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RESEARCH ON IMMUNIZATION AGAINST AFRICAN SLEEPING SICKNESS, (U)

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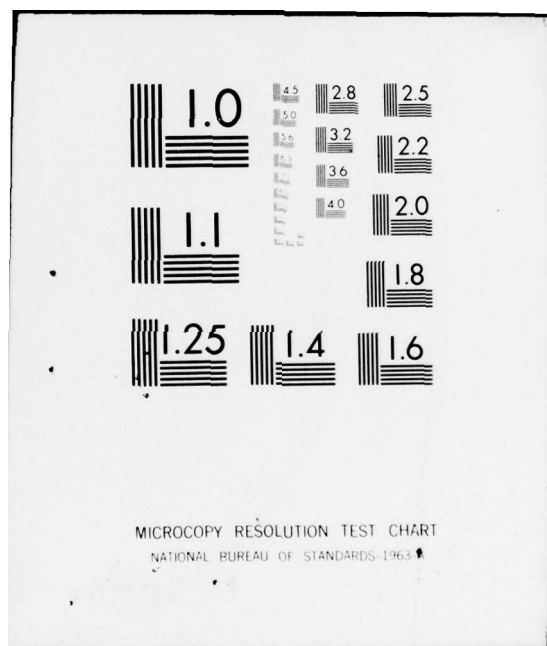


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RESEARCH ON IMMUNIZATION AGAINST
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African trypanosomiasis is an important disease of man and domestic animals on the African continent involving the area 15° North -29° South latitude (27). This Glossina (tsetse fly) infested area encompasses a land mass of approximately 4 million square miles. The disease of man, commonly referred to as African sleeping sickness, is present in endemic foci throughout this area making it hazardous or uninhabitable for humans. Insect vectors capable of transmitting the human disease, however, occur over a greater area increasing the potential for spread of this disease. Historically, large scale outbreaks occurred following the introduction of the trypanosomes into a previously unexposed population (5,15). During the period 1896-1906, over half a million people died in the Congo basin as the disease spread from the mouth of the river. During the epidemic of 1900-1910 in Uganda near Lake Victoria, an estimated 200,000 people were killed by the disease. More recently, in the 1930-1940 epidemic in East Africa, an estimated 11,500 human deaths were reported. Epidemics were followed by the establishment of persistent endemic foci. The current toll of more than 10,000 new cases reported annually, is considered to be an underestimate (3,4).

Animal trypanosomiasis is far more widely distributed. It may preclude or greatly reduce the production of domesticated animals for food or draft purposes. This deficit contributes to nutritional deficiencies and restricts social development (26).

In view of the increased awareness of our government in the African continent and the military mission to maintain a readiness

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capability to deploy and maintain effective military forces in any area of the world, the DOD supports research programs investigating various aspects of this problem.

There are 2 species of trypanosomes which cause human trypanosomiasis: Trypanosoma rhodesiense and Trypanosoma gambiense. Differentiation is based on the clinical syndrome as the parasites are morphologically indistinguishable (12,17). The Rhodesian form is a more acute disease which usually terminates in 1-12 months if not treated. It is characterized by recurrent fever, headache, irritability, insomnia, anemia, local edema with rapid development of congestive heart failure and often neurologic disorders. Gambian trypanosomiasis generally follows a less acute clinical course but terminates fatally when the central nervous system (CNS) becomes affected. Clinical features include marked lymph node enlargement, drowsiness, headache and ataxia. In some patients onset of CNS disease is delayed 2-6 years after demonstration of parasitemia.

A limited number of relatively toxic drugs administered by injection are available for treatment of the disease in man (1,19,20, 21, 31). Instances of drug resistance have been reported for all drugs now in use (31). Treatment with Suramin (Bayer 205) or pentamidine is fairly effective for either the Rhodesian or the Gambian form before CNS involvement has occurred. However, because CNS penetration of trypanosomes may occur early, additional follow-up treatment with drugs able to cross the blood - brain barrier is recommended. To cross this barrier, the arsenically based melaminyl compounds are now being used. Hospitalization is required for therapy because of the often severe nervous irritation and other toxic effects associated with this class of drugs (20,31).

