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Serological Identification of Reptile Feeds of *Glossina*

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Abstract

A method is described to prepare high titred antisera to reptile sera using small amounts of antigen. This involves injecting equal parts of reptile sera and Freunds complete adjuvant into the region of rabbit lymph nodes. Six antisera were prepared and, after absorption, they were tested against 24 different reptile sera to determine their specificity.

A survey of reptilian hosts of *G. fuscipes fuscipes* from two areas of South Busoga, Uganda, showed that 91 % of feeds were derived from monitor lizards, 6 % from snakes, possibly pythons, and 0.5 % from tortoises. Six *G. pallidipes* and two *G. brevipalpis* meals were also shown to have been taken from monitor lizards.

Introduction

It is well established that certain species of *Glossina* derive a major part of their bloodmeals from reptilian hosts. However, no definite data are available, from the analysis of the stomach contents of *Glossina,* as to the actual species of reptile involved. The main reason for this has been lack of a suitable method of preparing high titred antisera, using only small amounts of antigen for use in the precipitin and haemagglutination inhibition tests. The method of antisera preparation described by Weitz (1952) and Tempelis & Reeves (1962) require approximately 15 to 20 ml and 3.5 ml respectively. The method to be described here, and currently in use, utilises only 0.5–1 ml of serum.

There are a number of observations on the reptilian hosts of tsetse using other approaches in the literature.

1. Observation

Many workers have observed tsetse feeding on reptiles in the wild, or on recently killed reptiles. For example, Bruce et al. (1910), record that, in several parts of Africa, particularly in Uganda, tsetse were frequently observed sitting and apparently feeding on crocodiles, monitor lizards and tortoises. They also record an incident, when about 200 *Glossina palpalis* (= *G. f. fuscipes*) and 7 *G. pallidipes* were caught around the body of a recently shot crocodile. They concluded, from their observations, that snakes and most lizards are not very attractive to *G. palpalis,* at least if crocodiles and monitor lizards are available. Fiske (1920) reports having seen swarms of *G. palpalis* on *Varanus,* crocodile, tortoise, pigs, hippopotamus and sitatunga. Cott (1961), while studying the ecology of the Nile crocodile, observed *G. palpalis* feeding on crocodiles. He also states that the tsetse favour reptilian to mammalian blood since, at Magunga, where crocodiles are plentiful man is hardly attacked at all. An additional piece of evidence, that *G. palpalis* feeds readily on reptiles, was provided by Fiske (1920). He found that the largest number of pupae of *G. palpalis* were within a few yards

of a crocodile's nest, or close to places where monitors and sitatunga sun themselves. He suggested that *G. palpalis* feeds on a favoured host and then rests and deposits its larvae, without moving any great distance.

In laboratory studies SIMPSON (1918) records that *G. tachinoides* will feed readily on puff-adders *(Bitis arietans),* black cobra *(Naja nigricollis),* yellow-spotted monitor *(Varanus niloticus)* and crocodile *(Crocodilus niloticus).*

BRUCE and his co-workers (1910) found that, when various animals were tied in the haunts of *G. palpalis,* the number of bites per hour were as follows:

Varanus 49, crocodile 22.8 and goat 0.7.

2. Size and shape of cells

Reptilian erythrocytes may be easily separated microscopically from mammalian cells because they are nucleated. However, since avian, amphibian and fish cells are also nucleated, it is not always possible to state categorically that the nucleated cells are derived from reptiles, especially when it is considered that distortion may occur within the gut of a tsetse. The most extensive study of this type was carried out by LLOYD et al. in 1924. They measured the size of blood cells found in the gut of tsetse and were able to classify animals into groups, according to the diameter of the red cells. Four distinct groups of reptiles were found.

A. Range 13.7 to 14.8 μ crocodile, colubrine snakes and frog,
B. Range 14.9 to 16 μ *Varanus* and other lizards,
C. Range 16.1 to 17.2 μ chameleon,
D. Range 17.3 to 20 μ tortoise.

