one of 14 different areas within the Lambwe Valley or in the surrounding locations on the basis of their home or nearest village. A description of these areas can be found in Wellde *et al.* (1989*d*). Each patient's home, if located in the Lambwe Valley area, was visited by our field teams during the 1980–1984 period. Visits to patients' homes on the shore of Lake Victoria required the use of a Kenyan Government motor launch, as the area was inaccessible by road. Two journeys to Kisegi and also to Kisiambi on the lakeshore were made during 1981–1984. The location of villages is shown (Fig. 5A).

Population data collected in the demographic survey (Wellde *et al.*, 1989*d*) were used to calculate the incidence of sleeping sickness for the period 1980–1984 in eight study areas surrounding the Ruma National Park in the southern half of the Lambwe Valley. The incidence in these areas for the years prior to 1980 was calculated from a population growth curve derived from survey data recording the year of the family's arrival in the study sites (Areas 1–8). The population data also provided the basis for calculation of attack rates by age, tribal group and duration of residence (Wellde *et al.*, 1989*d*).

To relate tsetse fly density and disease prevalence to rainfall, monthly rainfall data collected by Forestry Department personnel at their camp on the Kaniamwa Escarpement, the eastern boundary of the Lambwe Valley, were obtained at the Department of Meteorology in Nairobi.

Characterization of *T. b. rhodesiense* Stocks

Methods of biochemical analysis and determination of zymodemes for the Lambwe Valley *T. b. rhodesiense* stocks have been published recently (Gibson and Wellde, 1985). To determine whether or not the trypanosome isoenzyme type was related to antigenic composition, antisera were prepared against 10 different stocks of *T. b. rhodesiense*. Nine stocks isolated in the Lambwe Valley represented eight different zymodemes. Another stock, isolated in 1978 in Samia, Kenya, along the Uganda border, represented a zymodeme not found in the Lambwe Valley. Each stock was injected intraperitoneally (ip) into rats which were subsequently bled by cardiac puncture into heparin. Trypanosomes were counted, and after dilution in phosphate buffered saline (PBS) containing 10% foetal calf serum 1×10^4 trypanosomes from each stock were injected intravenously (iv) into a yearling Boran steer (the steers were purchased from a tsetse fly free area) which was shown to be negative for trypanosomiasis by both blood smears and subinoculation. Six weeks after infection each steer was bled and serum collected. Neutralization tests (Soltys, 1957) were done by mixing serum from each steer with each of 10 different stocks of *T. b. rhodesiense* for one hour at room temperature. The mixtures, containing 1×10^3 trypanosomes in 0·2 ml, were subsequently injected into mice (Walter Reed—ICR strain) which were monitored for parasitaemia by wet blood smears daily for the next 30 days.

Statistical Methods

Analysis of data by statistical formulas was as follows: Analysis of variance, Chi Square (χ^2), Poisson Goodness of Fit, Protected Least Significant Difference, and the Students' *t*-Tests were done as described by Snedecor and Cochran (1967). Bartholomew's Test for Order and the calculation of 95% confidence limits of attack rates were done as described by Fleiss (1981) and Coulton (1974) respectively. Calculations were based on population data collected during a demographic and disease survey (Wellde *et al.*, 1989*d*).

RESULTS

Section I:
Descriptive Aspects of Rhodesian Sleeping Sickness in the Lambwe Valley

Reported Numbers of Sleeping Sickness Cases

The annual number of reported sleeping sickness patients from the Lambwe Valley area is presented in Fig. 1. A total of 912 primary cases have been reported during the period 1959–1984, with an average of 35 each year. The highest number of cases (98) was reported in 1967 and the lowest number (3) in 1979. Ninety-seven individuals with relapse infections detected within a three-year interval after treatment of their primary infections are also shown in Fig. 1. Of the 45 relapse cases detected during 1980–1984 most had been treated with suramin and were found as a result of systematic follow-up. Ten patients were found to have been reinfected after periods ranging from 3·2 to 14 years following a successfully-treated primary infection. Four reinfections occurred during 1980–1984, and treatment records were available from each patient for both primary infection and reinfection.

Sex Ratios (M/F)

The sex ratios of sleeping sickness patients calculated for the years 1963–1984 are shown in Fig. 1. Infected males predominated in 19 of the 22 years, equal numbers of males and females occurred in one year (1981), and infected females predominated in two years (1965, 1975). An increased M/F ratio was most prominent in the years 1971–1973 reaching levels of 4·0. In the early stages of the most recent outbreak (1980–1984) ratios approaching 1·0 were found.

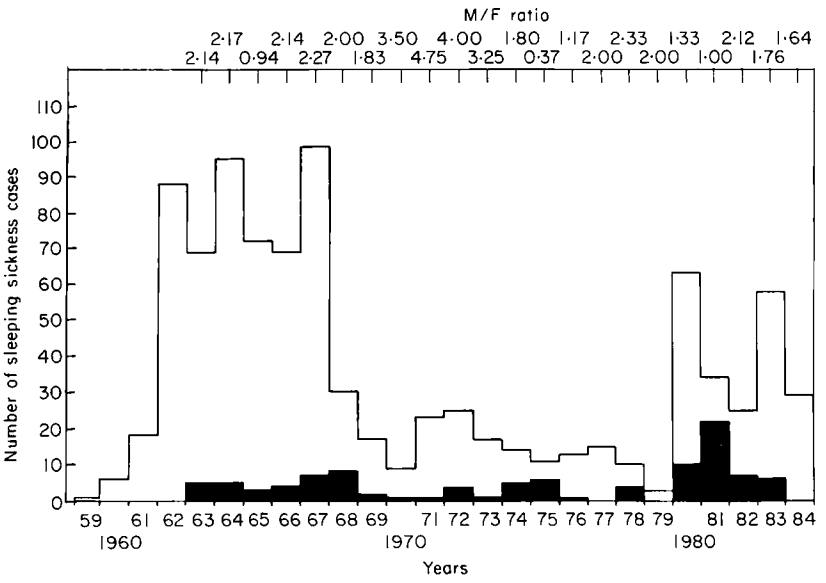

Fig. 1. Reported sleeping sickness cases by year (1959–1984) in the Lambwe Valley. □, primary infections; ■ relapse infections.

TABLE 1

Distribution of 758 Rhodesian sleeping sickness patients from the Lambwe Valley by age and sex (1963–1984)*

	Age groups						
	0–9	*10–19*	*20–29*	*30–39*	*40–49*	*50–59*	*60+*
Male	11	107	95	86	96	59	40
Female	14	55	74	64	37	16	4
Male and Female	25	162	169	150	133	75	44
Ratio (M/F)	0·78	1·94	1·28	1·34	2·59	3·69	10·00

*41 adults (21 male and 20 female) whose exact age was unknown, have been excluded.

Distribution by Age and Sex

Table 1 shows the distribution of 758 patients for whom records were available, by age and sex over the period 1963–1984. Relatively small numbers of children under 10 years of age were infected and numbers of patients declined in the older age groups. Sex ratios (M/F) increased with age.

