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Heterophile antibodies to chicken erythrocytes in sheep infected with *Trypanosoma congolense*

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ABSTRACT

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High hemagglutinin titres against chicken erythrocytes were detected in the sera of *Trypanosoma congolense*-infected sheep. Adsorption of sheep sera with solubilized *T. congolense* resulted in marked reduction of hemagglutinitin titre. Heat inactivation of the sera at 56 °C for 30 min had no demonstrable effects on the hemagglutinin titre. Sera collected from the sheep before trypanosome infection did not agglutinate chicken erythrocytes. On the other hand, erythrocytes of horse, donkey and dog were agglutinated at very high titres by sera collected both pre-infection and during the course of infection. Erythrocytes from bovine and caprine species were not agglutinated by contemporaneous sera at both low and high dilutions. Hemagglutinin titres for chicken erythrocytes returned promptly to pre-infection levels in chemotherapeutically terminated infections. The brand of trypanocide used, had no effect on the course of the hemagglutinin titre's return to a normal level.

Keywords: Chicken erythrocytes, heterophile antibodies, sheep, *Trypanosoma congolense*

INTRODUCTION

Trypanosome infections are often diagnosed only by the demonstration of antigen or antibody to the parasite because, at the very late stage of infection, parasites are not detectable by routine diagnostic methods. The concentration method then becomes necessary. (Paris, Murray & McOdimba 1982). The haematocrit technique is increasingly being used for surveillance, and this has the additional advantage of providing the packed-cell-volume readings.

The level of disease diagnosis will have to be intensified to improve treatment and disease surveillance. Despite the improvement in the concentration technique, many infected animals still go undetected (Woo 1970; Joshua 1985; Nantulya, Musoke, Rurangirwa, Saigar & Minja 1987).

In time, the emerging field of molecular biology might supply investigators with immunological reagents for detection of blood-stream trypanosomes and differentiation of epidemiologically important species. Owing to difficulties in detecting the parasites in chronic carriers, indirect parasite identification or immunodiagnosis is essential for the recognition of infected hosts (Houba & Allison 1966).

A good diagnostic method is also a reliable adjunct for measuring the chemotherapeutic and chemoprophylactic efficacy of drugs. During trypanosome infection, some parasites are destroyed by the host-immune responses which attempt to eliminate the predominant populations of trypanosome-variable antigen types (VATs) that arise through antigenic variation (Vickerman 1978). This leads to the release of several soluble antigens in the blood and other

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tissue fluids of the infected hosts (Barry & Emery 1984). Besides the surface antigens which stimulate specific immunity, some trypanosomes stimulate heterophile antibodies during infections (Henderson-Begg 1946; Houba & Allison 1966; Joshua 1984) and in other malignancies (Kano & Milgrom 1977; Kano, Mori, Katagiri & Makara 1985). Heterophile antibody in trypanosomosis was first described in 1946. It has not found much practical application in trypanosomosis surveillance. It is unlikely that such a test might find wide-scale use in the field. However, laboratory application might be illuminating in limited cases.

In a serological study of hosts infected with trypanosomes, Parratt & Cobb (1978) applied the haemagglutination test to differentiate *T. gambiense* infection from *T. rhodesiense*. On the other hand, Klein & Mattern (1965) could not find any heterophile antibodies in the sera of patients infected with *T. gambiense*.

Observations of elevated heterophile antibodies to chicken erythrocytes in sheep infected with *Trypanosoma congolense*, are reported.

MATERIALS AND METHODS

Trypanosome infection

Trypanosoma congolense was isolated from a cow at the Bodija abattoir, Ibadan, in 1990. Its primary isolation number has been constructed as Ibadan/90/VM/1. Primary isolation was carried out in mice following intraperitoneal inoculation of citrated bovine-infected blood. The stock produced a chronic infection in mice, lasting 45–63 d, but induced an avirulent infection in sheep.

