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SEROLOGIC EVIDENCE OF TOXOPLASMOSIS IN CAPTIVE AND FREE-LIVING WILD MAMMALS IN KENYA [□]

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Abstract: Sera from 27 captive and 132 free-living mammals in Kenya were tested for antibodies to *Toxoplasma* by the Sabin-Feldman dye test. Of these 8 of 8 (100%) captive carnivores, 14 of 19 (74%) captive herbivores, 11 of 14 (79%) free-living carnivores and 97 of 118 (82%) free-living herbivores were found to have *Toxoplasma* antibodies. The feeding and drinking habits of the various herbivore species were considered as possibly facilitating exposure to *Toxoplasma* oocysts; also the feeding on prey in the wild and on meat in captivity as favoring exposure to the cyst form of *Toxoplasma*.

INTRODUCTION

In spite of the fact that one of the first observations of *Toxoplasma gondii* was in an African rodent, the gundi (*Ctenodactylus gundi*),¹⁵ there is relatively little known about the occurrence of toxoplasmosis in African wild mammals. Ratcliffe and Worth¹⁸ reported toxoplasmosis in a rock hyrax (*Procavia capensis*) in the Philadelphia Zoo; Hofmeyr⁶ described toxoplasmosis in the Cape hunting dog (*Lycan pictis*) in a zoo in Pretoria; Neitz¹⁴ reported *Toxoplasma* in the black-backed jackal (*Canis mesomelas*) in South Africa; and recently in Kenya, Olubayo and Mwangela¹⁶ noted cysts typical of *T. gondii* in a captive colobus monkey (*Colobus abyssinicus*) and Olubayo and Karstad (unpubl.) diagnosed toxoplasmosis as a cause of death in a captive tree hyrax (*Dendrohyrax arboreus*). In a zoo in California, Riemann *et al.*¹⁹ found serologic evidence of toxoplasmosis in several species of African origin, particularly in the felidae.

There is even less information about toxoplasmosis in free-living African wildlife. McConnell and co-workers¹³ found serologic evidence of toxoplasmosis in 11 of 94 free-ranging baboons (*Papio ursinus*) in Kruger National Park, and demonstrated cysts in the heart, brain and skeletal muscles in four of the baboons. Riemann *et al.*²⁰ published results of a survey for toxoplasmosis in free-living mammals in eastern Africa. Using the indirect haemagglutination test, antibodies were detected in 20 of 157 sera, including among the antibody-positive species, zebra (*Equus* sp.), hippopotamus (*Hippopotamus amphibius*), elephant (*Loxodonta africana*), waterbuck (*Kobus* sp.), lion (*Felis leo*), and rock hyrax.

The occurrence of toxoplasmosis in humans in tropical Africa has been studied by several workers. Evidence of infection, latent or acute, was reported across the continent in: Senegal,²² Nigeria,^{7,9} the Congo,^{4,17} Sudan,¹ Uganda^{10,21} and Kenya.¹² However, the

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prevalence of infections in wild and domestic mammals, which may act as reservoirs for human infection, has not been carefully or extensively examined. For this reason, and in view of the recently diagnosed cases in captive wild animals in Kenya (mentioned above), a serologic survey for *Toxoplasma* antibodies in captive and free-living wild animals was initiated.

MATERIALS AND METHODS

Blood specimens from 132 free-living and 27 captive mammals were collected in Kenya. As soon as possible the serum was separated and stored at -20 C until sent by air on dry ice to Leiden, the Netherlands, to be examined for antibodies to *Toxoplasma*. The Sabin-Feldman dye test (DT)^{11,23} was used for serological examination. The serum samples were inactivated for 90 min in a water bath at 56 C before dilutions were prepared.[□] Dilutions of 1/4, 1/64 and 1/512 were prepared with physiological saline and a titre of 1/4 (final dilution) was considered as negative.

RESULTS

The results obtained in the DT are shown in Tables 1 and 2. The over-all prevalence of *Toxoplasma* antibodies was 81.5% in the 27 captive animals and 81.8% in the 132 free-living animals. The percentage positive in the 22 carnivores was 86.4% and in the 137 herbivores, 81%.

In further comparing prevalence rates between carnivores and herbivores in captivity and free-living, all eight captive carnivores were positive, while 79% of the 14 free-living carnivores were positive. In the herbivores, 74% of the 19 captive animals were positive as compared with an antibody prevalence of 82% in the 118 free-living herbivorous animals. There was, therefore, no significant difference of *Toxoplasma* antibody between carnivores and herbivores living free and in captivity.

In comparing the antibody prevalence among herbivorous species in which there were at least 10 free-living individuals tested, 50% of the giraffe had antibody as compared with 80-83% of the hartebeest, topi, impala and Grant's gazelle, while 90% of the Thomson's gazelle and zebra were positive.

There was no significant difference in antibody prevalence between males and females in the herbivores.

The age distribution of DT antibodies in 123 herbivores was examined, - the individuals grouped for convenience in three broad categories "young" (suckling), "adult" (sexually mature) and "subadult" (animals between weaning and sexual maturity). Eighty two per cent of the young, 73% of the subadult and 85% of the adult animals had *Toxoplasma* antibodies.

The numbers of specimens from the carnivores were too few to warrant consideration of sex and age distribution.