Distribution by Month

Figure 2 shows the monthly distribution of cases over a 22-year period (1963–1984). The peak number of cases (13·6%) was reported in June, followed by July (11·9%) and January

(10·8%). April had the lowest number of reported cases (4·7%) followed by December (5·6%). The distribution of male and female patients by month of diagnosis was similar and males predominated in every month. The number of months when patients were recorded was lowest in February (13) and highest in July and August (19 each) over the 22-year period. Figure 2 also illustrates the 10-year (1971–1980) average calculated rainfall with peak months of April, May and November.

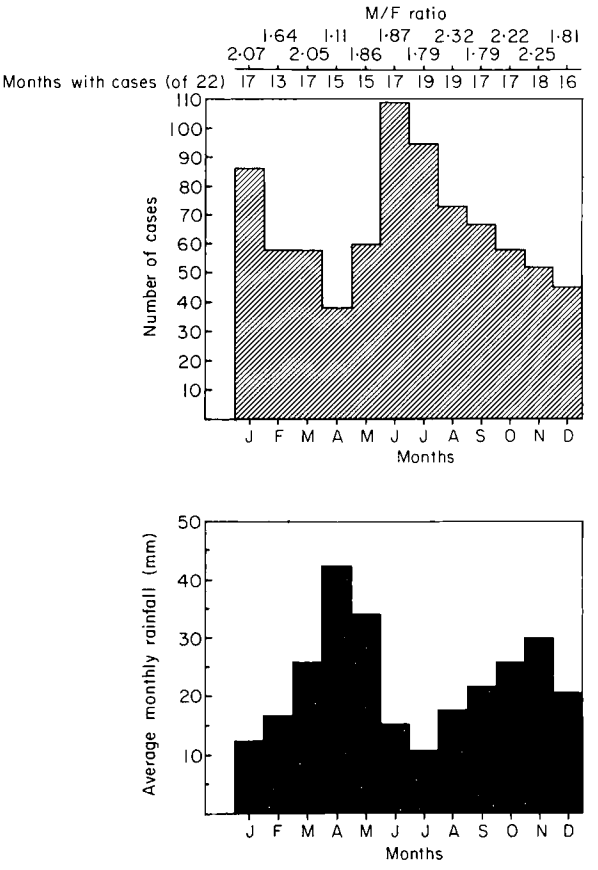

Fig. 2. Sleeping sickness cases by month of diagnosis from 1963–1984 in the Lambwe Valley, and average monthly rainfall for the 10-year period (1971–1980).

Geographical Distribution

Figure 3 shows the distribution of sleeping sickness patients in 14 areas in the Lambwe Valley. These areas are shown (Fig. 5A), and have been defined by Wellde *et al.* (1989*d*). Two groups of patients whose homes were not in the Lambwe Valley but who had visited the valley (Group 15) or who worked for the Kenya Government in the valley (Group 16) are also included. Four time periods during 1963–1984, each with different characteristics, were selected for description.

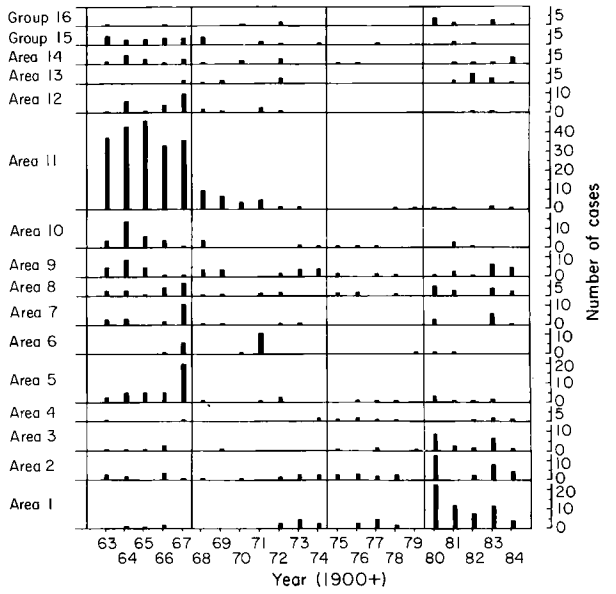

Fig. 3. Distribution of sleeping sickness patients in 14 areas within or near the Lambwe Valley (1963–1984). Group 15 represents visitors to the Lambwe Valley who developed sleeping sickness upon returning home. Group 16 represents Kenya Government employees who developed sleeping sickness while working in the Lambwe Valley.

1963–1967

In this early period the largest number of cases were reported from Area 11, the area in which Rhodesian sleeping sickness first appeared, and adjacent Areas 10 and 12. Few infections were found in the southern area of the Lambwe Valley (Areas 1–4) during this time period. Relatively low numbers of cases in Areas 5–8 over this period culminated in 1969 with an outbreak in these areas. Area 9, on the top of Kaniamwa escarpment, had reported cases every year during this period. Area 14, the most southerly area of the Valley, also reported cases each year. Group 15 contained patients from distant areas who had visited the Lambwe Valley area, or who, in the early years, possibly may have contracted Gambian sleeping sickness which was endemic at low levels on the lakeshore and along the Kuja River. Area 13 comprised the coastal area of Lake Victoria south of the village of Sindo, and only a few cases were reported in 1967 from areas near Sindo infested with *G. pallipides*.

Three Kenya Government personnel who were in the Lambwe Valley on official business also became infected (Group 16). Minimal efforts at tsetse control measures were made during this period.

1968–1974

Vector control operations and settlement of immigrants during this period reduced the major focus of disease in Areas 10, 11 and 12 to low levels. Towards the end of this period (1972–1974) increased numbers of cases began to appear in the populations in Areas 1 and 2 at the southern end of the Lambwe Valley. Areas 6 and 12 experienced an outbreak of disease in the Nyakia and Opuch sections in 1971. Cases continued to to be reported from Area 9 on the escarpment, with the exception of 1970 and 1971. Transmission continued in the northern aspect of Area 13 in *G. pallidipes*-infested areas away from the lakeshore. Area 14 had intermittent cases

and infected individuals from outside the area continued to be found. Three more Kenyan Government employees were found to be infected during this period.

1975–1979

Vector control programmes in the Lambwe Valley were discontinued in 1974. During this five-year period the majority of cases detected came from the population living in close proximity to the National Park (Areas 1–9). Only five cases were reported from Areas 10, 11 and 12 combined during this period. Two cases were detected from Area 14 and one outsider (Group 15) was found to be positive. No infected individuals were found in Area 13 on the Lake Victoria shoreline, and no government employees became infected. In 1979 only three cases were reported, one each from Areas 3, 6 and 11, despite the fact that tsetse control efforts had ceased in 1974.