LLOYD and his co-workers measured the size of the erythrocytes inside the gut of 550 *G. tachinoides* and 215 *G. morsitans* and concluded that for *G. tachinoides* 54.5% of the feeds were from mammals, 4.9% from birds, and 40.8% from reptiles. Reptile feeds were composed of 46.8% of group A, crocodile and colubrine snake, 46.8% group B, *Varanus* and other lizards, 5.9% group C, chameleons and 0.5% group D, tortoises. A number of other workers have calculated the percentage of non-mammalian bloodmeals by the presence or absence of nucleated red cells, for example, BRUCE & NABARRO (1908), CARPENTER (1913), SIMPSON (1918), JOHNSON & LLOYD (1923) and TAYLOR (1930).

3. Crystals

DUKE (1935) reported that when *G. palpalis* had fed on *Varanus* lizards, green crystals were present in the intestinal contents. The significance of these crystals for diagnosis of host-source is unknown. DUKE also reported that *G. morsitans* and *G. palpalis,* which had fed on baboon blood, also contained green crystals 20 to 200 μ in length. These crystals also occur in other blood-sucking insects which have fed on baboon blood, e.g. Culicine mosquitoes, *Stomoxys,* tabanids and bed-bugs.

4. Parasites

A number of blood-parasites have been found in tsetse bloodmeals which are also known to occur in reptiles. JACKSON (1945) found a *G. palpalis* which contained a haemogregarine parasite which corresponded to that found in the blood of terrapins. He showed that *G. palpalis* would feed on the terrapin readily in the

Table 1. Reptilian feeds on tsetse as determined by the precipitin test (WEITZ, 1963)

Species	Location	No. tested	No. reptile	% reptile
G. palpalis fuscipes	Kenya, Uganda, Tanganyika	590	203	34.4
G. palpalis palpalis	Nigeria	371	101	27.2
G. tachinoides	Nigeria	424	35	8.3
G. morsitans submorsitans	Nigeria, Uganda	1,342	8	0.6
G. morsitans morsitans	Uganda, Tanganyika	3,778	10	0.3
G. pallidipes	Kenya, Uganda, Tanganyika	2,687	5	0.2
G. swynnertoni	Kenya, Tanganyika	5,531	9	0.2
G. morsitans orientalis	Tanganyika, Northern and Southern Rhodesia	2,367	1	0.04
G. austeni	Kenya, Zanzibar	394	0	
G. fuscipleuris	Nigeria, Uganda	553	0	
G. tabaniformis	Nigeria	253	0	
G. longipalpis	Nigeria	1,069	0	
G. fusca	Nigeria	707	0	
G. longipennis	Kenya, Tanganyika	1,422	0	
G. brevipalpis	Kenya, Uganda	1,151	0	

laboratory. JOHNSON & LLOYD (1923) occasionally found haemogregarine parasites in the nucleated red cells found in *G. tachinoides,* which were characteristic of blood parasites of monitor lizards and crocodiles. HOARE (1962) has shown that *G. palpalis* is the intermediate host of two protozoon parasites specific to the Nile crocodile. *Hepatozoon pettiti* and *Trypanosoma grayi.* He noted that the infection rate with *T. grayi* among wild tsetse was comparable to that among experimental flies, hence he inferred that the crocodile was the main source of food for *G. palpalis* in that area.

5. Serological identifications

Precipitin tests have been carried out by WEITZ (1963) using antisera prepared by injecting rabbits intramuscularly with alum precipitated mixed reptile sera. No attempt was made to distinguish different groups of reptiles. The results of the identifications, using these serological tests, obtained by WEITZ, are summarised in Table 1.

WEITZ concluded that "The feeding patterns of the *palpalis* group are consistent with its habitat. Hosts (including man) frequenting the water's edge, are attacked. In areas with no domesticated animals the majority of feeds are from crocodiles or monitor lizards."

Materials and methods

Preparation and testing of antisera

The method used to prepare antisera has been adapted from that described by NEWBOULD (1965). An emulsion of equal parts of Freunds complete adjuvant and serum was prepared and aliquots injected into four sites, as close as possible

to lymph nodes, of adult New Zealand white rabbits, weighing 2.5–3 kg. The lymph nodes chosen were two axillary and two popliteal nodes. In most healthy rabbits the lymph nodes can be felt as small, hard moveable lumps in the tissues and, with practice, injections can be made close to the nodes.

