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An investigation into the seroprevalence of *Toxoplasma gondii*, *Bartonella* spp., feline immunodeficiency virus (FIV), and feline leukaemia virus (FeLV) in cats in Addis Ababa, Ethiopia

N. TIAO[1], C. DARRINGTON[1], B. MOLLA[1], W. J. A. SAVILLE[1], G. TILAHUN[2], O. C. H. KWOK[3], W. A. GEBREYES[1], M. R. LAPPIN[4], J. L. JONES[5], J. P. DUBEY[3]*

[1] *Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*
[2] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*
[3] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Parasite Biology, Epidemiology and Systematics Laboratory, Beltsville, MD, USA*
[4] *Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado, USA*
[5] *Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA*

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SUMMARY

Toxoplasma gondii and *Bartonella* spp. are zoonotic pathogens of cats. Feline immunodeficiency virus (FIV), and feline leukaemia virus (FeLV) are immunosuppressive viruses of cats that can affect *T. gondii* oocyst shedding. In this study, the prevalence of antibodies to *T. gondii*, *Bartonella* spp., FIV, as well as FeLV antigens were determined in sera from feral cats (*Felis catus*) from Addis Ababa, Ethiopia. Using the modified agglutination test, IgG antibodies to *T. gondii* were found in 41 (85·4%) of the 48 cats with titres of 1:25 in one, 1:50 in one, 1:200 in six, 1:400 in six, 1:800 in six, 1:1600 in eight, and 1:3200 in 13 cats. *Toxoplasma gondii* IgM antibodies were found in 11/46 cats tested by ELISA, suggesting recent infection. Antibodies to *Bartonella* spp. were found in five (11%) of 46 cats tested. Antibodies to FIV or FeLV antigen were not detected in any of the 41 cats tested. The results indicate a high prevalence of *T. gondii* and a low prevalence of *Bartonella* spp. infection in cats in Ethiopia.

Key words: *Bartonella*, cats, epidemiology, Ethiopia, humans, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is a worldwide zoonosis [1]. In general its seroprevalence is very high in South America and low in Asia. Fragmentary reports indicate a high incidence of *T. gondii* infections in Africa [1]. Toxoplasmosis is usually asymptomatic in immunocompetent adults, but can cause mortality in the very young and the immunocompromised. Many patients infected with human immunodeficiency virus (HIV) who are untreated die of toxoplasmosis. This is of particular concern in many African

* Author for correspondence: Dr J. P. Dubey, APDL, ANRI, BARC, Building 1001, USDA, Beltsville, MD 20705, USA. (Email: jitender.dubey@ars.usda.gov)

countries because of the high prevalence of HIV and lack of resources to manage it.

Humans and animals become infected with *T. gondii* mostly by ingesting uncooked meat of infected animals or by ingesting soil, food or water contaminated with oocysts. Cats are essential in the life cycle of *T. gondii* because they are the only hosts that can excrete the environmentally resistant oocysts in nature. Prevalence of *T. gondii* antibodies varies with age, lifestyle of the cat (stray *vs.* pet), the serological test utilized, the screening dilution, and other undefined factors. In general, infection in cats increases with age and the prevalence is higher in stray cats. Infected cats can shed millions of oocysts in a matter of a few days, and after sporulation oocysts can survive in the environment for months or even years depending on the moisture and ambient temperature. Cats are thought to become infected by ingesting infected prey, often soon after they begin to hunt. Cats usually shed oocysts only for a short time and once in their life. However, poor nutrition, concurrent infections, and immunosuppression may affect the immune status of the cat and lead to increased oocyst shedding.

Cats have been shown by culture or DNA amplification to be infected by a number of *Bartonella* spp. [2–7]. *Bartonella henselae*, *B. clarridgeiae*, and *B. koehlerae* are transmitted among cats by the cat flea, *Ctenocephalides felis*. *B. henselae* is the main aetiological agent associated with cat scratch disease in immunocompetent people, as well as bacillary angiomatosis and peliosis hepatis which are common disorders in people with AIDS. *Bartonella* spp. infections have also been associated with a number of other chronic disease syndromes in immunocompetent people [8]. *B. quintana* is known to be present in lice in Ethiopia [9] and in addition, *C. felis* has been reported in the country [10]. Thus, while prevalence of *Bartonella* spp. infections of cats has not been reported, there is potential for these infections to exist.

Feline immunodeficiency virus (FIV) is a retrovirus related to HIV, and is known to cause immunosuppression in some cats, depending on the stage of infection. Feline leukaemia virus (FeLV), is related to human T-lymphocytic virus, and can also cause immunosuppression in cats. Here we investigated serological prevalence of *T. gondii*, *Bartonella* spp., and FIV and FeLV infections in cats in Ethiopia for the first time.