1980–1984

In June 1980 increased numbers of sleeping sickness cases were reported from areas in close proximity to the National Park. Areas 1, 2 and 3 were the most severely affected. Despite major vector control measures during subsequent years, these areas continued to yield sleeping sickness cases. Increased numbers of cases also appeared at the northern boundary of the Ruma National Park (Areas 7 and 8) and on top of the escarpment (Area 9). Area 14 also had increased numbers of cases. Three visitors to the Lambwe Valley developed infection after returning to their homes in distant locations. Kenyan government personnel working in the Valley also became infected in greater numbers (11). After a long period with no reported cases, infections began to appear in Area 13 along the lakeshore. Previous sleeping sickness cases reported from Area 13 had usually originated from *G. pallidipes*-infested sections north of Ragwe village. These sections were cleared of *G. pallidipes* in the late 1960s or early 1970s. In 1981–1984, however, cases of Rhodesian sleeping sickness appeared along the Lake Victoria shoreline outside *G. pallidipes* areas but in areas with light reinfestations of *G. fuscipes*. A total of 11 cases in all were detected from this coastal region (Area 13) during the period. Figure 4 shows the distribution of patients in the 14 geographical areas and in the other two groups during the most recent outbreak in 1980–1984.

Factors Associated with a Disease Outbreak in 1980–1984

The distribution of Rhodesian sleeping sickness patients in relation to their place of abode during the period 1980–1984 is shown (Tables 5A, B).

The Rhodesian sleeping sickness outbreak in 1980 followed the heaviest rainfall in the months of April and May in the previous 10 years. This period of heavy rainfall was followed by the driest June in the same time period. Under these apparently favourable conditions, high levels of tsetse flies dispersed from the thickets in the National Park into areas adjacent to the National Park. The human and domestic animal population had increased markedly in the previous 10 years, especially in areas surrounding the National Park (Wellde *et al.*, 1989*d*). Thus the reservoir of infection was extended outside the game animals in the National Park, and *T. brucei* ssp. organisms were isolated from man, cattle, goats and dogs. Trypanosome stocks isolated from man and animals indicated, after analysis, that most of them represented zymodemes which had not been identified in the Lambwe Valley during the previous 10-year period.

Tsetse control and eradication attempts with Endosulfan in early 1981 were partially successful, but after a period with no reported cases transmission of Rhodesian sleeping sickness resumed in July 1982. This resumption of disease was not as explosive in Areas 1, 2 and 3 as in the original outbreak. Figure 4 graphically depicts the outbreak of Rhodesian sleeping sickness in the Lambwe Valley by monthly occurrence during the period 1980–1984.

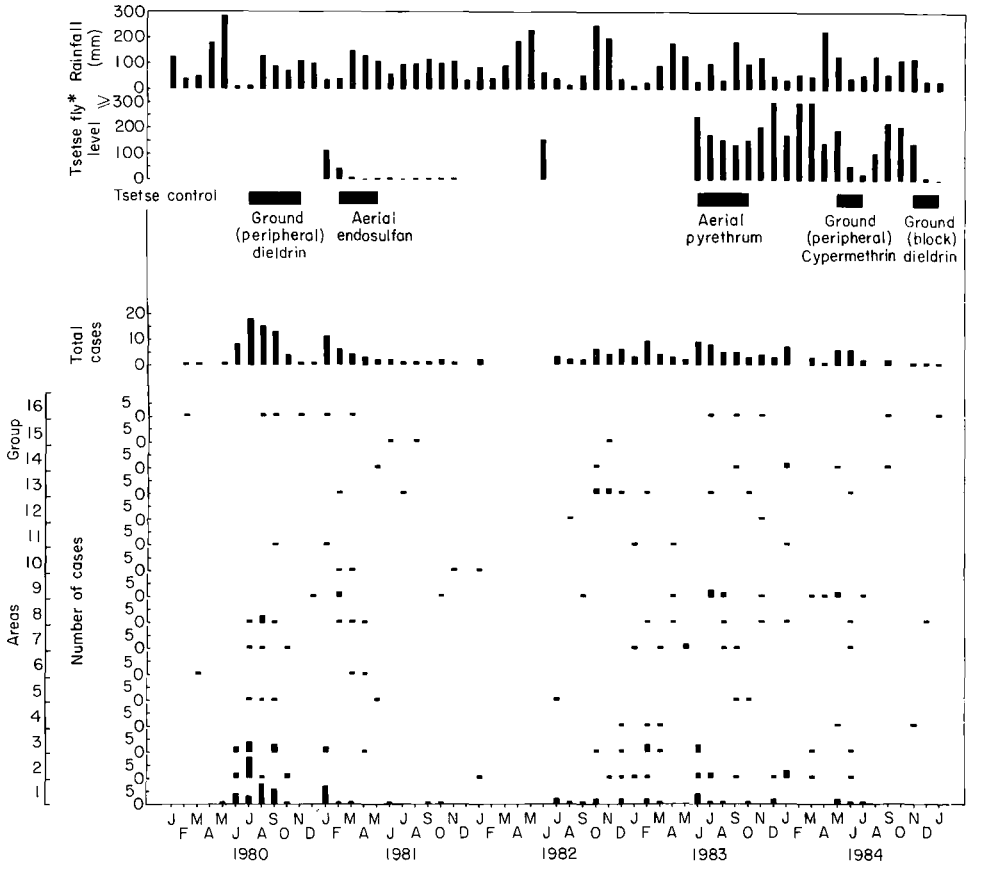

Fig. 4. Graphic description of Rhodesian sleeping sickness in the Lambwe Valley 1980–1984. *Average no. flies per trap day^{-1}.

Section II: Population-Based Epidemiological Studies

Annual Incidence of Sleeping Sickness in the Study Site (Areas 1–8)

The annual incidence of sleeping sickness was based on the estimated total population determined in Areas 1–6 and the number of sleeping sickness cases which occurred in each area (Fig. 6). Generally, a higher incidence occurred during the early years when the population was lowest. In 1967 a higher incidence was due to increased numbers of sleeping sickness cases in Areas 4–7, and in 1971 a small outbreak in Area 6 again caused increased incidence in that area. During the most recent outbreak in 1980, the overall incidence in the study site reached a level of 5·5, approximately one-third the incidence calculated for 1967. Incidence in 1980 was highest in Area 1 (21·5), followed by Area 3 (19·2), while the lowest incidence occurred in Area 4 (0·0) and Area 5 (0·7).

Sleeping Sickness in Areas 1–8 (1980–1984)

Using population data collected during a demographic survey in 1981–1982 (Wellde *et al.*, 1989*d*), attack rates for individuals in seven different age groups in eight different areas were calculated for the period 1980–1984 (Table 2A).