Control of the disease has been directed toward reduction of contact between flies and man and by chemoprophylaxis. Fly numbers have been reduced by bush clearing and insecticide application. Avoidance of endemic areas has been practiced, though population movements away from such areas may be responsible for early dissimulation of the human reservoir (14).

Chemoprophylaxis has been widely used in West and Central Africa with pentamidine being the drug of choice. Because of its serum binding property, pentamidine prophylaxis for the Gambian type is effective for at least 6 months (23). Suramin also has prophylactic properties for approximately 3 months but because of its nephrotoxicity and lack of effectiveness for the Gambian form, its usage has been restricted. In any case, following the introduction of trypano-

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somiasis into a new area it continues in an endemic form irrespective of the control measures used (5,15).

The study of immunologic aspects of the disease which could possibly lead to a means of control have received increasing attention in recent years. Several workers have had limited success in immunizing animals using killed suspensions of trypanosomes (13,22) or extracts of trypanosomes as immunogens (2,28). Recent experiments have shown that immunity can be induced in rodents, cattle and monkeys against blood form trypanosomes by immunization with live parasites attenuated by ionizing radiation (8,29). Experimental work with Trypanosoma congolense and Trypanosoma brucei has not been as promising. Attempts to immunize cattle with irradiated parasites did not result in a sterile immunity but immunized animals mounted a humoral response and prepatent periods were extended (6,7). A major problem with the irradiated vaccine model is that immune animals are not protected against heterologous strain or variant challenge (30) and no information exists on whether these animals are protected from a natural tsetse fly challenge.

Our studies describe immunization trials with T. congolense in bovines in the laboratory. We also report on a field study, which has taken place over the last 8 years, in which we determined the antigenic similarities of T. rhodesiense occurring in an endemic area of western Kenya.

An animal pathogen, T. congolense has been used in the immunization trials to preclude the possibility of accidental human infection in the laboratory or release of infected vectors into a potential endemic area. Animal models are not unusual in the study of human disease and we believe the immunologic similarities in terms of antigenic variation between T. rhodesiense and T. congolense warranted its use to preclude unnecessary risk for these studies. If anything, our work indicates that immunization with T. rhodesiense is less difficult than that of T. congolense (6,7,29).

MATERIALS AND METHODS

Trypanosoma rhodesiense serodemes in Lambwe Valley

To determine the antigenic composition and number of serodemes active in an endemic area (Lambwe Valley), isolates of T. rhodesiense were collected from patients at the Homa Bay Hospital near Lake Victoria, western Kenya, by members of the Kenya Medical Department. Blood samples from the patients were then injected intraperi-

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toneally into rats and transported to our laboratory in Nairobi for study. The trypanosome isolates were tested by neutralization (25) with two separate antisera. The antisera were developed by long term infection of two bovines, one with an isolate collected in 1972 and the other with an isolate obtained in 1974. The duration of the infection in these animals was 227 and 279 days, respectively. To date, 45 T. rhodesiense isolates over 8-year period have been tested.

Acquired immunity to trypanosomiasis

Animals - Bovines used in these studies were a predominantly Hereford breed and obtained from the Veterinary Department at Kabete or from other trypanosomiasis free areas of Kenya. All animals were dipped or sprayed with an acaricide weekly and received periodic treatment with Ranizole (Merck Sharpe and Dohme, B.V., Haarlem - Netherlands) to limit helminthic infections. During periods of poor pasture supplemental food was provided.

Parasites - The Trans-Mara I strain of T. congolense was used in these experiments. It was first isolated from an infected bovine in the Trans-Mara area near the Kenya-Tanzania border in 1966 and has subsequently been maintained in stabulate form at -80°C with occasional passage in bovines or rodents. White albino mice obtained from the Veterinary Laboratory rearing facility were infected with this stabulate. When parasitemias reached satisfactory levels the mice were anesthetized with ether and bled by cardiac puncture. Trypanosomes were counted in a hemocytometer and diluted with phosphate buffered saline (pH 7.8) containing 5% glucose and 10% fetal calf serum.