A second injection was given seven days later, when the nodes had swollen and it was usually possible to inject the emulsion directly into the nodes. After a further period of ten days, a 2 ml sample of blood was taken from the ear vein of the rabbit and the serum, containing antibodies, separated.

The titre of the antisera was determined by preparing dilutions of the serum, and testing it by the capillary ring precipitin test against the neat antiserum (WEITZ, 1956). If the titre was satisfactory, 50 ml of blood were removed from the ear vein, but if the titre was low, a further injection was given. It was found that, in most cases, two injections each containing 0.5 ml of serum and 0.5 ml of Freunds complete adjuvant, were sufficient to produce a high-titred antiserum. The specificity of the antisera was checked using the capillary ring test. Dilutions of different reptile sera representative of the different groups were used as antigens. In cases where the antisera was not entirely specific, absorptions were carried out as described by WEITZ (1952). Antisera were prepared against monitor lizard *(Varanus niloticus),* crocodile *(Crocodilus niloticus),* python *(Python sebae),* tortoise (Family Chelonidae), chameleon (Family Chamaeleonidae) and agama lizards *(Agama agama).*

After preparation the antisera were ether extracted to remove lipids (MCFARLANE, 1942), freeze-dried in small aliquots and sealed in ampoules under nitrogen. The antisera were then stored at room temperature.

Bloodmeal samples

As part of a wider study into possible seasonal changes in the host-selection pattern of tsetse in two areas of South Busoga, Uganda, Dr. S. K. Moloo has sent blood smears of 2,240 tsetse, comprising 1,212 *G. fuscipes fuscipes,* 373 *G. pallidipes* and 655 *G. brevipalpis.* These bloodmeals were tested by the procedure outlined by WEITZ (1963). All feeds derived from reptiles were separated and subsequently extensively tested by the precipitin test using the antisera prepared as above.

Results

The titres of the six reptile antisera are shown in Table 2. Three of the antisera-crocodile, agama lizard and chameleon, were considered to be satisfactory, in that homologous titres ranged from 32,000 to 128,000 and did not cross-react with heterologous sera at dilutions of 1 in 10. The monitor lizard antiserum had a titre of 1 in 256,000, but cross-reacted with python serum at 1 in 2,000 and boa-constrictor serum at 1 in 1,000. Similarly, the anti-tortoise serum titre, 1 in 128,000, cross-reacted with crocodile serum at 1 in 32,000. Both these antisera were not considered suitable for precipitin testing and absorptions were carried out, as described by WEITZ (1956), in order to improve the specificity.

The antiserum prepared against python serum cross-reacted with other snakes, but not with antigens of other orders of reptile. This antisera was satisfactory for testing snakes, but would not distinguish species.

The absorbed antisera were tested for specificity against 24 reptile sera and representatives of the other main classes. The results are shown in Table 3.

Table 2. Precipitin titres of reptile antisera prepared by lymph node injections before absorptions

Sera	Antisera					
	Monitor	Python	Tortoise	Crocodile	Chameleon	Agama
Monitor	*256,000*	0	0	0	0	0
Python	2,000	*256,000*	0	0	0	0
Tortoise	0	0	*128,000*	0	0	0
Crocodile	0	0	32,000	*128,000*	0	0
Chameleon	N.T.	N.T.	0	0	*32,000*	0
Agama	0	0	0	0	N.T.	*64,000*
Boa	1,000	16,000	N.T.	N.T.	N.T.	N.T.
Bothrops jararaca	0	8,000	N.T.	N.T.	N.T.	N.T.

N.T. = Not tested.

These results indicate that the antisera we have prepared could be used as follows:

The tortoise antisera could be used to detect feeds on the Anapsida sub-class of reptiles which includes turtles, terrapins and tortoises. The anti-python serum reacts with a number of different snakes, while crocodile antiserum reacts with members of the Family Crocodilia. Specific antisera to the Agamids, Varanids and Chameleons were also available.