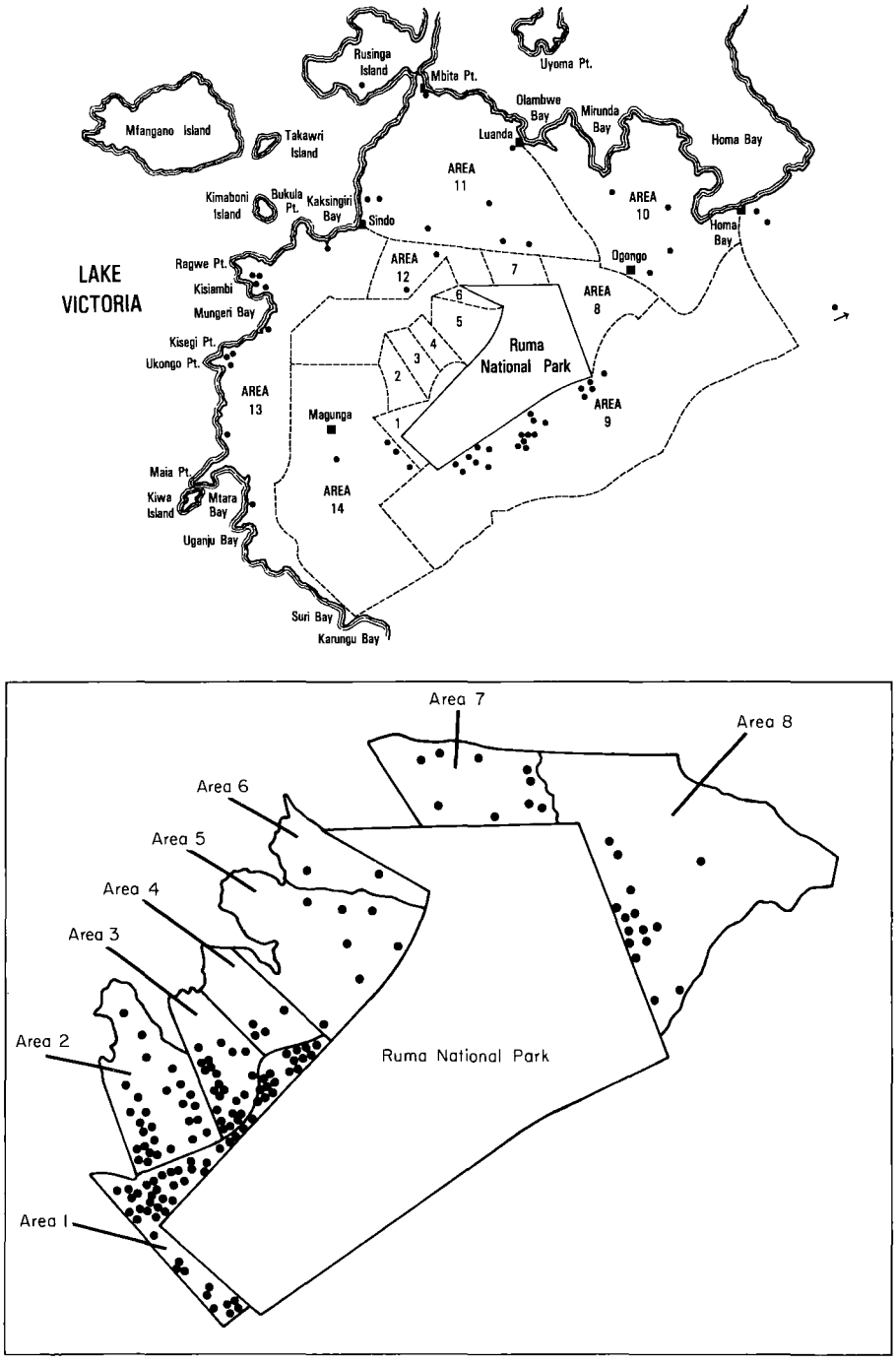

Fig. 5A and B. Distribution of Rhodesian sleeping sickness patients by place of abode during the most recent disease outbreak (1980–1984). Fig. 5A shows patients from Areas 9–14 and outlying areas, while B shows patients from within our study site, Areas 1–8. Each point represents a patient. → represents patient from Kamagambo.

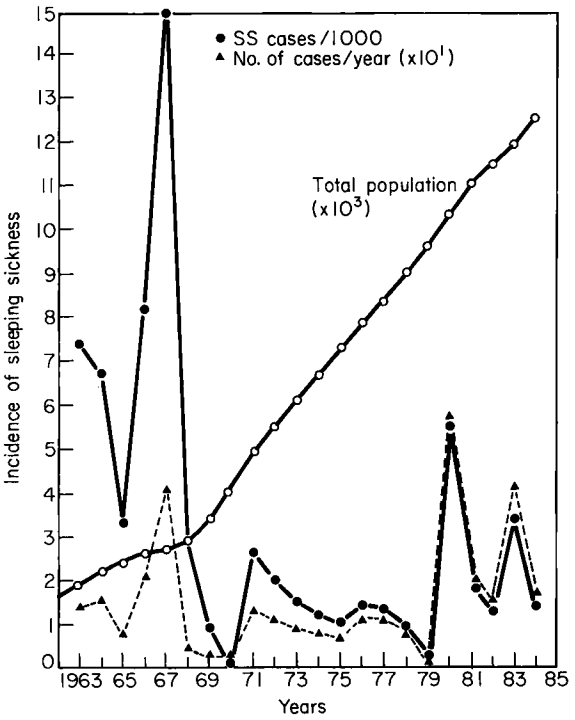

Fig. 6. Annual incidence of sleeping sickness in the Lambwe Valley study site (Areas 1–8). (●—●—●), SS cases; (▲—▲—▲), no. of cases/year ($\times 10^{-1}$).

Area 1, which had the longest border with the Ruma National Park and which was in closest proximity to the thicket, had the highest disease incidence of the eight areas (53·9‰) over the five-year period. In Area 1 the lowest incidence occurred in those under nine years of age (8·4‰) while the highest incidence of 125‰ was recorded in those individuals over 50 years of age. To test the significance of these observations, individuals were further grouped into three categories and Bartholemew's Test for Order was applied to the fractions (Table 2B). The results rejected the hypothesis that the three proportions were equal in favour of the ordered alternative hypothesis ($F_1 < F_2 < F_3$) at the $P < 0{\cdot}05$ level.

Incidence was also high in Area 3 where individuals in younger age groups (20–49) had the highest attack rates of sleeping sickness. Areas 4, 5 and 6, most distant from the *G. pallidipes*-infected thicket, had lower incidence. Area 7, on the northern perimeter of the National Park, had increased incidence partially due to the establishment of *G. pallidipes* in thickets outside the boundary of the National Park along the Lambwe River. While cases of sleeping sickness occurred on the perimeter of Area 8 and the Ruma National Park, the incidence of disease in this area was low because of the substantial human population located at a considerable distance from the thicket. Attack rates for the five-year period in the eight study areas combined were highest in the 40–49 year group (35·6‰), followed by the 30–39 year group (28·8‰). The lowest attack rate occurred in the 0 to nine year age group (1·6). In general, age distribution of sleeping sickness cases in males and females followed a similar pattern.

The ratios of infected males to infected females was less than one in Areas 1 and 7 and slightly over one in Area 8. All three Areas border on the National Park perimeter and are closest to the infected thicket. Areas more distant from the thicket had higher M/F ratios (Table 3). The overall ratio for the five-year period was 1·33.