MATERIALS AND METHODS

Naturally infected cats surveyed

The cats surveyed were from the Addis Ababa area. Addis Ababa is the dense urban capital city of Ethiopia with an estimated population of 3 million in 2010. It experiences generally two main climates: the dry season between October and June and the rainy season between July and September. A total of 48 feral cats were used in this study. The cats were acquired by volunteers during the entire 2011 rainy season. The study was endorsed by the appropriate bodies of the Aklilu Lemma Institute of Pathobiology (ALIPB). Thirty-one of the cats were from the subcity of Lideta (latitude 9°1′21.8496″ N, longitude 38°44′48.4764″ E) of Addis Ababa, which is where ALIPB is located. Twenty-eight cats were female and 20 were male. Age was estimated by dental maturity, status and health; 41 of 48 cats were aged $\geqslant$6 months (adults). Most cats had fleas but appeared to be in good physical condition.

Blood was collected from either a jugular or a femoral vein after mild sedation following the guidelines of the local institutional Animal Care Policies. The serum was separated and kept cold during air transport to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland; 3 days elapsed between collection of sera and transport to APDL. Sera were subsequently stored at −20 °C until assayed.

Testing for *T. gondii* antibodies

All cat sera were initially tested for *T. gondii* IgG antibodies at 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600 and 1:3200 dilutions using the modified agglutination test (MAT) as described previously [11]. A cut-off point of 1:25 dilution was used as indicative of *T. gondii* infection [1]. This MAT detects only IgG antibodies because the mercaptoethanol used in the test destroys both specific and non-specific IgM.

Examination for *T. gondii* IgM and concurrent infections

After MAT testing at APDL, leftover serum from the cats were shipped to the Colorado State University (CSU), Fort Collins, Colorado, USA and stored at −80 °C until assayed for *T. gondii* IgM antibodies, *Bartonella* spp. antibodies, FIV antibodies and FeLV antigens at CSU. *T. gondii*

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Table 1. *Serological examination of cats for* T. gondii, Bartonella, *FIV, and FeLV infections*

		No. of positive cats				
Cats tested for:	No.	IgG	IgM	*Bartonella*	FIV	FeLV
T. gondii IgG, IgM, *Bartonella*, FIV, FeLV	41	37	11	4	0	0
T. gondii IgG, IgM, *Bartonella*	5	3	0	1		
T. gondii IgG only	2	1				
Total	48	41	11	5	0	0

IgM antibodies were assayed as described previously [12]. *Bartonella* spp. IgG antibodies in serum were detected using a previously reported technique with a titre of $<1:64$ considered negative [13]. While this assay uses *B. henselae* as the capture antigen, it appears to detect antibodies against *B. clarridgeiae*, and *B. koehlerae*; the lowest positive titre is defined as 1:64 [13]. It is unknown whether antibodies against *B. quintana* are detected. Forty-six of the 48 cats were tested for *T. gondii* IgM and *Bartonella* spp. antibodies (Table 1). Forty-one of the 48 cat sera were assayed for FeLV antigen and FIV antibodies (Table 1) using a commercial kit (SNAP® FeLV/FIV®, IDEXX Laboratories, USA).

RESULTS

T. gondii IgG antibodies (MAT, 1:25) were found in 41 (85·4%) of the 48 cats; in 24 (85·7%) of 28 females and 17 (85·0%) of 20 males. The age distribution and titres are shown in Table 2. Subsequently, six of the seven sera negative at 1:25 dilution (sample for cat no. 7 was exhausted) were tested at 1:5, 1:10, and 1:20 dilutions. Four 2- and 4-month-old kittens had no detectable antibodies even at 1:5 dilution, two 2-month-old kittens had a titre of 1:5, and one 4-month-old kitten was seropositive at 1:10 dilution. Thirteen cats had titres of $\geqslant 1:3200$ (Table 2).

T. gondii IgM antibodies were found in 11 cats; all of them were aged $\geqslant 3$ months; all IgM-positive cats also had IgG antibodies (Table 3).

Antibodies to *Bartonella* spp. were found in five cats [aged 8 months ($n=1$), 1 year ($n=2$), 3 years ($n=1$), 8 years ($n=1$)], with titres of 1:64 in four and 1:128 in one (Table 2). All five *Bartonella*-infected cats also had *T. gondii* IgG antibodies.

None of the 41 cats tested were positive for FeLV or FIV, and the negativity was not related to the age of the cat (Tables 1, 2).

DISCUSSION

A high percentage of cats in the present study had *T. gondii* antibodies. The cats that we sampled represented both young and old cats, and both genders. All four 2-month-old cats were seronegative at 1:25 dilution; the low titre of 1:5 in two of these kittens was probably a result of colostrally acquired *T. gondii* antibodies. *T. gondii* transcolostral antibodies disappear in cats usually by age 3 months [14–16]. Both 3-month-old cats had relatively high titres of 1:200 and 1:3200, and thus probably had active infections. Of the four 4-month-old kittens, two were clearly seronegative at 1:5, one had a high IgG titre of 1:1600; the fourth kitten had a titre of 1:10 that could have been recently infected, but it was negative for IgM antibodies. Cats in the present study were tested for *T. gondii* IgM antibodies to detect any additional infected cats missed by IgG antibody screening, but all IgM-positive cats also had IgG antibodies. IgG antibodies can be detected by MAT as early 10 days post-inoculation [17]. A very high IgM titre of 1:16 384 and low IgG titre of 1:50 in cat no. 7 (Table 3) suggests a recently acquired *T. gondii* infection.