From the study of bloodmeals of *Glossina* collected in South Busoga, Uganda, a total of 186 feeds were identified as being derived from reptiles (8.3 %). These consisted of 178 *G. f. fuscipes* (14.7 %), 6 *G. pallidipes* (1.6 %) and 2 *G. brevipalpis* (0.3 %). One hundred and eighty two of these feeds were tested using the six reptile antisera, and it was possible to identify the hosts of 179 feeds (98 %).

The results are summarised in Table 4.

The major reptilian host of *Glossina* in South Busoga appears to be the monitor lizard (93.3 %). However, 11 (6.1 %) were identified from snakes and a single feed (0.6 %) from a tortoise.

Discussion

Extensive information has been obtained about the feeding patterns of tsetse in many parts of Africa from the serological identification of bloodmeals (see WEITZ, 1963, 1970). The whole technique depends upon the production of high titred specific antisera. The technique for the preparation of antisera described by WEITZ (1956) has the disadvantage of using large quantities of serum. The method described here utilizes much smaller amounts (1 ml) and also improves the specificity, necessitating less absorptions, which can be time-consuming.

The original method described by NEWBOULD (1965) involves making an incision, dissecting out the lymph node and injecting directly into the node. In our experience this laborious procedure is unnecessary since injections of antigen and adjuvant into the region of the nodes is usually sufficient. Even if the first injection is not actually into the lymph node it causes hyperplasia of the node, so that a subsequent injection seven days later, can be made with more accuracy.

Class	Species	Location	Antisera					
			Monitor Lizard	Python	Tortoise	Croco-dile	Chame-leon	Agama
Reptilia								
a. Anapsida	Turtle							
	Fam. Testudinae	E. Africa	0	0	128,000	0	0	0
	Terrapin							
	Order: Chelonia	Uganda	0	0	1,000	0	0	0
	Water Tortoise							
	Fam.: Chelonidae	Tanzania	0	0	10	0	0	0
	Florida soft shell							
	Turtle: *Trionyx ferox*	U.S.A.	0	0	128,000	0	0	0
	Diamond back turtle							
	Malaclemys terrapin tequesta	U.S.A.	0	0	256,000	0	0	0
	Florida red bellied turtle							
	Pseudemys nelsoni	U.S.A.	0	0	32,000	0	0	0
b. Diapsida								
i. Lacertilia	Monitor Lizard							
	Varanus niloticus	Uganda	128,000	0	0	0	0	0
	Iguana							
	Iguana iguana	Colombia	1,000	0	10	0	100	10
	Chameleon							
	Fam.: Chamaeleonidae	Uganda	0	0	0	0	16,000	0
	Tegu							
	Tupinambis nigropunctatus	Trinidad	0	0	0	0	0	0
	Agama agama	Nigeria	100	0	0	0	2,000	64,000
ii. Ophidia								
	Boa constrictor							
	Constrictor constrictor	Trinidad	0	32,000	0	0	0	0
	Python							
	Python sebae	Uganda	0	64,000	0	0	0	0

	Puff adder							
	Bitis arietans	E. Africa	0	64,000	0	0	0	0
	Spitting cobra							
	Naja nigricollis	Nigeria	0	0	0	0	0	0
	Bothrops jararaca	Brazil	0	1,000	0	0	0	0
	Xenodon merremii	Brazil	0	1,000	0	0	0	0
	Eastern Indigo Snake							
	Drymarchon corai couperi	U.S.A.	0	32,000	0	0	0	0
	Southern blue racer							
	Coluber constrictor	U.S.A.	0	0	0	0	0	0
	Eastern Garter Snake							
	Thamnophis sirtalis sirtalis	U.S.A.	0	0	0	0	0	0
	Dryadophis biforsatus	Brazil	0	0	0	0	0	0
c. Archosauria								
Crocodilia	Crocodile							
	Crocodilus niloticus	Uganda	0	0	0	256,000	0	0
	Crocodilus niloticus	Nigeria	0	0	0	128,000	0	0
	Alligator							
	Alligator mississipiensis	U.S.A.	0	0	100	128,000	0	0
Choanichthyes								
Dipnoi	Lungfish							
	Protopterus annectans	Uganda	0	0	0	0	0	0
Amphibia								
Salientia	*Bufo marinus*	Fiji	0	0	0	0	0	0
	Toad *Bufo* sp.	Uganda	0	0	0	0	0	0
	Bullfrog *Rana* sp.	Uganda	0	0	0	0	0	0
Aves								
Galliformes	Chicken							
	Gallus domesticus	England	0	0	0	0	0	0
Mammalia	Man							
Primates	*Homo sapiens*	England	0	0	0	0	0	0

