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Trypanosomiasis in domestic livestock in the Lambwe Valley area and a field evaluation of various diagnostic techniques

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A preliminary survey of 2 073 domestic animals in the Lambwe Valley, Kenya, showed a 7.4 % rate of infection with Trypanosoma congolense *and* T. vivax. *In comprehensive surveys covering 6 384 domestic stock, pathogenic trypanosomes were found in 17.0 % of cattle, 5.0 % of sheep, and 2.1 % of goats. Adults were more often infected than young animals, and males more often than females.* T. congolense *was the trypanosome most frequently diagnosed, followed by* T. vivax *and the* T. brucei *subgroup.* T. theileri *was also found. The examination of wet blood films in the field as a means of diagnosing trypanosome infections was shown to be valueless. More infections were detected in peripheral blood films than in systemic blood films, but both should be examined. An examination of smears of glandular fluid is essential for the diagnosis of* T. vivax *in cattle, while mouse-inoculation tests are necessary for the diagnosis of the* T. brucei *subgroup. The detection of* T. vivax *was improved by the high-speed centrifugation of blood samples in capillary tubes.*

The tsetse fly *Glossina pallidipes* Aust. was first recorded in the Lambwe Valley area of western Kenya in 1910, probably in the western end of the Roo Valley (E. A. Lewis, quoted by Ford, 1971). At that time there were large populations of man and cattle in the Lambwe Valley, and it is presumed that infestations of *G. pallidipes* were limited to suitable habitats on high ground above the lowland settlement areas.

Some time after 1915 much of the area was abandoned, probably mainly because the human population was severely exposed to *Trypanosoma gambiense* infection, transmitted by lacustrine populations of *G. fuscipes*, and to malaria. A continuing consequence of this depopulation was the spread of *G. pallidipes* into the lowland area, particularly the Olambwe River flood plain, where thickets providing suitable habitats for *G. pallidipes* were colonizing abandoned farmland. That is the situation at the present time. The residual cattle population appears to have been very severely affected by animal trypanosomiasis (see Ford, 1971).

During the period 1955–57, Gambian sleeping sickness was largely eradicated from the Lambwe area (Glover, 1962) and, under a 1959 settlement scheme, people and livestock started to repopulate the area; this trend is still continuing.

This paper presents the results of surveys for animal trypanosomiasis carried out in the Lambwe Valley area during the period 1968–71. The surveys were designed to provide data on the incidence and epizootiology of trypanosomiasis in the large and increasing livestock population. It must be borne in mind, however, that, throughout the course of the investigation, domestic animals were indiscriminately treated with trypanocidal drugs both by staff of the Veterinary Services Division of the Ministry of Agriculture for Kenya, and, illegally, by stockowners themselves.

The comparative merits of the various diagnostic techniques employed to detect trypanosome infections in livestock are also discussed.

THE STUDY AREA

Surveys for animal trypanosomiasis were made in the Lambwe Valley area of the South Nyanza District of western Kenya. A general description of the area as

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a whole is given by Allsopp & Baldry (1972) and the boundaries of the survey areas are shown on a map accompanying that article.

MATERIALS AND METHODS

Sampling methods

During the preliminary survey of the north-eastern end of the Lambwe Valley, settlements with livestock were sampled at random, half the total stock being examined in each selected settlement. Subsequently, when investigations were conducted in the defined survey areas, all settlements were visited and, as far as possible, all livestock were examined.

Specimens examined

For the preliminary survey, a smaller range of samples was collected from livestock than during the subsequent more detailed surveys. Specimens taken during the preliminary survey were as follows: (1) ear-vein blood for the preparation of thick and thin blood films (cattle, sheep, and goats); (2) ear-vein blood for examinations of wet blood films (cattle only); and (3) prescapular gland fluid for the preparation of stained smears (cattle only).

Thick and thin blood films and glandular smears were stained with Giemsa stain. Using a microscope fitted with a × 100 oil-immersion objective, 200 fields were examined for trypanosomes. No attempts were made to isolate trypanosomes by animal inoculation techniques.

Specimens collected from stock during the first three detailed surveys were as follows: (1) ear-vein blood for the preparation of thick and thin blood films (cattle, sheep, and goats); (2) 0.5 ml of jugular blood for inoculation into mice (cattle, sheep, and goats); and (3) prescapular gland fluid for the preparation of stained smears (cattle only).