Induction of immunity to blood form trypanosomes - Bovines were infected intravenously with 1×10^4 trypanosomes per 500lb body weight unless otherwise noted. When animals became weak, severely anemic, and were near death, treatment was initiated with Berenil (Farbwerke Hoechst, Frankfurt (M) Germany) at levels of 1.05g or 2.10g active ingredient per 660lb of body weight. This procedure of infection and therapeutic cure was repeated until these animals were resistant to an intravenous challenge of 1×10^4 trypanosomes per 500lb body weight.

Tsetse fly challenge - Infected adult Glossina morsitans were used to induce metacyclic infection. The tsetse fly colony was established from shipments of puparia supplied by Dr. A.M. Jordan, Tsetse Research Laboratories, Bristol, England. Standard rearing procedures were utilized (16). Newly emerged (< 24 hours post-emergence) flies were allowed to feed on a bovine infected with the Trans-Mara I strain of

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T. congolense. They were exposed to the donor animal each morning for 14 consecutive days and then allowed to feed for 5 consecutive days on each of two non-infected animals. Infection in the flies was verified by dissecting proboscis, midguts and salivary glands from a random sample of flies. Infected flies were allowed to feed on cattle immune to syringe challenge with blood stage trypanosomes of T. congolense and control animals not previously exposed to T. congolense. Each animal received an average of 468 tsetse fly bites over a two day period.

Monitoring of Infections - The temperatures, parasite levels and hematologic parameters of experimental animals were monitored. Blood for Giemsa stained thick and thin films were obtained from the tip of the tail six days per week and blood for routine hematology was obtained twice weekly from the jugular vein in disodium ethylenediamine tetracetic acid (EDTA). The hematology performed included erythrocyte, leucocyte and thrombocyte counts as well as determination of packed cell volumes and hemoglobin concentrations. Parasitemias were estimated by counting the number of trypanosomes per 100 leucocytes on Giemsa stained thick blood films and relating these values to the total leucocyte count per mm³.

Verification of Immune Status - When animals which "self-cured" or were repeatedly infected and cured with Berenil developed no detectable parasitemia or clinical evidence of disease after a blood induced challenge, they were considered immune. Partially immune refers to those animals which received a single infection and treatment. Blood was subinoculated from immune animals into mice as an adjunct to determining their immune status.

In conducting the research in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences National Research Council.

RESULTS

Survey of antigenic specificity of T. rhodesiense - During the past eight years 45 isolates of T. rhodesiense were collected from patients and tested against two different antisera in an attempt to determine the extent of antigenic variation occurring in the Lambwe Valley, western Kenya, over a period of time. As can be seen in Table 1, antisera against two different isolates of T. rhodesiense reacted with 60 and 61 percent of the isolates, respectively. Only 9 isolates, distributed over the 8-year period, were not neutralized by

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TABLE 1

RESULTS OF NEUTRALIZATION TESTS OF ISOLATES
OF TRYPANOSOMA RHODESIENSE COLLECTED BETWEEN
1970 AND 1978 IN LAMBWE VALLEY, KENYA

Antiserum	No. Isolates tested	No. Neutralized*	Percent
Anti LVH-1	45	27	60
Anti LVH-2	44	27	61

* 9 isolates (20%) were not neutralized by either antiserum.

TABLE 5

RESULTS OF CHALLENGE BY INFECTED TSETSE FLIES OF ANIMALS
IMMUNIZED AGAINST BLOOD FORMS OF TRYPANOSOMA CONGOLENSIS

Number of Animals	Group ¹	RESULT			
		Median ² P.P. (Days)	No Detectable infection	Self Cure	Treatment
9	Immune	180	5	4	0
3	Partially Immune	14.0	0	3	0
9	Control	10.0	0	2	7

1. Immune - resistant to last blood challenge.
Partially Immune - one infection and treatment.
2. Prepatent period.

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either antiserum.

Immunization Experiments - As shown in Table 2, there is a marked age resistance to primary infection with T. congolense in bovines. Eighty-five percent of 13 animals less than 1 year of age and 11% of the animals between one and two years old survived and "self-cured" the initial infection. All animals two years or older required treatment to survive. No differences in survival times were found between male and female animals of the same age group.

