Prevalence of echinococcosis in dogs and wild carnivores in selected Serengeti ecosystem areas of Tanzania

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SUMMARY

A prevalence study on echinococcosis in dogs and wild carnivores was conducted in northen Tanzania. Copro-antigen ELISA was used to screen 442 dog faecal samples from Magu, Bariadi and Ngorongoro districts, together with 88 wild carnivore samples from Serengeti National Park. Overall prevalence of *E. granulosus* in dogs was 12.4%. Magu (14.6%) and Ngorongoro districts (10.0%) had higher prevalence than Bariadi district (6.3%). The prevalence of echinococcosis in wild carnivores was 13.6%. Species which were positive to Copro-antigen ELISA test included bat eared fox (*Otocyon megalotis)* (14.3%), cheetah (*Acinonyx jubatus*) (16.1%), spotted hyaena (*Crocuta crocuta)* (37.5%) and lion (*Panthera leo*) (10.0%). The findings uncover that dogs and wild carnivores from northern Tanzania are infected with *E. granulosus*, a situation which may pose a risk of infection to other hosts including human. Therefore, more epidermiological investigation is needed to understand the dynamics of the disease in human, domestic animals and wildlife.

Key Words: *Echinococcus granulosus*, dogs, wild carnivores, Tanzania

INTRODUCTION

Echinococcus granulosus has a cosmopolitan distribution (Eckert *et al.*, 2000) and is a problem in Asia, Mediterranean, South America and Africa. Recently, prevalence of the disease has increased in Europe and North America (Khuroo, 2002). Previous and recent reports described endemic occurrence of *E. granulosus* in dogs and livestock from many African countries (Eckert and Deplazes, 2004; Magambo *et al.*, 2006). Human cystic echinococcosis is important from public health and economic perspectives in Africa especially in countries with many livestock (Macpherson *et al.*, 2004; Magaji *et al.*, 2004; Azlaf and Dakkak, 2006).

Currently, *E. granulosus* is the only species of the genus *Echinococcus* that has been reported in sub-Saharan region, and domestic dogs and possibly wild carnivores serve as definitive hosts of adult tapeworm (Eckert and Deplazes, 2004). Transmission of echinococcosis depends largely on carnivores as definitive hosts and herbivores as intermediate hosts. Human and other intermediate hosts get infected when they ingest eggs of *E. granulosus* from materials contaminated with infected carnivore faeces (Torgerson and Heath, 2003). The infection pressure and intensity of *E. granulosus* in dogs have been studied in several areas of Africa and indicated significant prevalence (Lamar *et al.,* 2001; Buishi *et al.,* 2005; Magambo *et al.,* 2006). However, few studies on the status of *E. granulosus* in African wild carnivores have been conducted and showed existence of the parasite (Jenkins and Macpherson, 2003).

Knowledge on *Echinococcus* infection in definitive hosts is important component for establishing epidemiological parameters and preventing spread of infection (Prathiush *et al.,* 2008). Unfortunately, in Tanzania the status of echinococcosis in definitive hosts both dogs and wild carnivores is not well documented. The country has a wide diversity of wild carnivores in many protected areas where some of them have adequate contact with domestic animals. Hence, it is essential to obtain baseline data concerning prevalence of *E. granulosus* before contemplating any rational control programmes. Therefore, the aim of this study was to determine the magnitude of echinococcosis in dogs and wild carnivores using copro-antigen assay in selected Serengeti ecosystem areas of Tanzania.

MATERIALS AND METHODS

Study areas and animals

Coproantigen screening for *E. granulosus* in dogs was conducted in three districts; Ngorongoro, Magu and Bariadi which are the wildlife/livestock interface areas within Serengeti ecosystem. Geographically, Ngorongoro is one of the districts in Arusha region, Tanzania and is positioned between longitude 35° and 36°E and latitude 2° and 4°S. The district is inhabited predominantly by Maasai tribe who practice pastoralism and of recent limited cultivation. Magu district is in Mwanza region and located at 2° 30' 0 S and 33° 30' 0 E while Bariadi is in Shinyanga region located at 2°48'00"S and 33°58'48"E. Magu and Bariadi borders Serengeti National Park (SNP) to the western side, inhabited predominantly by Sukuma tribe. The major economic activity is agropastoralism with low unreliable rainfall. Climatically, the districts are semiarid areas with bimodal rainfall pattern, whereby short rains fall between October and December and long rains start in March and end in May/June. There are significant variations in rainfall distribution between highlands and lowlands. Magu and Bariadi are mainly plain land areas and the annual rainfall ranges from 600 to 800 mm whereas in Ngorongoro district, the highlands have bimodal rainfall and receive between 800 to 1,200 mm and lowlands receive unimodal rainfall of 500 to 700 mm. The mean monthly temperatures in the three districts fluctuate from 25ºC to 30ºC during warm months (September to December) to between 20ºC to 11ºC in cool dry seasons (June to August). SNP is located at 1°30'-3°20'S, 34°00'-35°15'E and it is positioned between the Great Rift Valley and Lake Victoria in northern Tanzania, 200 km northwest of Arusha. Climatically, the park is in the same ecological zone as Ngorongoro district.