During the Masangala survey, and subsequently, ear-vein blood was used only for preparing thick blood films, and jugular vein blood was used for preparing both thick and thin blood films. Blood films and smears were stained and examined as described above.

Mice inoculated with blood from livestock were examined on alternate days for a period of at least 40 days.

RESULTS OF THE PRELIMINARY SURVEY IN THE NORTH-EASTERN LAMBWE VALLEY

Between August 1967 and April 1968, survey teams visited 146 homesteads and examined 1 407 cattle, 161 sheep, and 505 goats, in an area of some 150 km² lying between the Olambwe River and the Kaniamwia Escarpment, north of the Lambwe Field Station.

The overall rate of infection with pathogenic trypanosomes in the three species of domestic animal was 7.4% (154 infections in 2 073 animals). This level of infection would be expected in a locality where *G. pallidipes* infestations were limited to a small part of the whole sample area. Individual infection rates were as follows: cattle, 9.6% (135 infections); sheep, 1.9% (3 infections); goats, 3.2% (16 infections).

Of the 135 infected cattle, 81 (60.0%) were infected with *T. congolense*, 52 (38.5%) with *T. vivax*, and 2 (1.4%) with mixed *T. congolense* and *T. vivax*. Since it was not possible to carry out animal inoculation tests, no data on *T. brucei* infections were obtained. Of the 3 infections found in sheep 2 were caused by *T. vivax* and 1 was by *T. congolense*. Among the goats, 12 were infected with *T. congolense*, 3 with *T. vivax*, and 1 with mixed *T. congolense* and *T. vivax*.

During this investigation, the opportunity was taken to make a preliminary assessment of various diagnostic techniques. Since infections in sheep and goats were diagnosed only by means of blood examinations, diagnostic techniques were evaluated for cattle only. Although 137 infections were detected in cattle by a combination of diagnostic techniques, only 114 infections (83.1%) were found by the examination of thick blood films, and only 32 (23.3%) by the examination of wet blood films. These data show clearly that the examination of wet blood films in the field has little value in the diagnosis of bovine trypanosomiasis.

The number and proportions of *T. congolense* and *T. vivax* infections diagnosed in cattle by means of various techniques (Table 1) demonstrated that examinations of glandular fluid are essential. If glandular fluid had not been examined during the survey, 31.5% of all *T. vivax*, and 7.2% of all *T. congolense*, infections would have been missed.

RESULTS OF COMPREHENSIVE SURVEYS IN DEFINED AREAS OF THE LAMBWE VALLEY

The eight areas involved in these surveys have been defined by Allsopp & Baldry (1972); they were surveyed between 1968 and 1970 on the dates shown in the following tabulation:

area A	Obaluanda	18 June–10 September 1968
area B	Kaksingiri	10 September–20 December 1968

Table 1. Preliminary assessment of different methods for detecting trypanosomes in cattle, northern Lambwe Valley, August 1967 to April 1968

Sample	*T. congolense*		*T. vivax*		All infections	
	No.	%	No.	%	No.	%
blood alone	35	42.2	18	33.3	53	38.7
blood + glandular fluid	42	50.6	19	35.2	61	44.5
glandular fluid alone	6	7.2	17	31.5	23	16.8
totals	83	100.0	54	100.0	137	100.0

area C West Ruma 16 April–3 May 1969
area D Otuok 16 April–11 June 1970
area E Wiga 21 August 1969–30 January 1970
area F Magunga 3 March–29 July 1970
area G Masangala 23 July–31 July 1969
area H Escarpment 5 August–6 August 1970

All homesteads with domestic stock were visited, and all stock were examined. The total number was 6 384, of which 3 695 were cattle, 402 sheep, and 2 287 goats. Of the 6 384 animals examined, 723 (11.3%) were infected with pathogenic trypanosomes, including those of the *T. brucei* subgroup, and 37 (0.6%) were infected with the nonpathogenic species *T. theileri*. The latter was only found in cattle and the incidence was 1.0%. Individual infection rates with pathogenic trypanosomes were as follows: cattle, 17.0% (654 infections); sheep, 5.0% (20 infections); goats, 2.1% (49 infections).