□ To inactivate heat-labile systems in human blood serum, e.g. complement, 30 min in a water bath at 56 C is sufficient. Sera from several animal species require a longer period of time to inactivate a heat-labile, nonspecific serum system that is capable of affecting the *Toxoplasma* organism *in vitro*, resulting in the loss of its affinity to methylene blue, even to such a degree that the organism can no longer be recognized.^{4,5,8,11} This very loss of affinity to stain is the phenomenon observed in the DT when antibodies to *Toxoplasma* are present in the serum under examination, provided a so-called accessory factor has been added, i.e. normal human blood serum free from *Toxoplasma* antibodies. In other words, if this nonspecific serum system is not completely inactivated, a false positive DT will be the result; furthermore, a certain titre level can be established not only due to the actual presence of specific antibody, but increased by a nonspecific serum system not completely inactivated. For cattle sera 30 min inactivation are not sufficient, while 90 min are sufficient and 150 min is superfluous; the titre level, however, remains unchanged, even after the last mentioned period of time.¹¹ Ignorant of the time required concerning the animal species at issue, we first examined a serum sample of each species, after, respectively, 30 and 90 min of inactivation. The results made us decide to apply 90 min of inactivation before the serum samples were diluted for the DT.

DISCUSSION

Although we have not had opportunities to collect blood samples and/or feces from free-living Felidae to be examined for antibodies and oocysts, the antibodies detected in the blood of 11 of 14 carnivores and 97 of 118 herbivores (Table 1) is evidence of spread of *Toxoplasma* among free-living wildlife. It is presumed that the herbivorous animals must have been exposed by ingestion of oocysts.

The finding of antibodies in 82% of 118 free-living herbivorous animals indicates that the droppings of free-living Felidae must be a source of infection. In other words, flooded grassland, muddy places, waterholes, brooklets, etc., appear to present sufficiently favourable places for the oocyst to survive. Feeding and drinking habits of certain of the species examined may predispose to infection.

If we consider the antibody prevalence in herbivorous species in which we had at least 10 sera of free-living individuals for testing (Table 2), the percentage of DT positives correlate in general with what might be expected from their feeding habits.² Animals with the highest antibody prevalence (90%) include the strict grazers, the Thomson's gazelle and zebra. Eighty to 83% antibody prevalence was found in the hartebeest, topi, impala and Grant's gazelle. These last two species are browsers as well as grazers,

while the hartebeest and topi graze but utilize the longer, coarser grasses. Giraffe feed on bushes and the leaves and twigs of trees, and bring their mouths to ground level only occasionally and with difficulty, in drinking and utilizing mineral licks. It is perhaps these latter activities that permit exposure and account for the 50% antibody prevalence in the giraffe. In view of the above, one might postulate that grazing facilitates exposure to *Toxoplasma* on grass contaminated with oocysts. Regular visits to ponds, lakes, springs or streams, where cool, moist conditions might be expected to favour survival of oocysts, might also favour exposure to *Toxoplasma*. Similarly, wallowing or mud bathing as practiced by animals like buffalo, male wildebeest, male hartebeest, and warthog, might allow exposure to oocysts. Sera from larger groups of free-living buffalo, waterbuck and warthog may be useful to support or reject these hypotheses. Our findings on the Grant's gazelle, that can go without water for long periods,² is difficult to explain, unless it is assumed that this species does drink regularly if water is available, though it may survive long waterless periods.

The percentages of serologically positive animals in the three age categories are what one would expect with an infectious agent which, once acquired, is believed to be carried for the

TABLE 1. *Toxoplasma* dye test titres* found in 159 captive and free-living animals in Kenya.

	Number	Negative	4<T≤64	64<T≤512	T<512	% Pos.
27 captive:						81
carnivores	8	0	2	3	3	100
herbivores	19	5	11	3	0	74
132 free-living:						82
carnivores:	14	3	3	5	3	79
herbivores	118	21	73	13	11	82
TOTALS	159	29	89	24	17	82

*reciprocal value

TABLE 2. Taxonomic distribution of dye test results from free-living (f) and captive (c) carnivorous and herbivorous animals in Kenya.

	Number	Negative		Positive	
		f	c	f	c
Order Carnivora					
Family: Canidae					
Species: <i>Canis familiaris</i>	8				8
Species: <i>Canis mesomelas</i>	6	2		4	
Family: Viverridae					
Species: <i>Ichneumia albicauda</i>	2	1		1	
Family: Hyaenidae					
Species: <i>Crocuta crocuta</i>	6			6	
Order Perissodactyla					
Family: Equidae					
Species: <i>Equus burchelli</i>	10	1		9	
Order Artiodactyla					
Family: Suidae					
Species: <i>Phacochoerus aethiopicus</i>	2			2	
Family: Giraffidae					
Species: <i>Giraffa camelopardalis</i>	10	5		5	
Family: Bovidae					
Species: <i>Taurotragus oryx</i>	10			4	6
Species: <i>Tragelaphus scriptus</i>	10	2		7	1
Species: <i>Oryx beisa callotis</i>	4	1	1	1	1
Species: <i>Kobus spp.</i>	11	2	4	3	2
Species: <i>Redunca fulvorufula</i>	1	1			
Species: <i>Alcelaphus buselaphus</i>	12	2		10	
Species: <i>Damaliscus korrigum</i>	11	2		9	
Species: <i>Connochaetes taurinus</i>	10	2		9	1
Species: <i>Aepyceros melampus</i>	10	2		8	
Species: <i>Gazella granti</i>	10	2		8	
Species: <i>Gazella thomsonii</i>	10	1		9	
Species: <i>Raphicerus campestris</i>	1			1	
Species: <i>Rhynchotragus sp.</i>	5			5	
Species: <i>Syncerus caffer</i>	10			7	3

life of the individual. Suckling young would have received antibody via the colostrum; these antibody titres, waning with time, would then be replaced by an active immune response as a result of infection with *Toxoplasma*.

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