Five bovines, 25 to 54 weeks after "self-curing" the initial infection, received a primary challenge. All five animals were immune and showed no detectable evidence of infection while the control animals became patent and required treatment within 9 weeks (Table 3). Older animals did not "self-cure" the infection and required treatment to survive. As seen in Table 4, nine animals over one year of age that received an initial infection but required Berenil therapy, were challenged 28-128 weeks after treatment. Five of these animals "self-cured" and 4 required treatment but at times extended beyond that of the initial infection. "Self-cure" of a primary challenge seems to be dependant on a longer initial exposure to the parasite and rechallenge within a year of the last detectable parasitemia. However, there was detectable resistance even in the animals challenged over two years after the treatment of the initial infection. Five of the animals which had either "self-cured" or required treatment of the primary challenge were then given a secondary and subsequently a tertiary challenge. No detectable infections resulted in the experimental animals from either challenge while controls developed typical infections and all required treatment.

Twelve bovines, immune or partially immune to blood form trypanosomes, were subsequently challenged with T. congolense infected G. morsitans (metacyclic trypanosomes from tsetse flies). This fly-borne challenge was 8-9 months after the animal's last exposure to blood form trypanosomes. Eight control animals, not previously exposed to T. congolense, were similarly challenged. As seen in Table 5 and Fig. 1, 55% of the animals immune to blood stage trypanosomes were also completely resistant to metacyclic challenge. Though 45% became patent, they exhibited a significantly lengthened prepatent period and reduced parasitemia when compared to controls. These animals manifested no clinical evidence of disease and ultimately, all "self-cured". The partially immune animals which had received only a single exposure to blood trypanosomes, demonstrated a lengthened prepatent period and reduced parasitemia when compared to controls. They also "self-cured". Of the control group, 7 of 9 animals required treatment for survival

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TABLE 2
THE EFFECT OF AGE ON TRYPANOSOMA CONGOLENSE INFECTIONS IN CATTLE

Age (Years)	Number of Animals	Median Survival* Time (Weeks-Days)	Range (Weeks-Days)	No. Self Cured (%)
0.5-1	13	> 78-0	8-6 to > 78-0	11 (85)
1-2	11	24-4	5-5 to > 78-0	2 (11)
2-3	11	11-5	5-5 to 30-6	0 (0)
3-4	5	6-3	6-1 to 13-6	0 (0)
4-5	2	6-8	4-2 to 9-0	0 (0)
5-6	2	8-1	8-0 to 8-3	0 (0)

* Based on time to treatment or day of death.

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TABLE 3
RESULTS OF PRIMARY CHALLENGE OF PREVIOUSLY INFECTED, SELF-CURED ANIMALS

An. No.	Age (Yrs)	Sex ¹	Dose per 500lbs.	P.P. ² (Days)	Last patent Parasitemia (Wks-Days)	Interval ³ (Wks-Days)	Primary Challenge (1x10 ⁴ /500lbs.)		
							Age (Yrs)	P.P. ² (Days)	Result (Wks-Days)
1	0.5	M	2.8x10 ³	8	54-4	25-0	2.0	Not Patent	No Detect- able Infection
2	1.3	MC	1.0x10 ⁴	5	61-1	31-6	3.0	"	"
3	0.3	F	1.0x10 ⁴	5	56-2	36-5	2.0	"	"
4	0.5	M	2.9x10 ³	6	30-5	48-5	2.0	"	"
5	1.4	MC	1.0x10 ⁴	5	38-2	54-5	3.1	"	"
Average of 3 control animals for primary challenge							3.1	4.7	T (9-3) ⁴

1. F - Female; MC - Male Castrated; M - Male. 3. Time between last patent parasitemia and challenge.
2. Prepatent period. 4. Treated (Time since challenge).