T. congolense was found in 6.4% of all animals examined and was the most common trypanosome; *T. vivax* was found in 4.0% and the *T. brucei* subgroup in 3.0% of all animals. There were 107 cases of double infection and 13 cases of triple infection.

The detailed results, by trypanosome species and by age and sex of the animals, are given in Table 2 (cattle), Table 3 (sheep), and Table 4 (goats). The tables show that adult cattle had higher infection rates than did calves (19.7% against 12.0%). However, this was to be expected since adult cattle tended to graze further from the homestead and were probably more exposed to *G. pallidipes* populations. Male cattle had a higher infection rate than females (19.6% against 15.8%). This was particularly noticeable in the adults, probably because adult male and castrated cattle were subjected to more physical stress by being used for ploughing. The overall infection rate of 17% in cattle was rather higher than expected, considering the widespread indiscriminate treatment of cattle with trypanocidal drugs.

Table 2. Pathogenic trypanosome infections found in cattle in defined survey areas in the Lambwe Valley

	T. congolense alone	*T. vivax* alone	*T. brucei* alone	*T. congolense* + *T. vivax*	*T. congolense* + *T. brucei*	*T. vivax* + *T. brucei*	*T. congolense* + *T. vivax* + *T. brucei*	Total infected	Total examined
Calves									
male	16	30	8	1	1	4	0	60	440
female	19	22	5	3	3	7	0	59	547
total	35	52	13	4	4	11	0	119	987
Adults									
male	74	49	28	3	16	10	4	184	805
female	128	62	33	9	23	15	6	276	1 568
castrates	49	11	8	0	3	2	2	75	335
total	251	122	69	12	42	27	12	535	2 708
Grand total	286	174	82	16	46	38	12	654	3 695

Table 3. Pathogenic trypanosome infections found in sheep in defined survey areas in the Lambwe Valley

	T. congolense alone	*T. vivax* alone	*T. brucei* alone	*T. congolense* + *T. vivax*	*T. congolense* + *T. brucei*	*T. vivax* + *T. brucei*	*T. congolense* + *T. vivax* + *T. brucei*	Total infected	Total examined
Lambs									
male	1	1	0	0	0	0	0	2	39
female	0	0	0	0	0	0	0	0	45
total	1	1	0	0	0	0	0	2	84
Adults									
male	2	1	0	1	1	0	1	6	89
female	7	2	2	0	1	0	0	12	229
total	9	3	2	1	2	0	1	18	318
Grand total	10	4	2	1	2	0	1	20	402

Adult sheep and goats were also more frequently infected than their young, i.e., infection rates in adult sheep and lambs were 5.7% and 2.4%, respectively, and in adult goats and kids 2.8% and 0.3%, respectively. In sheep and goats there was very little difference in infection rates between the sexes, being 6.2% and 4.4% in male and female sheep, and 1.8% and 2.3% in male and female goats, respectively.

Trypanosome infections in cattle, sheep, and goats are analysed according to defined survey areas in the Lambwe Valley in Tables 5, 6, and 7. The data show that, in general, infection rates rise towards the southern end of the valley, with the Otuok and Escarpment areas having very high (over 60%) infection rates. These findings are discussed in more detail later.

AN ASSESSMENT OF THE VALUE OF DIFFERENT METHODS USED TO DETECT TRYPANOSOME INFECTIONS IN LIVESTOCK

Results from surveys in defined areas

During the surveys of animal trypanosomiasis in defined areas of the Lambwe Valley, the opportunity was taken to make final comparisons of the various diagnostic methods used during the preliminary survey in the northern part of the valley, including also the mouse-inoculation technique (Robson & Ashkar,

Table 4. Pathogenic trypanosome infections found in goats in defined survey areas in the Lambwe Valley

	T. congolense alone	*T. vivax* alone	*T. brucei* alone	*T. congolense* + *T. brucei*	Total infected	Total examined
Kids						
male	1	0	0	0	1	231
female	0	0	1	0	1	379
total	1	0	1	0	2	610
Adults						
male	7	2	0	0	9	373
female	25	5	6	1	37	1 283
castrates	1	0	0	0	1	21
total	33	7	6	1	47	1 677
Grand total	34	7	7	1	49	2 287

Table 5. Trypanosome infections in cattle analysed according to defined survey areas in the Lambwe Valley