TABLE 2A

*Age specific rates for Rhodesian sleeping sickness in study Areas 1–8 (1980–1984)**

Area	*0–9*	*10–19*	*20–29*	*30–39*	*40–49*	*50–59*	*60+*	*All ages*	*95%† C.L.*
1	8·4	70·9	39·4	87·4	116·9	125·0	125·0	53·9	40·5–67·3
2	2·7	16·3	27·7	25·0	19·6	13·2	13·0	13·8	8·80–18·9
3	0·0	41·3	115·9	98·0	161·3	0·0	0·0	44·5	26·7–62·3
4	0·0	21·4	8·7	0·0	0·0	0·0	0·0	6·1	0·1–12·1
5	0·0	0·0	4·1	28·1	19·2	0·0	0·0	4·7	1·2–8·2
6	0·0	5·1	0·0	0·0	0·0	30·3	0·0	2·6	0·0–6·2
7	3·8	10·3	9·2	39·5	19·2	0·0	31·2	11·9	4·1–19·7
8	0·0	5·2	4·5	5·4	8·2	11·1	5·9	3·9	2·0–5·9
Total	1·6	15·6	16·7	26·9	29·7	18·7	19·7	13·3	
95% C.L.†	0·3–2·8	11·0–20·2	13·7–19·7	17·1–36·7	17·2–42·2	6·6–30·8	6·9–32·5	11·2–15·4	

*Combined sexes—No. cases ‰ individuals.
†95% confidence limits.

TABLE 2B

Attack rates for Rhodesian sleeping sickness in three age groups in Area 1, 1980–1984 (pooled data)

	Age groups			
	0–9 (F_1)	*10–39 (F_2)*	*40+ (F_3)*	*Total*
Total population	355	574	165	1094
No. cases	3	36	20	59
Attack rates*	8·4	62·7	121·2	53·9

$\chi^2 = 29{\cdot}8$, C = 0·292, $P = < 0{\cdot}05$, ($F_1 < F_2 < F_3$).
* = ‰ individuals.

Distribution by Household

Figure 7 shows the distribution of sleeping sickness patients during 1963–1984, identified by household in the eight demographically defined study areas. Some households appeared to contain a disproportionate number of sleeping sickness patients. To test the significance of this observation, the actual distribution of patients was compared to an expected random distribution by the Poisson Goodness of Fit test (Table 4). The distribution of patients in four of the eight areas (1, 2, 5 and 6) was non-random. Data for Area 3 approached significant values, but in Areas 4, 7 and 8 the distribution of patients followed an expected distribution in households. The distribution in the eight areas combined was non-random. Analysis showed fewer households than expected with 0 and one patient, and more households than expected with three or more patients. Some households had histories of sleeping sickness extending over a 20-year period, while other households contained multiple cases who were all diagnosed within a relatively short period.

Distribution by Tribal Group (1980–1984)

The population in the study area consisted of two large tribal groups, Nilotic and Bantu, and attack rates for each group were compared in Areas 1–8. The chi square (χ^2) test was employed

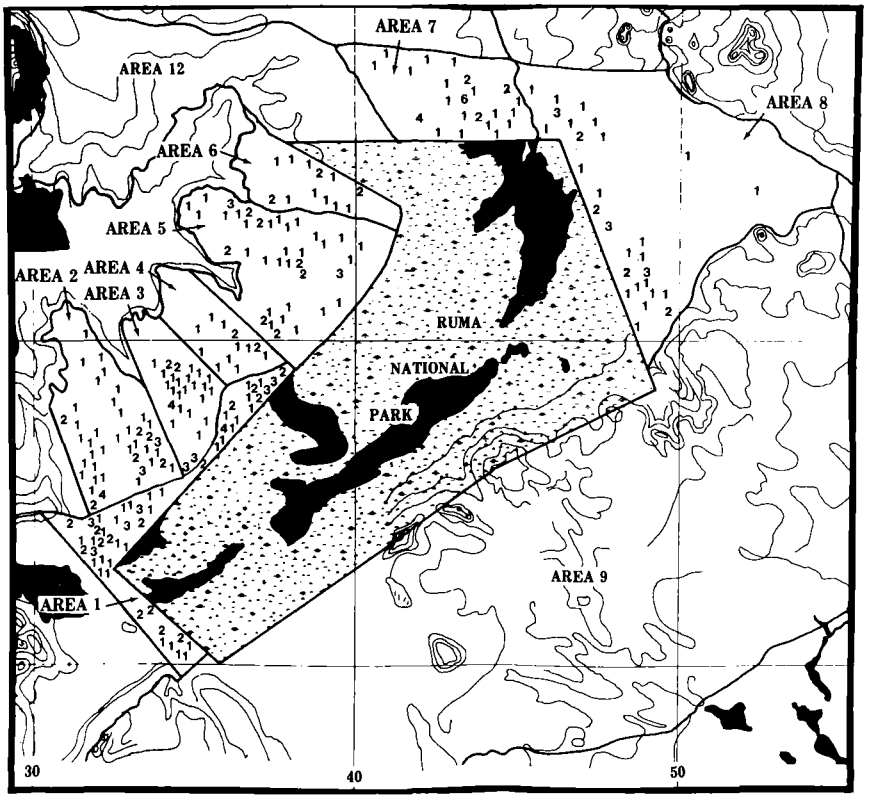

Fig. 7. Distribution of sleeping sickness patients within households (1963–1984). The numbers indicate patients in a single household. Households without patients have not been plotted.

TABLE 3

Sex ratios of Rhodesian sleeping sickness patients in Areas 1–8 (males/females) 1980–1984

	Age groups								
Area	*0–9*	*10–19*	*20–29*	*30–39*	*40–49*	*50–59*	*60+*	*Total*	*M/F ratio*
1	1/2	10/9	3/5	3/6	5/4	1/4	4/2	27/32	0·84
2	0/2	7/1	7/2	2/3	1/1	0/1	1/0	18/10	1·80
3	0/0	4/1	4/4	3/2	3/2	0/0	0/0	14/9	1·55
4	0/0	3/0	1/0	0/0	0/0	0/0	0/0	4/0	und.*
5	0/0	0/0	1/0	3/1	2/0	0/0	0/0	6/1	6·00
6	0/0	1/0	0/0	0/0	0/0	1/0	0/0	2/0	und.*
7	0/1	1/1	0/1	1/2	1/0	0/0	1/0	4/5	0·80
8	0/0	3/2	2/1	1/1	2/0	0/2	1/0	9/6	1·50
Total	1/5	29/14	18/13	13/15	14/7	2/7	7/2	84/63	1·33
M/F ratio	0·20	2·07	1·38	0·87	2·00	0·28	3·50	1·33	

*und. = undefined.