The study involved local breeds of dogs and selected wild carnivores namely bat eared fox (*Otocyon megalotis*), cheetah (*Acinonyx jubatus*), genet (*Genetta tigrina*), spotted hyaena (*Crocuta crocuta*), black backed jackal (*Canis mesomelas*), lion (*Panthera leo*), banded mongoose (*Mungos mungo*) and serval cat (*Leptailurus serval*). Dogs in study areas were managed under free range system with minimal veterinary attention. Individual dogs from different households were purposively sampled based on owner’s willingness to participate in the study. The study villages from the districts were conveniently chosen based on accessibility and availability of dogs. Wild carnivores were opportunistically sampled from different areas of the park.

Sampling and handling of faecal samples

Following appropriate physical restraint of dogs, faecal samples were collected per rectum using gloved fingers. Faecal samples from wild carnivores were not invasively collected. Each time samples from wild carnivores were collected once in different locations of the park (more than 50 kilometers apart) to avoid sampling the same animals multiple times. Only freshly defecated faecal samples were collected for Copro-antigen ELISA assay. Collected samples were mixed with an equal volume (w/v) of 0.15 M phosphate buffered saline (PBS) (pH 7.2) containing 0.3% Tween 20 (Sigma) and shaken vigorously to mix (Craig *et al.,* 1995). Samples were stored under refrigeration temperature in the cool box with ice packs during the field work.

Copro-antigen ELISA

The copro-antigen ELISA was performed as described by Craig *et al.,* (1995). Briefly, the faecal samples were mixed by hand shaking then centrifuged at 1000 rpm for 10 min at room temperature. Faecal supernatants were aliquoted and tested for presence of *Echinococcus* coproantigens. The assay was optimised as described by Allan *et al.* (1992). Briefly, approximately 5 µg ml^{-1} of hyperimmune rabbit IgG against *E. granulosus* proglottis somatic extract diluted in BCB was used to coat (100µl per well) microtitre plates (Immulon 1, Dynatech) overnight at 4ºC. Plates were washed three times with PBS/0.1% Tween (20 PBS-0.1% TW) and "blocked" with 100 µl per well of PBS-0.3% TW for 1 hour at room temperature. Fifty microlitres per well of faecal supernatant plus 50 µl per well of heat inactivated foetal calf serum (FCS, Seralab) were added to each well and incubated (in duplicate) for 1 hour, then washed three times in PBS-0.1% TW. One hundred microlitres per well of peroxidase conjugated rabbit IgG anti-E, *granulosus* proglottis somatic extract (diluted 1:200) was incubated for 1 hour at room temperature (25ºC) and plates subsequently washed as above. Substrate solution was 5 aminosalicylic acid (Sigma) in 0.1 M phosphate buffer pH 6.0 containing 1 mM Na_2 EDTA and 0.005% hydrogen peroxide and placed in a dark cupboard for 25 minutes to allow reaction to take place and colour development. Absorbance was measured at a wavelength of 450 nm (Dynatech MR500 microplate reader). Each plate incorporated known reactive and negative dog faecal supernatants and a positive-negative cut-off of 0.09 (i.e. mean ± 3 SD) was employed based on uninfected and heterologous species infections (Allan *et al.,* 1992).

Data were analysed using Epi Info version 6 statistical software (Coulombier *et al.,* 2001). Using statcalc, proportions of categorical variables were computed and further compared using chi-square test at critical probability of $P < 0.05$. The strength of associations between dependent and independent variables were determined using 2 x 2 contingency tables.

RESULTS

A total of 442 dogs were screened for *E. granulosus* coproantigen. The overall prevalence of echinoccocosis in dogs was 12.4%. Individual district prevalence is shown in Table 1. Magu and Ngorongoro districts had almost the same level of prevalence (14.6% and 10.0% respectively) while Bariadi had a prevalence of 6.4%. Of the 88 wildlife samples screened, 13.6% tested positive. Species which were positive to copro-antigen included bat eared fox (14.3%), cheetah (16.1%), spotted hyaena (37.5%) and lion (10.0%) (Table 2).