Survey area	No. of cattle examined	Total no. and percentage infected with pathogenic trypanosomes		*T. congolense*		*T. vivax*		*T. brucei* subgroup		*T. theileri*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Obaluanda	411	49	11.9	26	6.3	16	3.9	12	2.9	0	0
Kaksingiri	516	48	9.3	22	4.3	15	2.9	13	2.5	1	0.2
W. Ruma	118	24	20.3	12	10.2	13	11.0	5	4.2	0	0
Otuok	177	115	65.0	68	38.4	41	23.2	69	39.0	4	2.3
Wiga	1 553	183	11.8	102	6.6	69	4.4	25	1.6	26	1.7
Magunga	887	218	24.6	122	13.7	84	9.5	49	5.5	5	0.4
Masangala	14	5	35.7	0	0	5	35.7	0	0	1	7.1
Escarpment	19	12	63.1	8	42.1	0	0	5	26.3	0	0
totals	3 695	654	17.0	360	9.7	243	6.6	178	4.8	37	1.0

1972). Of all *T. congolense* infections diagnosed, 87.5% were detected by blood examination alone. Only 43.3% of all *T. congolense* strains became established in mice, but if this test had not been made, 12.0% of infections would have been missed.

The value of glandular smear examinations in the detection of *T. vivax* infections was very evident. If this technique had not been employed in cattle, 27.6% of all infections would have been missed. Altogether, 79.8% of the *T. vivax* infections were diagnosed from glandular smears. No infections were diagnosed by mouse inoculation, thus emphasizing once again the difficulty of infecting mice with *T. vivax*.

In an attempt to improve the detection of *T. vivax*, blood from cattle, sheep, and goats was centrifuged at high speed in microcapillary tubes before it was examined. This treatment resulted in a 46.1% improvement over the routine examination of blood from ear veins and the jugular vein of cattle. There was a 200.0% improvement in sheep and goats, but as the numbers involved were very small, this result should be accepted with some reservation (Robson & Rickman, 1972).

Since 94.8% of all the *T. brucei* subgroup infections

Table 6. Trypanosome infections in sheep analysed according to defined survey areas in the Lambwe Valley

Survey area	No. of animals examined	Total no. and percentage infected		*T. congolense*		*T. vivax*		*T. brucei* subgroup	
		No.	%	No.	%	No.	%	No.	%
Obaluanda	64	2	3.1	2	3.1	0	0	0	0
Kaksingiri	42	0	0	0	0	0	0	0	0
W. Ruma	3	0	0	0	0	0	0	0	0
Otuok	33	11	33.3	8	24.2	5	15.1	3	9.1
Wiga	118	1	0.8	0	0	0	0	1	0.8
Magunga	133	6	4.5	4	3.0	1	0.7	1	0.7
Masangala	9	0	0	0	0	0	0	0	0
Escarpment	0	0	0	0	0	0	0	0	0
totals	402	20	5.0	14	3.5	6	1.5	5	1.2

Table 7. Trypanosome infections in goats analysed according to defined survey areas in the Lambwe Valley

Survey area	No. of animals examined	Total no. and percentage infected		*T. congolense*		*T. vivax*		*T. brucei* subgroup	
		No.	%	No.	%	No.	%	No.	%
Obaluanda	512	8	1.6	7	1.4	1	0.2	0	0
Kaksingiri	433	7	1.6	7	1.6	0	0	0	0
W. Ruma	71	2	2.8	1	1.4	0	0	1	1.4
Otuok	86	9	10.5	5	5.8	1	1.7	4	4.6
Wiga	802	7	0.9	6	0.7	0	0	1	0.1
Magunga	368	15	4.1	9	2.4	5	1.4	1	0.3
Masangala	6	0	0	0	0	0	0	0	0
Escarpment	9	1	11.1	0	0	0	0	1	11.1
totals	2 287	49	2.1	35	1.5	7	0.3	8	0.3

were diagnosed by the inoculation of blood into mice, and 81.7% by mouse-inoculation tests alone, the great value of mouse inoculations in the detection of this trypanosome is apparent.

A total of 37 cases of *T. theileri* infection were diagnosed, all in cattle. All but one case (97.3%) were diagnosed by the examination of blood films alone; the remaining case was detected by examining glandular fluid alone.