Experimental sheep

All sheep used for this investigation, were observed clinically and screened for blood and gastrointestinal parasites by microscopic examination of blood and faeces, for 3 weeks before the commencement of the experiment. No protozoan or helminth parasites were detected in the animals. Six Yankassa sheep were each infected with parasitaemic mouse blood via the jugular vein. A trypanosome concentration of $10^{7.8}$ was used. Three sheep that were free of trypanosome infections served as controls.

Blood samples for serological tests were collected from all the sheep before and during the course of infection.

Haemagglutination test

Blood was obtained from each of domestic fowl, cattle, goat, horse, donkey and dog. Sodium citrate was used as anticoagulant. Erythrocytes were washed separately in phosphate-buffered normal saline, and a 2% suspension of each erythrocyte species was prepared in physiological saline. Serial twofold dilution of each sample of inactivated sera was prepared in saline (range 4–1024) with the use of a microtitre pipette. Sera from infected sheep were heat-inactivated at 56 °C for 30 min and then cooled to room temperature. Agglutinin in the sera was assayed as previously described (Parratt & Cobb 1978).

Adsorption of sera with Trypanosome congolense antigen

It is necessary to ascertain the specificity of this test since heterophile antibodies have earlier been demonstrated in *Trypanosoma brucei gambiense* infection in monkeys (Houba, Brown & Allison 1969) and *T. rhodesiense* infection in man. *Trypanosoma congolense* was separated from rat cells by ion-exchange chromatography (Lanham 1968). Approximately 10^{8-1} parasites were solubilized per 1 mℓ of distilled water, the total volume used being 5 mℓ. Samples were then dispensed in 1 mℓ volumes and subsequently frozen and thawed three times to ensure proper homogenization of the trypanosomes. Equal volumes (0,5 mℓ) of solubilized *Trypanosoma congolense* and neat sheep serum were mixed in a test tube. The mixture was incubated at 56 °C for 30 min and then centrifuged at 1 500 *g* for 10 min. Serial dilutions were prepared from the supernatant.

A contemporaneous haemagglutination test was carried out on chicken erythrocytes.

Chemotherapeutic termination of infection

It is important to evaluate the status of heterophile antibodies after the chemotherapeutic termination of infection. Six sheep were infected with *T. congolense*; three (A11, A21 and A31) were treated with diminazene, while the remaining three (A44, A55 and A66) were treated with isometamidium chloride. Diminazene aceturate was injected at 7 mg/kg of body mass, while isometamidium chloride was used at 0,5 mg/kg. Infected animals were treated 20 d post infection.

RESULTS

Clinical observations in infected sheep

Microscopically patent parasitaemia was detected in all infected animals on day 10. By day 13 post infection, most of the infected animals had pale mucous membranes with poor appetite. A marked decrease in packed-cell volume (PCV) was a common feature in all infected sheep from day 7 (Table 1). A consistent decrease in PCV was maintained as the infection persisted. Treatment of infection resulted in a dramatic improvement of the PCV. (Table 1). In all infected animals, pale mucous membranes were consistent findings, even when parasitaemia was microscopically latent. Parasitaemia was consistently

TABLE 1 Effect of *T. congolense* infection on PCV of sheep

Sheep no.	Days post inoculalation													
	–21	–10	–6	0	3	7	10	13	17	20	24	27	34	45
A1	33	32	33	33	30	29	24	18	19	18	20	22	27	32
A2	32	32	31	32	30	28	23	19	17	D				
A3	30	31	31	31	29	26	25	21	19	19	21	23	25	30
A4	31	30	31	31	28	25	22	20	18	18	21	24	26	31
A5	32	32	33	33	30	27	24	20	19	19	20	22	25	32
A6	33	32	33	33	30	28	26	23	19	19	21	23	27	32
	Controls													
A7	32	32	33	33	32	32	33	32	33	32	33	32	33	32
A8	30	29	30	30	31	31	31	32	31	32	31	32	31	32
A9	33	32	32	33	33	33	32	33	33	33	32	32	33	32