Table 1. Prevalence of *E. granulosus* in dogs from Magu, Ngorongoro and Bariadi districts

District	Villages	Number of dogs screened	Number of dogs infected in a village (%)	Number of infected dogs in a district (%)
Magu	Imalamate	45	4 (8.9)	14.6
	Kijilishi	40	11 (27.5)	
	Lamadi	40	11 (27.5)	
	Mayega	12	1 (8.3)	
	Mwakiloba	47	8 (17.1)	
	Ng'wangika	38	0 (0.0)	
	Mwanhale	38	3 (7.9)	
Ngorongoro	Olbalbal	13	1 (7.7)	10.0
	Nainokanoka	22	2 (9.1)	
	Makao	28	2 (7.2)	
	Endulen	39	4 (10.3)	
	Loliondo	48	6 (12.5)	
Bariadi	Somanda	15	2 (13.3)	6.4
	Sanungu	17	0 (0.0)	

Table 2. Wild carnivores from Serengeti National Park screened for *E. granulosus*

Species	Number of wild carnivore screened	Number of wild carnivore infected (%)
Bat eared fox	7	1 (14.3)
Cheetah	31	5 (16.1)
Genet	2	0 (0.0)
Spotted hyaena	8	3 (37.5)
Black backed jackal	4	0 (0.0)
Lion	30	3 (10.0)
Banded mongoose	5	0 (0.0)
Serval cat	1	0 (0.0)

DISCUSSION

The genus *Echinococcus* is of great importance because it contains a number of zoonotic species that can cause serious ill health in human. The findings of the current study have shown that *E. granulosus* is prevalent in both domestic dogs and wild carnivores of northern part of Tanzania. Overall prevalence of the disease in dogs (12.4%) is comparable to that reported by Ernest (2004) in Ngorongoro district. Studies from other countries in Africa have reported high prevalence of 18-29% in northern Kenya, 22% in Ethiopia, 58.8% in Morocco, 21.6% in Libya and 21% in Tunisia (Macpherson *et al.*, 1985; Dakkak, 1992; Mersie, 1993; Lamar *et al.*, 2001; Buishi *et al.*, 2005; Buishi *et al.*, 2006). Lower prevalence of 1% has also been reported in Zambia (Islam and Chizyuka, 1983). This trend gives the evidence of endemicity of *E. granulosus* in many African countries with domestic dogs playing important role on perpetuation of domestic cycle.

According to Gemmel *et al.* (2001), 8470 eggs of *E. granulosus* are shed in faeces from infected dog per day. Thus, high prevalence of echinococcosis in dogs at any locality may lead to parasite load on specific environment resulting to more new incidences. This may be the case with Magu, Bariadi and Ngorongoro districts where dogs are managed freely with minimal or no veterinary services. Ernest (2004) reported that only 19.3% of dogs in Ngorongoro district had ever received antihelmintic at least once in life time and majority were under free management system, fed on condemned offals from backyard slaughters. A study conducted by Buishi *et al.* (2005) in Libya reported that dogs fed on condemned raw offal were eight times more likely to be infected with *E. granulosus*. Findings of the present study suggest that backyard slaughters, poor veterinary services and large dog population, which is the typical characteristic of agropastoral and pastoral areas, confounded by wildlife interface could be among the factors responsible for maintaining the *Echinococcus* infection in the districts.

In wild carnivores, the prevalence of *E. granulosus* was 13.6%. A study by Muller-Graf (1995) in SNP and Ngorongoro Conservation Area reported Taeniidae eggs prevalence of 58% in lions. Furthermore, Engh *et al.* (2003) reported Taeniidae eggs prevalence of 12.9% in spotted hyaena (*Crocuta crocuta*) from Mara ecosystem. Similarly, a study by Macpherson *et al.* (1983) reported echinococcosis prevalence of 28.3% in black backed jackals from the pastoralist areas in Turkana district, Kenya. The findings of our study suggest the existence of sylvatic cycle of *E. granulosus* in Serengeti ecosystem, involving wild carnivores and possibly some wild and domestic herbivores species. Other studies elsewhere reported cystic echinococcosis in wild ungulates which are important intermediate hosts of the parasite in different African countries (Boomker *et al.*, 1989; Krecek *et al.*, 1990; Sierfert *et al.*, 2004; Ocaido *et al.*, 2004). Since the incidence of livestock depredation from villages bordering SNP is common (Holmern *et al.*, 2007); possibility of scavenging dead carcasses from livestock and wild herbivores by dogs and wild carnivores may also exist. Such situation suggests that both domestic and sylvatic cycles may operate together in the ecosystem.

Interestingly, recording a prevalence of 14.3% in bat eared fox in the current study poses a researchable theme when considering their diet. This species is known to be insectivores and sometimes feeds on rodents, birds and fruits. Most of its water intake comes from the food it eats. It may be assumed that the foxes probably were infected as a result of feeding on infected rodents. However, more work need to be done since the most commonly known method for *E. granulosus* transmission from intermediate hosts (herbivores) to definitive hosts (carnivores) is through predation.

In view of *E. granuslosus* being prevalent in dogs and wild carnivores in Serengeti ecosystem, the risk of infection to human and animals in other parts of the country is also likely. Such a risk justifies for further epidemiological work in human, domestic animals and wildlife in Tanzania.

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