Table 4. Identification of reptile feeds from two areas of South Busoga, Uganda

	G. f. fuscipes				
	Bukunya		Bunyundo		Total
	♂	♀	♂	♀	
Monitor Lizard	74	11	61	13	159
Snake	7	2	2	0	11
Tortoise	1	0	0	0	1
Unidentified	1	1	1	0	3
Total	83	14	64	13	174

	G. pallidipes				
	Bukunya		Bunyundo		Total
	♂	♀	♂	♀	
Monitor Lizard	4	0	2	0	6

	G. brevipalpis				
	Bukunya		Bunyundo		Total
	♂	♀	♂	♀	
Monitor Lizard	0	1	1	0	2

The volume of serum used is 0.5 ml per injection by the lymph node method, as opposed to 5 ml using alum precipitated serum (WEITZ, 1956) and 3.5 ml injected intravenously into chickens without an adjuvant (TEMPELIS & REEVES, 1962). The titres of antisera prepared in this way are comparable to those using the standard method of WEITZ (1956). It is believed that this technique would have wide applications where only small amounts of antigen are available, since the antigen is presented to the major sites of antibody production. A number of antisera to insect antigens have recently been prepared using this technique (BOREHAM, unpublished).

It is our experience that antisera prepared in the manner described above, tend to produce an antiserum of at least an equivalent titre to the alum precipitated method and the specificity is much greater, especially if it is harvested ten days after the second injection. As already mentioned, the major advantage is that much less antigenic material is required.

The antisera prepared by the lymph node method have been successfully used to elucidate the reptilian hosts of *Glossina* in two areas of South Busoga. The overall percentage of reptile feeds of 14.7% in South Busoga found in this survey compares with 28% found at Lugala from 321 bloodmeals (SOUTHON, 1964), 63% on Uhaya peninsula from 27 bloodmeals (LUMSDEN et al., 1963), 15% of 201 bloodmeals at Alego in 1964 (VAN VEGTEN, 1971) and 17.5% at Samia Bugwe, 481 meals tested (VAN VEGTEN, 1971). It is clear from these earlier results and the present investigations that reptiles form important food sources of *G. f. fuscipes* around the Northern shores of Lake Victoria.

In 1920, FISKE recorded the biting behaviour of *G. fuscipes* from South Busoga and found 100 bites per hour on *Varanus* and 47 bites per hour on crocodile. FISKE concluded that reptiles formed an important host source of *G. fuscipes.*

Crocodiles have been exterminated from this area but monitor lizards are abundant, especially in the reed beds of the edge of Lake Victoria and, together with snakes, form the major reptilian fauna of the area. The commonest species of snake seen at Lugala were pythons, black mambas, puff-adders and various small species of tree snake. Agamids are not present in the bush areas, but are found around habitation not far from the collection sites (ROGERS, D., personal communication).

It is not possible to say which snakes the tsetse were feeding on since our antisera reacted with python and puff-adder sera equally well. No black mamba serum was available for testing.

All six *G. pallidipes* feeds from reptiles were derived from monitor lizards, as were the two *G. brevipalpis* feeds. To our knowledge, these are the first records of *G. brevipalpis* feeding on reptiles.

The number of engorged female *G. f. fuscipes* caught in this study were small compared with males, probably reflecting the "non hungry picture" (FISKE, 1920; JACKSON, 1933). The numbers tested were too small to come to any definite conclusions, but there appears to be little difference to the feeding patterns in the two areas, although 9 out of 97 (9.3%) feeds at Bukunya were from snakes, and only 2 out of 77 (2.6%) at Bunyundo were derived from this source. This could reflect differences in availability. Further tests would be required to confirm this point.

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