TABLE 4

Distribution of Rhodesian sleeping sickness patients by household within the eight study areas in the Lambwe Valley (1963–1984)

Area	No. households	No. of sleeping sickness patients per household							Statistics		
		0	1	2	3	4	5	6	χ^2	df	P value
1	125	72	31	15	6	1	0	0	6·99	2	0·030
2	220	179	32	6	2	1	0	0	4·77	1	0·029
3	64	38	21	2	2	1	0	0	5·20	2	0·074
4	71	59	10	2	0	0	0	0	0·71	1	0·400
5	195	158	28	7	2	0	0	0	5·79	1	0·016
6	101	89	9	3	0	0	0	0	5·16	1	0·023
7	88	66	17	3	0	1	0	1	1·94	1	0·164
8*	107	86	16	3	2	0	0	0	2·77	1	0·096
Total 1–8	971	747	164	41	14	4	0	1	74·8	2	0·000

*To prevent bias due to skewed distribution of cases in Area 8, only 107 households in closest proximity to the Ruma National Park were included.

except in areas where small numbers of patients required the use of Fisher's Exact Test (Table 5). The skewed distribution of the tribal groups within the study site and the higher attack rates in the population living at the southern end of the study site negated a reliable overall comparison between the two groups. The fairest comparison appeared to be that done for Area 1, where peridomestic transmission occurred throughout the area and where both tribal groups were represented by a substantial number of individuals. In this area there was no statistical difference in attack rates between the two ethnic groups ($\chi^2 = 0{\cdot}02$, $df = 1$, $P = 0{\cdot}89$).

The Relationship of Duration of Residence and Infection

The duration of residence in the Lambwe Valley prior to infection was studied in a group of former sleeping sickness patients who were either born in the Lambwe Valley or whose date of arrival in the area was known. Table 6A provides information regarding this group by age at the time of immigration. Older individuals appeared to acquire infection more rapidly, and to examine this finding a further age grouping of individuals was done (Table 6B) and an Analysis of Variance (Anova) performed. At least two of the three mean values differed. A comparison by the Protected Least Significant Difference Test showed all three means to be significantly different at the $P < 0{\cdot}05$ level. Thus, in this series of patients, older individuals appeared to require a shorter period of residence before acquiring sleeping sickness than did younger immigrants or those born in the Lambwe Valley.

Section III: Biochemical and Serological Analysis of Trypanosome Stocks

Trypanosome stocks isolated from man and from domestic and game animals in the Lambwe Valley have recently been analysed by isoenzyme electrophoresis (Gibson and Wellde, 1985). Twelve zymodemes were found in 136 stocks of *T. b. rhodesiense* which were examined, and 21% of the domestic cattle tested in the recent outbreak also harboured trypanosomes of these same zymodemes. Twenty-four other zymodemes of *T. brucei* ssp. were found in animals and tsetse flies but not in man. A comparison of zymodemes of *T. b. rhodesiense* which occurred in man

TABLE 5

Distribution of Rhodesian sleeping sickness patients between two tribal groups in the Lambwe Valley (1980–1984)*

Area		*Nilotic*	*Bantu*	*Total*	χ^2	*Fisher's exact test*	P
1	Total pop.	196	898	1094	0·02	—	0·89
	no. cases	11	48	59			
	attack rate†	56	53	54			
2	Total pop.	41	1981	2022	Invalid	Yes	0·11
	no. cases	2	26	28			
	attack rate†	49	13	14			
3	Total pop.	118	399	517	5·19	—	0·02
	no. cases	10	13	23			
	attack rate†	85	33	44			
4	Total pop.	217	439	656	0·12	—	0·73
	no. cases	1	3	4			
	attack rate†	4	7	6			
5	Total pop.	1011	462	1473	0·43	—	0·51
	no. cases	4	3	7			
	attack rate†	4	6	5			
6	Total pop.	501	265	766	Invalid	Yes	1·0
	no. cases	1	1	2			
	attack rate†	2	4	3			
7	Total pop.	719	34	753	Invalid	Yes	1·0
	no. cases	9	0	9			
	attack rate†	12	und.§	12			
8	Total pop.	3741	57	3798	Invalid	Yes	1·0
	no. cases	15	0	15			
	attack rate†	4	und.§	4			
8‡	Total pop.	848	7	855	Invalid	Yes	1·0
	no. cases	14	0	14			
	attack rate†	16	und.§	16			

*Nilotic = Luo, Bantu = Abasuba, Kisii and Luyha.
†Cases ‰ individuals.
‡Inhabitants of 100 households in closest proximity to the National Park in Area 8.
§und. = undetermined.

during the period 1970–1979 and those found during the recent disease outbreak (1980–1984) can be found in Table 7.

To better define relapse infections as opposed to reinfections, *T. b. rhodesiense* isolated during primary infections in 11 patients were compared to trypanosome stocks isolated at relapse. Enzyme profiles of trypanosomes from each patient were identical at primary infection and at relapse. Three zymodemes were represented in the trypanosome stocks (Table 8).

Prior to biochemical characterization, neutralization of *T. b. rhodesiense* stocks by specific antibody had been attempted. The results indicated that an antiserum prepared in cattle against one stock would always neutralize the homologous stock but would also on occasion neutralize other stocks of *T. b. rhodesiense* (Wellde *et al.*, 1983*a*). Once the zymodemes of these stocks became known, it appeared that the zymodeme classification was closely related to the neutralization test. In an attempt to investigate this relationship, 10 different trypansomes stocks comprising nine different zymodemes of *T. b. rhodesiense* were each inoculated into

TABLE 6A

Development of Rhodesian sleeping sickness in 174 individuals who were born in the Lambwe Valley or who immigrated there from other areas of Kenya

	Age at immigration						
	0–9	*10–19*	*20–29*	*30–39*	*40–49*	*50+*	*Mean*
Years to diagnosis of sleeping sickness (mean)	11·6*	6·9	6·8	6·0	3·1	4·9	7·3
s.d.	7·8	4·8	5·6	4·8	4·3	4·1	6·3
Range	1–48	1–17	0·5–23	0·5–18	0·5–16	0·5–13	0·5–48
No.	42	24	41	29	15	23	174

*Includes individuals born in the Lambwe Valley study site.

TABLE 6B

Pooled data from Table 6A

	Age at immigration		
	0–9	*10–39*	*40+*
Years to diagnosis of sleeping sickness (mean)	11·6	6·6	4·2
No.	42	94	38

young Boran steers and antisera were collected six weeks after infection. Neutralization tests were then done using each antiserum against the homologous and heterologous stocks (Table 9). With very few exceptions antiserum was specific for each of the separate zymodemes. Homologous mixtures of parasites and antiserum yielded no infections in mice. A partial reaction appears to have occurred between two antisera to zymodeme 74 and trypanosomes of zymodeme 23, and this requires further definition. However, the only complete cross-reaction occurred with antiserum to two different stocks of the same zymodeme (Z-74). It appeared from these preliminary results that in the Lambwe Valley most individual zymodemes of *T. brucei rhodesiense* represented separate serodemes.