Infected sheep treated with diminazene aceturate on day 20 post infection

TABLE 2 Titres of Heterophilic antibodies in sheep sera[a] to the erythrocytes of different species of animals

Source of erythrocytes	Infected sheep[b]						Controls		
	1	2	3	4	5	6	7	8	9
Domestic fowl	512	256	512	512	128	256	8	8	8
Cattle	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Goat	Nil	Nil	Nil	Nil	NIl	Nil	Nil	NIl	Nil
Horse	512	512	1 024	1 024	512	1 025	64	128	64
Donkey	128	128	256	128	256	128	128	512	128
Dog	512	512	512	1 024	512	512	128	128	128

[a] Sera collected 10 days post infection
[b] Reciprocal of highest dilution of sera that agglutinated RBC

low, but was detected regularly by mouse inoculation of ovine blood. The buffy-coat examination gave inconsistent positive results, even when mouse inoculation consistently depicted sustenance of trypanosome infection.

Hemagglutinins to heterologous erythrocytes

Preliminary studies were carried out to test hemagglutinins on non-sensitized erythrocytes from cattle, goat, donkey, horse, dog and domestic fowl. Erythrocytes from horse, donkey and dog were agglutinated at high dilution by both the preinfection and sera of experimentally infected sheep. (Table 2). Chicken erythrocytes were agglutinated only by sera from infected sheep. Bovine and caprine erythrocytes were not agglutinated at both low and high dilutions by infected sheep sera (Table 2).

Hemagglutinin titres during the course of infection

In all infected sheep, the hemagglutinin titre remained low (8) until about day 5, when a very high titre (64) was noticed in all the infected animals. As the infection progressed, the antibody response of the sheep became very active and the hemagglutinin titre rose steadily and remained high as long as the infection was maintained (Tables 3 and 4). The hemagglutinin titre remained high in the reinfected sheep, even during the period of latent parasitaemia. Serum inactivation was not found to exert any influence on the titre of the agglutinin, thus showing that heterophile antibody is not affected by heat inactivation of serum.

Observations on adsorbed sera

Titres of the hemagglutinins were strikingly reduced in the sera adsorbed with trypanosome antigen (Table 5). Nevertheless, the adsorbed sera still showed agglutinin titres far above those of sera from control sheep.

Serological reaction after chemotherapeutic termination of infection

Sera from infected sheep treated with either diminazene aceturate or isometamidium chloride were assayed for hemagglutinin. A sudden increase in titre was observed in all animals 5 d after treatment. This

TABLE 3 Haemagglutination[a] of sheep sera by non-sensitized erythrocytes

Source of erythrocytes	Days post infection							
	–15	–6	–0	3	7	10	17	20
Horse	64	64	64	64	128	512	1 024	512
Donkey	64	64	64	64	512	128	128	128
Cow	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Goat	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Chicken	8	8	8	8	64	128	512	512

[a] Mean titre for six samples tested on each day shown

TABLE 4 Haemagglutinin against non-sensitized chicken erythrocytes

Sheep no.	Days post infection								Days post treatment							
	–6	0	3	5	7	10	17	20	5	10	15	25	91	35		
	Hemagglutinin titres															
A11	8	8	8	64	128	512	512	512	1 024	1 024	512	64	64	8	8	8
A21	8	8	8	128	128	256	256	256	512	512	512	64	64	16	8	8
A31	4	4	4	16	64	512	512	512	1 024	1 024	1 024	256	64	8	8	8
A44	4	4	4	64	128	512	512	512	1 024	512	54	16	8	4	4	4
A55	8	4	8	64	128	128	256	256	512	256	64	8	8	8	8	8
A66	4	4	4	32	128	256	256	512	1 024	1 024	256	64	4	4	4	4
	Controls															
A7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	4	8
A8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	4
A9	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

high agglutinin titre was maintained in all treated sheep. Sheep treated with isometamidium returned to preinfection level by day 31, while sheep contemporaneously treated with diminazene aceturate, had pre-infection titres of hemagglutinin by day 35 (Table 4).