Hornby & Bailey (1931) showed that more trypanosomes could be detected in the peripheral blood than in the systemic blood of livestock. During the Lambwe Valley surveys it was possible to confirm this observation on a larger scale. Thick films were made from ear-vein (peripheral) and jugular vein (systemic) blood from 2 641 cattle, 288 sheep, and 1 259 goats. Examination of the blood films showed that 8.6% more infections could be diagnosed from an examination of ear-vein blood. There were variations according to the species of trypanosome involved, but more infections were always detected in peripheral blood than in systemic blood (Robson & Ashkar, 1972).

DISCUSSION

Trypanosomiasis in domestic stock in the Lambwe Valley

Broadly speaking, the farther south in the valley, the higher the overall trypanosome infection rate in livestock, with the Otuok and Escarpment areas having infection rates exceeding 60%. At the time the surveys were conducted, settlements in both these areas were comparatively recent and the degree of stock–tsetse contact was probably higher than in any of the other areas surveyed. In this connexion, England & Baldry (1972) have found that trypanosome infection rates in *G. pallidipes* were higher in the southern than in the northern part of the valley. They report that there was a mean infection rate for the Obaluanda/East Ruma area of 10.4% and infection rates varying from 17.6% to 30.9% in the vicinity of the Rari and Otuok thickets, both of which were inside the Otuok/Escarpment survey areas. Both the veterinary and the entomological findings indicate that stock–tsetse contact in the southern areas was very much closer than it was farther north.

Within the survey areas it was not possible to define any grouping of homesteads with infected animals except that, as would obviously be expected, animals belonging to settlements closer to the Otuok thicket, with its large population of *G. pallidipes*, tended to have higher infection rates than did those of more distant settlements.

T. congolense was the most prevalent trypanosome found in cattle and was present in all the areas surveyed, except in the Masangala area where there were very few cattle. The overall infection rate was 9.7% varying from 4.3% to 42.1%. Adults were more frequently infected than calves (11.3% and 4.3%, respectively). Adult male and castrated cattle were more often infected than adult females, probably

because these animals spent more time close to *G. pallidipes* habitats while being used to plough the more fertile, but more heavily tsetse-infested, lowland regions of the valley.

T. vivax was diagnosed in cattle in all areas except the Escarpment and the overall infection rate was 6.6%, varying from 2.9% to 35.7%. A slightly higher proportion of calves than of adults was infected, suggesting that the calves may have acquired some of their infections by mechanical transmission from biting flies other than tsetse as it is well known that *T. vivax* can be transmitted by mechanical means. Calves were kept closer to the homestead than were adult cattle, and consequently were more exposed to *Stomoxys* spp. and Tabanidae, which were often very numerous in and around unhygienic cattle kraals and the shelters used for sheep and goats.

The *T. brucei* subgroup was diagnosed in cattle in all areas except the Masangala area. The overall infection rate was 4.8% varying from 1.6% to 39.0%. The latter figure, for the Otuok area, is extremely high. Adult cattle in the valley as a whole were more heavily infected than calves (5.5% and 2.8%, respectively).

Since the *T. brucei* subgroup was more frequently diagnosed in double infections than would have been expected, and was found in single infections less frequently than expected, it would appear that single infections with the *T. brucei* subgroup are normally present at too low a grade to be found in a single day by one subinoculation. But when another infection is superimposed on the *T. brucei* infection the animal's resistance is lowered, and the *T. brucei* subgroup infection reaches a level at which it can be diagnosed by the methods used during the surveys reported here.

After the blood incubation infectivity test (BIIT) was evolved by Rickman & Robson (1970a, 1970b), it was possible to differentiate *T. brucei* (*sensu stricto*) from *T. rhodesiense*. Out of 133 *T. brucei* subgroup isolates from cattle in the Lambwe Valley tested by this method, 14 (10.5%) were shown to be *T. rhodesiense* (Robson et al., 1972), thus not only confirming the findings of Onyango et al. (1966) but also emphasizing the fact that cattle can constitute a very serious reservoir of human sleeping sickness.