The 85% seroprevalence of *T. gondii* in cats from Addis Ababa suggests a high level oocyst contamination in the environment, because by the time cats become seropositive they have already shed oocysts [1]. In addition, a number of cats shedding oocysts are likely to be seronegative. There are no data on the feral cat population in Ethiopia but they are common in public places; most of these stray cats are very wild and difficult to catch. Thus, we could survey only 48 cats.

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Table 2. *Seroprevalence of* T. gondii, Bartonella *spp., FIV, and FeLV in cats from Ethiopia*

Age group	No. of cats	*T. gondii* (no. of cats with IgG titre of*)								No. IgM positive/no. tested	No. *Bartonella* positive/no. tested	No. FIV, FeLV positive/ no. tested
		<25	25	50	200	400	800	1600	3200			
2 mo.	4	4†	0	0	0	0	0	0	0	0/3	0/3	0/2
3 mo.	2	0	0	0	0	1	0	0	1	1/2	0/2	0/2
4 mo.	4	3‡	0	0	0	0	0	1	0	0/4	0/4	0/4
6–10 mo.	6	0	0	0	0	1	2	1	2	3/6	1/6	0/5
1 yr	15	0	1	0	3	2	3	4	2	3/14	2/14	0/12
2 yr	7	0	0	1	1	1	0	1	3	2/7	0/7	0/7
3–6 yr	4	0	0	0	1	0	0	0	3	0/4	1/4	0/3
>8 yr	6	0	0	0	1	1	1	1	2	2/6	1/6	0/6
Total	48	7	1	1	6	6	6	8	13	11/46	5/46	0/41

* Of 48 cats tested.
† Two were <1:5, two were 1:5.
‡ Two were <1:5, one was 1:10.

Table 3. *Details of Ethiopian cats positive for* Toxoplasma gondii *IgM antibodies*

Cat no.	Age	Sex	IgG titre	IgM titre
3	8 mo.	F	1600	128
4	8 yr	M	400	2049
6	9 mo.	F	800	64
7	2 yr	F	50	16 384
11	1 yr	F	200	64
17	2 yr	M	400	64
22	1 yr	F	800	256
27	8 yr	F	200	128
32	3 mo.	M	400	4096
37	1 yr	F	800	64
44	8 yr	F	800	64

F, Female; M, male.

There is considerable variability with respect to *T. gondii* prevalence and concurrent infections [18, 19]. There could be many reasons for this observation, including different serological tests, cut-off titres, and lifestyle of cats surveyed [18, 19]. Witt *et al.* [20] and Childs *et al.* [21] tested stray and pet cats from Baltimore, Maryland, USA and found that 14·7% had *Bartonella* spp., 2·4% had FIV, and 15·2% were seropositive to *T. gondii*. Both the magnitude of *T. gondii* titre and the seroprevalence were higher in FIV-infected cats [20]. In the present study, there was no evidence of FIV and FeLV infection and seroprevalence of *Bartonella* spp. was low.

To our knowledge, this is the first report of *Bartonella* spp. infections in cats from Ethiopia. Most of the cats tested in this study were infested by fleas, but the genus and species was not determined. The seroprevalence of *Bartonella* was lower than most other studies of cats infested with fleas. For example, the *Bartonella* spp. seroprevalence rates using the assay described here were 37·8%, 59%, and 78·4% in studies performed in Australia, Egypt, and the USA, respectively [13, 22, 23]. The *Bartonella* assay performed here used *B. henselae* antigens. However, it appears that antibodies to *B. koehlerae* and *B. clarridgeiae* are also detected in the assay. It is unknown whether the assay detects *B. quintana* which is known to be present in lice in Ethiopia [9]. Thus, it cannot be stated with certainty which *Bartonella* sp. infected the cats in this study. In most studies around the world, *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* are most common in cats because of the association with *C. felis*. This ectoparasite is present in Ethiopia but prevalence rates in cats are unknown. Thus, the low *Bartonella* spp. prevalence rate may reflect infestation by a different flea genus and species.

This limited survey in cats indicates potentially high contamination of the environment by oocysts shed by infected cats, and is the first indication of the potential for ingestion of oocysts from the environment as a mode of *T. gondii* infection and high seroprevalence in humans and food animals in Ethiopia [24]. *Bartonella* spp. are likely to infect cats in the region and further work is required to document the infective species.

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DECLARATION OF INTEREST

None.

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