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TABLE 4
RESULTS OF PRIMARY CHALLENGE OF PREVIOUSLY INFECTED AND TREATED CATTLE

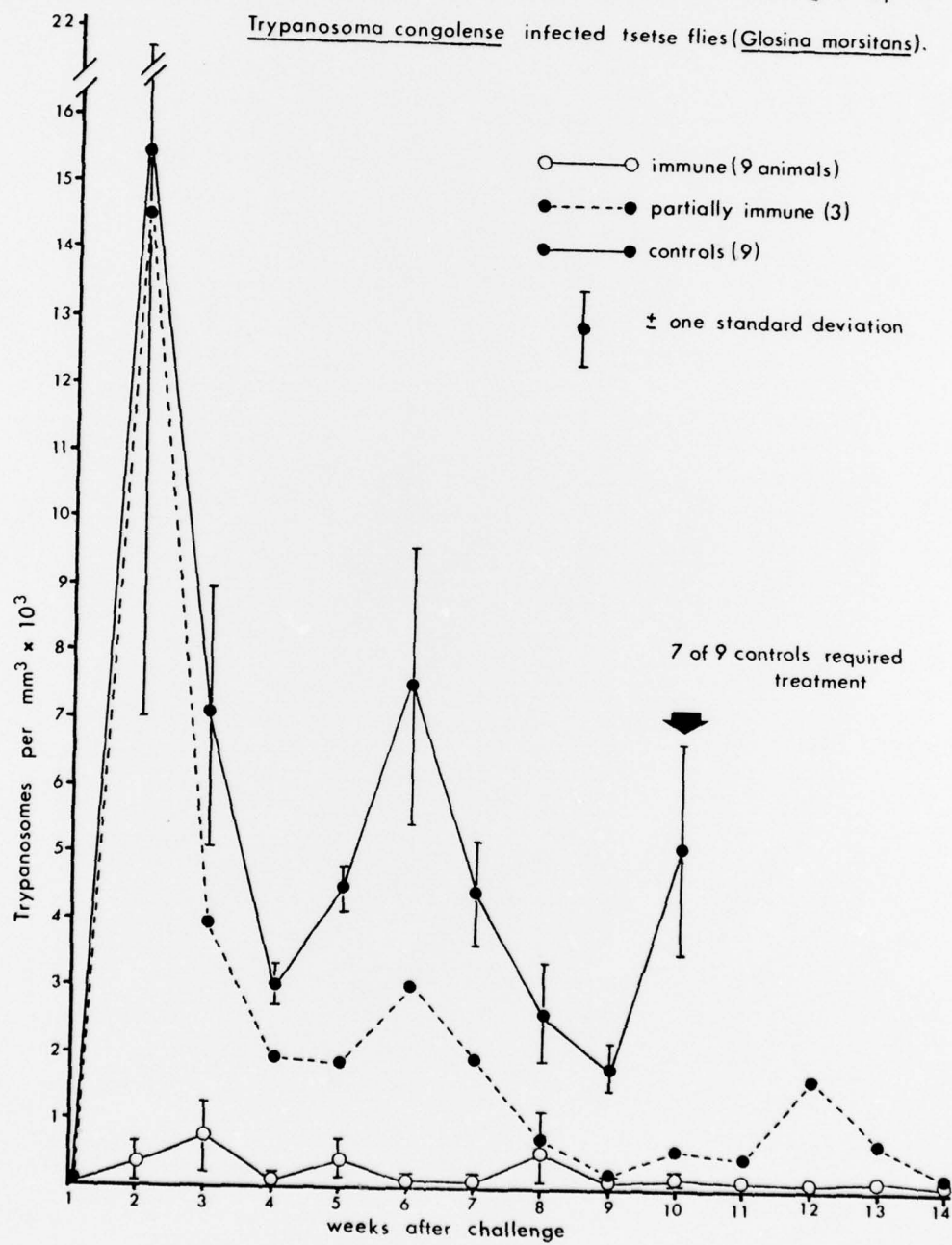
An. No.	Age (Yrs)	Sex ¹	Dose per 500lbs.	P.P. ² (Days)	Time to Treatment (Wks.-Days)	Interval ³ (Wks.-Days)	Primary Challenge (1x10 ⁴ /500lbs.)		
							Age (Yrs)	P.P. (Days)	Result (Wks.-Days)
6	1.0	F	6.8x10 ⁶	3	7-0	28-6	1.7	14	S.C. (17-0) ⁴
7	4.4	F	1.0x10 ⁴	6	9-0	30-0	5.2	10	S.C. (11-5)
8	2.7	F	1.0x10 ⁴	5	11-5	42-2	3.8	18	S.C. (15-5)
9	2.6	F	1.0x10 ⁴	5	6-6	47-1	3.7	13	T. (36-6) ⁵
10	1.9	F	1.3x10 ⁵	5	28-0	71-4	3.9	14	S.C. (4-4)
11	1.6	MC	8.4x10 ³	6	5-5	86-0	3.4	8	T. (21-3)
12	1.9	MC	1.0x10 ⁴	6	5-5	86-0	3.7	6	T. (11-5)
13	2.3	MC	1.9x10 ⁴	5	11-0	122-5	4.9	6	T. (27-0)
14	3.4	F	1.3x10 ⁴	5	5-1	128-4	6.0	6	S.C. (29-2)
Average of 8 control animals for primary challenge							4.1	5.5	T. (9-4)