The overall trypanosome infection rate in sheep was 5.0% and for the first time one *T. rhodesiense* infection was diagnosed by the BIIT. The data presented in Table 6 show that 55.0% of all ovine infections were in the Otuok area, where, according to England & Baldry (1972), the highest infection rates in *G. pallidipes* were recorded. If the number of infections in the Otuok area are subtracted from the total number of ovine infections, the overall infection rate decreases to 2.4%. This value is closer to the expected rate under the local conditions, where sheep probably rarely come into contact with tsetse and where, according to England & Baldry (1972), *G. pallidipes* rarely feeds on sheep.

In goats, the overall infection rate was 2.1%, the highest rates being in the Otuok and Escarpment areas. It is apparent that if goat–tsetse contact is sufficiently close, these animals could be important reservoirs of *T. rhodesiense*, since in the Otuok area a *T. brucei*/*T. rhodesiense* " intermediate " strain was identified by the BIIT (Robson et al., 1972).

Comparison of different diagnostic techniques

The results of studies on different techniques for the diagnosis of trypanosomiasis were so clear-cut that little discussion is required; some brief comments are, however, justified.

The investigations showed clearly that the microscopic examination of wet blood films as a routine diagnostic technique in the field is of very limited value and is probably only of use as a preliminary screening test for obviously sick animals. In contrast, the examination of stained smears of glandular fluid was particularly useful in detecting a great number of trypanosome infections, particularly those involving *T. vivax*. Since laboratory rodents are refractory to infection with *T. vivax*, this finding is of considerable importance and clearly indicates that, whenever possible, surveys for animal trypanosomiasis should include an examination of glandular fluid. With respect to the examination of blood films, a considerably higher number of *T. vivax* infections can be detected if the blood is first centrifuged in microcapillary tubes.

Our studies have confirmed the findings of other authors that more infections can be detected in peripheral than in systemic blood samples; and mouse-inoculation studies showed this method of diagnosis to be absolutely essential if all *T. brucei* and *T. rhodesiense* infections are to be detected.

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RÉSUMÉ

TRYPANOSOMIASE CHEZ LE BÉTAIL DANS LA VALLÉE DE LA LAMBWE; ÉVALUATION SUR LE TERRAIN DE DIVERSES TECHNIQUES DE DIAGNOSTIC

Au cours d'une enquête préliminaire portant sur 2073 animaux domestiques dans la vallée de la Lambwe, on a décelé 7,4% de cas d'infection par *Trypanosoma congolense* ou *T. vivax*. L'examen de préparations de sang non colorées s'est révélé sans valeur diagnostique; en revanche, l'étude de préparations de suc ganglionnaire colorées au Giemsa a fait la preuve de son efficacité pour la recherche des trypanosomes.

On a étendu les investigations à huit régions de la vallée où tout le bétail a été examiné. Sur 6384 animaux, 723 (11,3%) étaient porteurs de trypanosomes pathogènes. Les taux d'infection atteignaient 17,0% chez les bovins, 5,0% chez les moutons et 2,1% chez les chèvres. Parmi les trypanosomes identifiés, *T. congolense* était le plus fréquemment rencontré (6,4%), suivi par *T.* vivax (4,0%) et les trypanosomes du sous-groupe *T. brucei* (3,0%). *T. theileri* a été décelé chez 1% des bovins. Les animaux adultes étaient plus souvent parasités que les jeunes et, en général, les mâles que les femelles.

L'examen de frottis de sang colorés au Giemsa a permis à lui seul de diagnostiquer 87,5% des infections à *T. congolense*, 74,1% des infections à *T. vivax* et 18,3% des infections par des trypanosomes du sous-groupe *T. brucei*. Chez les bovins, l'examen du suc ganglionnaire a fait à lui seul découvrir 27,6% des infections à *T. vivax*. La centrifugation du sang a accru dans une proportion notable la fréquence des examens positifs. L'inoculation de sang à la souris a permis de déceler 94,8% de l'ensemble des infections par des trypanosomes du sous-groupe *T. brucei* et dans 81,7% des cas le diagnostic a été établi par cette seule technique. Par l'examen de frottis de sang périphérique (prélevé à l'oreille) on a diagnostiqué davantage (8,6%) d'infections que par l'examen de sang prélevé à la veine jugulaire.

L'épreuve d'infectivité après incubation en présence de sang humain a révélé la présence de *T. rhodesiense* chez 14 bovins et chez un mouton, cependant qu'une souche « intermédiaire » était isolée chez une chèvre.

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