DISCUSSION

The numbers of reported cases of Rhodesian sleeping sickness in the Lambwe Valley was markedly affected by tsetse control measures which included bush clearing, aerial and ground application of insecticides, and settlement of new immigrants whose agricultural methods prevented re-establishment of tsetse fly habitat in the area. The success of these methods in the late 1960s and early 1970s, especially in the northern Lambwe Valley and the westward extension (Roo Valley), gave impetus to the view that *G. pallidipes* could be eradicated from the entire valley. The establishment of a game reserve in an area scheduled for agricultural development in the southern half of the Lambwe Valley, however, thwarted the successful completion of an eradication programme. After the formation of the game reserve in the mid-1960s efforts were made to isolate the Park and its tsetse-infested thicket from the rapidly-developing human population in areas surrounding the game reserve. These efforts included

TABLE 7

Zymodemes of Trypanosoma brucei rhodesiense *found in a recent disease outbreak compared to those detected during the previous 10 years in the Lambwe Valley*

Zymodeme	*Pre-outbreak (1970–1979)*		*Recent outbreak (1980–1983)†*	
23	18*		4	
25	3		16	
74	0		35	
75	0	0%‡	18	73%‡
76	0		4	
77	0		5	
80	4		0	
87	4		1	
96	5		1	
98	4		0	
107	2		0	
130	1		1	
Total	41		85	

*No. of Rhodesian sleeping sickness patients with trypanosomes of particular Zymodeme.

†Isoenzyme types of trypanosome stocks isolated from man in 1984 were not determined.

‡Percentages of Zymodemes 74, 75, 76 and 77 in 1970–1979 and in 1980–1983.

TABLE 8

Isoenzyme type of T. b. rhodesiense *isolated from the blood during primary infection and at relapse in 11 sleeping sickness patients**

Patient no.	*Isoenzyme type at primary infection*	*Time to relapse (days)*	*Isoenzyme type at relapse*
LVH-61	Z-74	179	Z-74
LVH-62	Z-74	323	Z-74
LVH-75	Z-74	301	Z-74
LVH-76	Z-74	343	Z-74
LVH-77	Z-74	244	Z-74
LVH-78	Z-74	290	Z-74
LVH-79	Z-74	256	Z-74
LVH-82	Z-75	843	Z-75
LVH-89	Z-74	173	Z-74
LVH-94	Z-23	570	Z-23
LVH-112	Z-74	132	Z-74

*All 11 patients were treated with suramin, only one had analysis of CSF before treatment. All had abnormal CSF values at relapse.

TABLE 9

Results of neutralization tests: specificity of antisera for T. b. rhodesiense *stocks which represent homologous and heterologous zymodemes*

Anti-serum to stocks representing zymodeme nos	*Trypanosome stocks representing zymodeme nos*									
	96	*87*	*98*	*76*	*74*	*74*	*75*	*23*	*25*	*31*†
96	0–0*	3–5	2–5	3–5	3–5	2–5	4–5	3–5	4–5	2–5
87	3–5	0–0	3–5	4–5	3–5	2–5	3–5	3–5	2–5	3–5
98	4–5	3–5	0–0	6–5	3–5	3–5	4–5	3–5	5–5	3–5
76	3–5	3–5	2–5	0–0	3–5	2–5	4–5	3–5	3–5	2–5
74	4–5	3–5	2–5	4–5	0–0	0–0	4–5	7–3	4–5	2–5
74	4–5	4–4	3–4	4–5	0–0	0–0	4–4	8–4	4–5	2–5
75	3–5	3–5	2–5	3–5	3–5	2–5	0–0	3–5	4–5	4–5
23	3–5	4–5	3–5	6–5	3–5	3–5	4–5	0–0	3–5	2–5
25	4–5	4–5	3–5	4–5	3–5	3–5	5–5	6–5	0–0	5–5
31	3–5	4–5	3–5	4–5	3–5	4–5	4–5	5–5	3–5	0–0
Normal serum	3–5	3–5	2–5	4–5	3–5	2–5	3–5	3–5	2–5	2–5

*Median prepatent period—no. infected of five mice per group, homologous reactions underlined.

†Trypanosome stock isolated in Samia, Kenya, along the Uganda border. Trypanosomes representing Zymodeme 31 have never been found in the Lambwe Valley, but are common in Uganda.

the establishment of a perimeter barrier around the reserve and monthly applications of dieldrin along the perimeter. These measures limited the incidence of sleeping sickness in the human population to relatively low levels during the period from 1970 to 1974.

Reduced concern about the situation in the Lambwe Valley, due to the low incidence of disease and personnel changes in the tsetse control section, led to the cessation of control measures in 1974. The disease remained quiescent over the next five year period until an outbreak of sleeping sickness occurred in 1980. In general, poorly planned eradication and control programmes in subsequent years have also been constrained by the presence of the National Park, since ground control measures were not usually permitted within its perimeter (Wellde *et al.*, 1989*i*). Disease incidence in the 1980–1984 period was highest in areas in closest proximity to the tsetse-infested thicket along the perimeter of the National Park. The persistence of this focus of infection poses a danger to local farmers and tourists, and potentially to other areas in Kenya where suitable vectors of the disease are found.

Since the completion of our study in December 1984, there have been 26 reported cases of Rhodesian sleeping sickness in the Lambwe Valley regions. Eighteen cases were reported from the areas surrounding the Ruma National Park (Areas 1–9). The other eight cases reported from Area 13(2) and Area 14(6).

The classification of patients into three groups, primary, relapse and reinfection, was based on the patient's history, clinical parameters and, when possible, by the identification of the infecting trypanosome by determination of its isoenzyme profile. The distinction between primary infection and reinfection was relatively straight-forward. It seemed unlikely that patients would relapse as long as three years after treatment of their primary infections, on the basis of our patient follow-up during 1980–1984 (Wellde *et al.*, 1989*d*) and the experience of others (Apted, 1957). Most patients with relapse infections had been treated for sleeping sickness with suramin within the previous three years. The detection of trypanosomes in relapse patients was difficult and many were diagnosed on the basis of their clinical condition and CSF findings. In general, the laboratory findings regarding haematology and serum protein levels in relapse patients were milder than those of primary patients. However, as

opposed to primary patients, all relapse patients had abnormally high levels of leucocytes or total protein in their CSF, indicating central nervous system involvement which was usually accompanied by severe headache. Trypanosomes were isolated from 11 patients undergoing primary infection and again when these patients subsequently relapsed. When isoenzyme profiles were established for the stocks, we found that trypanosomes isolated during the primary infection in each patient were of the same zymodeme as those isolated at the time of relapse. Three different zymodemes were represented in the trypanosome stocks isolated from those 11 patients (Gibson and Wellde, 1985). This finding supports our contention that most relapse infections described in this study were true relapses and not reinfections.

In a study of the early outbreak of Rhodesian sleeping sickness in the Lambwe Valley, Southon (1962) reported that although the *G. pallidipes* was in a peridomestic habitat it appeared that the apparent risk of infection in man was about six times greater for males than females. In subsequent years, however, the ratios of male to female patients in the Lambwe Valley decreased. The sex ratio again increased during the period of major control operation in 1970–1973. This may have been the result of the destruction of much of the peridomestic habitat of the tsetse flies during this period and the transmission of disease in areas distant from homesteads. As the tsetse flies and disease were eradicated from the northern and western areas of the Lambwe Valley and became increasingly restricted within the boundaries of the National Park, increased numbers of individuals in the expanding population living in close proximity to the park became infected. The construction of a perimeter fence around the Park, and the more active pursuit of poachers who entered the National Park by increased numbers of game department staff, were probably responsible for lower sex ratios which occurred in mid-1970s.