1. F - Female; MC - Male Castrated. 4. S.C. - Self Cure (Time of last patent
 2. Prepatent period. parasitemia after challenge).
 3. Time between treatment and challenge. 5. T - Treated (Time since challenge).

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Figure 1
Average daily parasitemias of immune and control animals challenged by
Trypanosoma congolense infected tsetse flies (Glossina morsitans).



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while 2 "self-cured". The 2 that "self-cured" were 1-2 years old and reflected the age resistance to infection previously described.

DISCUSSION

Three basic problems must be resolved before immunization could be considered a method for control of trypanosomiasis. The first is concerned with the number and stability of serodemes persisting in an endemic area over a period of time. Gray has shown antigenic similarities in both T. brucei and T. gambiense isolates, respectively, collected from various geographic areas of Nigeria leading one to suspect that a limited number of serodemes were involved in each species (9,10). In our studies, the results of neutralization tests of T. rhodesiense isolates collected from individuals in Lambwe Valley, over an 8-year period, showed a marked similarity of antigenic composition. Both antisera used in the tests neutralized the same as well as different isolates which were presumably, all variants of the same serodeme. The 9 isolates which were not neutralized by either antiserum may belong to the same or to one or more different serodemes. These findings demonstrate the limited diversity and stable antigenic composition of the parasite population. This reduces the likelihood that erratic changes take place in the antigenic character of trypanosomes in a given area and indicates that a successful vaccine could retain its protective capacity over a relatively long period of time.

The second problem deals with antigenic variation; a process utilized by the parasite to evade the hosts immune response (11,18,24). Immunization by "self-cure" or infection and treatment results in exposure of the host to a broad spectrum of antigenic variants. The protection induced by these procedures is apparently manifested by an anamnestic response to most of the antigenic variants of one serodeme. While these methods of immunization are not practical procedures for man, it indicates the important finding that a finite number of antigenic variants are produced and that mutation is probably not involved to a great degree in the process of antigenic variation. Furthermore, it appears that exposure of a host to a significant proportion of variants of a serodeme results in protection against the entire serodeme.

The third and perhaps most important problem in the development of a vaccine involves the question of whether or not animals immune to blood forms are protected against tsetse fly challenge. While animals have been immunized against antigenic variants by various methods, (2,6,7,8,13,28,29), they are resistant only to homologous challenge and their resistance to tsetse fly challenge has not been

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tested. In our experiments employing natural tsetse fly challenge of animals previously shown to be immune to blood forms, we found a marked degree of immunity. Most immunized animals were completely protected and showed no parasitemia or clinical evidence of trypanosomiasis. The immunized animals which became patent did so at a later time than controls and only a transient low level parasitemia developed. These transient parasitemias were not accompanied by clinical symptoms. All controls developed typical T. congolense infections characterized by progressive weight loss, anemia, leucopenia and thrombocytopenia.

It has been shown
In summary, we have demonstrated that the antigenic character of the parasite population of T. rhodesiense from an endemic area was composed of perhaps as few as one serodeme which was antigenically stable over an 8-year period. We also found that immunity can be induced to blood and tsetse fly (metacyclic) forms by exposure of experimental animals to a broad spectrum of antigenic variants of the same serodeme. The sterile immunity described in our studies was long lasting. We believe these findings enhance the likelihood of immunologic control of trypanosomiasis.

It is believed that
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