DISCUSSION

The present investigation has shown that sera from sheep infected with *Trypanosoma congolense* agglutinate chicken erythrocytes at high dilutions. This cross-reactivity to antigens on cells such as erythrocytes, is termed heterophile reaction. Human heterophile antibodies have attracted the interest of immunologists for over eight decades.

The antibodies have been shown to have practical implications in the serodiagnosis of infectious mononucleosis (Parratt & Cobb 1978). Previous studies on the specificity of antibody molecules induced by trypanosomosis, have shown that B-cell stimulus is polyclonal and not antigen-specific (Greenwood 1970). Only a small proportion of immunoglobulin molecules induced, are antibodies directed against the parasites (Houba et al. 1969). Other heterophile antibodies were used to detect a variety of recent infections (Sheehan 1990). Henderson-Begg (1946) drew attention to high levels of serum heterophile agglutinins in humans with *T. brucei gambiense* infection. This phenomenon of non-specific stimulation by the trypanosomes, has since been observed in various domestic animals (Houba & Allison 1966; Greenwood 1970; Joshua 1984.) Heterophile antibodies have been intimately associated with IgM (McFarlane, Ojo, Houba & Akene 1970). African trypanosome infections in mammals are generally associated with enormous increases in serum IgM. Heterophile agglutinins of sheep erythrocytes were found in sera from malaria-infected patients (Greenwood 1970). The observation suggests that infections other than those by trypanosomes, might give rise to heterophile antibodies in Africa (Greenwood 1970). Nevertheless, the test could be of practical use in controlled laboratory investigations. Heterophile antibodies react with antigens found in species unrelated to stimulating antigens.

The present results suggest that the heterophile antibody in the sheep might be due to breakdown products from the trypanosomes. The sudden release of trypanosome antigen into the host, might be responsible for the sudden upsurge in hemagglutinin titre.

TABLE 5 A comparison of heterophile antibody titres before and after adsorption with trypanosomes

Sheep no.	Days post infection	Titre before adsorption		Titre post adsorption	
		Chicken	Horse	Chicken	Horse
A1	17	512	512	32	128
A2	17	256	256	16	64
A3	17	512	256	16	64
A4	17	512	512	32	64
A1	10	512	512	16	128
A2	10	256	512	16	128
A3	10	512	512	16	128
A4	10	512	512	16	128

The changes in the glycoprotein membrane of trypanosomes might be responsible.

Conventional methods of identifying the presence of trypanosomes in a sample generally involve microscopic examination of body fluid or infectivity of susceptible laboratory hosts.

In very low parasitaemia and in trypanosomes of low infectivity for laboratory animals, the time taken to detect the parasite, might take about one month (Masake & Nantulya 1987; Joshua, Obwolo, Bwangamoi & Evelyn Mandebvu 1985).

Numerous studies have evaluated different serological tests for the immunodiagnosis of African trypanosome infections in farm animals (Joshua 1985: Nantulya 1990). Persistent problems of non-specificity have been encountered even with the use of sensitive techniques like ELISA. (Nantulya *et al.* 1987). The heterophile antibody test is simple to perform, but lacks the specificity of the antigen ELISA (Nantulya & Lindquist 1989), as it cannot be performed on several animal species, since host-species-specific hemagglutinins are required.

The expression of heterophile antigens in various human malignancies has been demonstrated (Kano *et al.* 1985). The biological significance of heterophile antibodies in African trypanosomosis needs further investigation. Antibodies formed in response to serum of a different animal species, are found in serum sickness. These antibodies react with bovine, ovine and equine erythrocytes as well.

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