In the most recent period (1980–1984) almost equal numbers of females and males were infected in Area 1, consistent with the finding that peridomestic transmission occurred in this area for much of the outbreak period (Otieno and Darji, 1985). Areas 7 and 8, at the northern perimeter of the National Park in close proximity to tsetse infested thicket, had almost equal numbers of infected male and female patients, while areas more distant from the thicket had a predominance of infected males.

In Area 1 we also found that older individuals were more likely to be infected with sleeping sickness than younger members of the population. The reduced numbers of infected individuals in the 0 to nine year age group when compared to older individuals suggested a possible aversion of tsetse to feed on children, the possibility of a greater infectivity threshold in the young, or a more cryptic infection in children. The age-stratified incidence showed that the levels of infection were highest in the older age groups. Willett (1965), describing an outbreak of Rhodesian sleeping sickness in Alego, Kenya, mentioned the apparent disparity of infections in children even though transmission was by peridomestic populations of *G. fuscipes*. Low infection rates in young children have usually been attributed to their low level of contact with tsetse fly vectors (Apted, 1970*a,b*), but in our study, where all members of the population appeared to be at similar risk in Area 1, the low infection rates in children are more likely due to one or a combination of the above factors.

The *G. pallidipes* population in the Lambwe Valley fluctuates in a pattern related to the rainfall (Lewis, 1936). Fly populations generally increased in March, coinciding with the beginning of the long rains, and reached a peak in April, May and June. Another peak in fly concentrations occurs in November or December in relation to the short rainy season. Increased numbers of infections in man were found in June and July, approximately two months after the peaks of fly concentration, and again in January. This two-month period represents the incubation period and time to diagnosis. With the absence of control measures at the start of the 1980 outbreak in association with a very high rainfall in April and May followed by a very dry June, conditions were conducive for a greatly increased fly population which dispersed from the National Park into the surrounding areas. No one, including

members of the Ministry of Health and tsetse control staff who had worked in the Lambwe Valley area for 20 years, could remember similar high levels of tsetse infestation outside the National Park perimeter to that seen in May and June, 1980.

During the 1980–1984 period zymodemes of *T. b. rhodesiense* which had not been encountered in the Lambwe Valley during the previous 10 year period appeared in both cattle and man (Gibson and Wellde, 1985). The emergence of these new zymodemes could in part explain both the high mortality in domestic cattle and the occurrence of the outbreak in man where the majority (73%) of infections were caused by four new zymodemes of *T. b. rhodesiense.* There was a close correlation between zymodeme and serodeme, as shown by neutralization tests, and should this relationship we have shown for *T. b. rhodesiense* in the Lambwe Valley be common to other endemic areas, the isoenyzme electrophoresis technique may become a more important epidemiological tool than previously recognized. During the past 17 years it appears that 12 zymodemes/serodemes of *T. b. rhodesiense* have infected man in the Lambwe Valley. Four of these not found prior to 1980 appeared during the most recent outbreak and accounted for most infections in man. In these circumstances, development of a practical vaccine for Rhodesian sleeping sickness seems remote.

The occurrence of multiple cases of sleeping sickness within one household over short periods of time may be due to a number of interrelated factors. Proximity of the household to the National Park, as well as the propensity for relatives to enter the park together to collect firewood and water or to poach animals, are likely to be important. Consumption of raw infected animal tissue is also a possible explanation for multiple infections in a family occurring over a short period of time. Mechanical transmission (Roberts *et al.*, 1989), or more likely interrupted feeding of infected tsetse flies (Jenni *et al.*, 1980), may also account for multiple infections apparent within a short time span. Sleeping sickness occurring in families over many years, however, could be due to environmental factors or some undetermined genetic influence. Simple genetic markers such as blood group and haemoglobin type were no different in sleeping sickness patients from control values (Wellde *et al.*, 1989*g*). Wyatt *et al.* (1985) has reported similar findings in Rhodesian sleeping sickness patients in Zambia. Based on his work in rodents, Ormerod (1985) believes that dietary lipid may be important for resistance to human African sleeping sickness. A high-density lipoprotein (HDL) present in normal human serum has been shown to be cytotoxic to certain populations of *T. brucei* ssp. (Rifkin, 1978). It seemed possible that some genetic abnormality in lipid metabolism could be responsible for increased numbers of sleeping sickness patients occurring in certain households in the Lambwe Valley. For instance, Tangiers disease, a genetic disorder, may result in a decrease (heterozygous) or absence (homozygous) of HDL. Preliminary results from Lambwe Valley study, however, do not indicate lower levels of HDL in families having increased incidence of sleeping sickness (Wellde *et al.*, unpubl. res.). The status of HDL levels in individuals at the time of infection, however, could play a role in resistance. On the other hand, since only certain zymodemes/serodemes of *T. brucei* ssp. infect man, resistance to HDL by these trypanosomes may play a more important role than levels of HDL in the host.

No convincing differences in attack rates between Nilotic and Bantu tribesmen were demonstrated in our studies in 1980–1984. We recognize, however, that intermarriage had occurred between these tribal groups in the Lambwe Valley. Buyst (1977) suggests that in some areas where man has been in contact with tsetse throughout history, selection of people with a more efficient immune response to trypanosomes has occurred. He compared Europeans and Nilotics in some areas, who usually develop an acute course of disease, to Bantu-speaking people who more frequently develop a more subacute course of disease and who have had historical contact with tsetse fly areas and presumably trypanosomiasis. In the sleeping sickness patients from the Lambwe Valley we could not associate virulence of infection with ethnic origins since variation in duration of infection before diagnosis, the presence of trypanosomes of different zymodemes/serodemes of potentially different virulence, and the

patients' nutritional status and concomitant infections, made associations of this type tenuous in the Lambwe Valley patients.

Large numbers of domestic cattle in the study area had high infection rates of *T. brucei* ssp. (Wellde *et al.*, 1989*k*). Analysis of 51 trypanosome stocks isolated from cattle in the endemic area indicated that 21% were identical to trypanosome zymodemes found concurrently in man (Gibson and Wellde, 1985). Cattle were kept within each family boma at night in close proximity to family members and undoubtedly played a major role in the epidemiology of the disease in the area.

Cattle infected with *T. b. rhodesiense* (zymodeme 25) which were ear-tagged in the Lambwe Valley were found on the Lake Victoria shore outside the valley where 11 cases of Rhodesian sleeping sickness (all trypanosome stocks were zymodeme 25) occurred in areas devoid of *G. pallidipes* but which harboured low-level infestations of *G. fuscipes*. These cattle had been moved to the lake shore as a result of trading or movement of the animals for their protection. This illustrates the danger of spread of Rhodesian sleeping sickness by the movement of infected animals to other areas where appropriate tsetse fly vectors are common. To our knowledge no restrictions have been made to limit the sale and transportation of domestic animals from the Lambwe Valley to other areas in Kenya. The transfer of game animals captured in the Lambwe Valley to other game parks located in Kenya or other African countries should certainly not be contemplated or permitted.

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