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Bartonella and *Toxoplasma* Infections in Stray Cats from Iraq

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Abstract. Because of overpopulation, stray/feral cats were captured on military bases in Iraq as part of the US Army Zoonotic Disease Surveillance Program. Blood samples were collected from 207 cats, mainly in Baghdad but also in North and West Iraq, to determine the prevalence of *Bartonella* and *Toxoplasma* infections. Nine (4.3%) cats, all from Baghdad, were bacteremic with *B. henselae* type I. Seroprevalence was 30.4% for *T. gondii*, 15% for *B. henselae*, and 12.6% for *B. clarridgeiae*. Differences in *Bartonella* prevalence by location were statistically significant, because most of the seropositive cats were from Baghdad. There was no association between *T. gondii* seropositivity and either of the two *Bartonella* species surveyed. This report is the first report on the prevalence of *Bartonella* and *T. gondii* among stray cats in Iraq, which allows for better evaluation of the zoonotic risk potential to the Iraqi people and deployed military personnel by feral cat colonies.

INTRODUCTION

Feral cat overpopulation is a problem of global significance, and it has been an issue of concern in Iraq among both Iraqi residents and deployed US military personnel.[1–3] Feral cats roam freely and often form colonies of animals that live and feed in close proximity to humans.[1] Conditions in Iraq have enabled the proliferation of such colonies, because cultural and religious beliefs cause many Iraqis to forbid cats from entering their homes. In addition, there was no national government-backed control program to limit the feral/stray cat population before 2008.[3] During Operation Iraqi Freedom, the US military occupation of Army bases provided new sources of food and water for stray dogs and cats,[3] which elevated the concern that stray animals in close contact with military troops could serve as a reservoir for zoonotic agents, because high densities of feral cats have been shown to increase the incidence of such pathogens.[4,5] Studies have also shown that deployed military personnel in enzootic environments have a higher risk of contracting zoonotic diseases because of increased animal contact or associated animal exposure.[6] Therefore, the US Department of Defense established an animal control program early in the Iraqi campaign that involved the trapping and euthanasia of feral cats and dogs in Baghdad's Green Zone and on US military installations.[2,3] Although it is difficult to estimate the size of the feral/stray animal population in Iraq, 14,000 dogs and cats were euthanized under this program in 2005.[3] Despite the perceived threat of zoonotic diseases, few studies have aimed to determine the prevalence of zoonotic agents in stray animal populations in Iraq. The current study is, therefore, aimed at the estimation of the prevalence of *Bartonella* bacteremia and the seroprevalence of *Bartonella* spp. and *Toxoplasma gondii* in the stray cat populations captured on military bases in Iraq during the United States-funded animal control program.

Bartonella spp. are vector-borne gram-negative bacteria that infect the erythrocytes and endothelial cells of mammalian hosts.[7–9] Cats are the main reservoir for *B. henselae* and *B. clarridgeiae*, and coinfection has been shown.[7,10] These bacteria are transmitted between cats by the cat flea (*Ctenocephalides felis*), and this vector is essential for maintaining the infection within cat populations.[11] Most cats infected with *B. henselae* are asymptomatic or have mild self-limiting symptoms, such as fever or lymphadenopathy.[7,12,13] However, infected cats may be bacteremic for several months and contribute to the spread of the disease when fleas are present.[10] Humans can also be infected with *B. henselae* when flea fecal material is inoculated through the skin by a cat scratch or less likely, a cat bite.[13] The most common zoonotic disease caused by *B. henselae* in humans is cat scratch disease (CSD).[7] Serologic studies also suggest that *B. clarridgeiae* may serve as a minor causative agent of CSD.[8] *Bartonella* infection can cause culture-negative endocarditis, which is associated with 3% of all human endocarditis cases,[7,8] and infection in immunocompromised patients usually leads to systemic infections and hepatosplenomegaly, especially in acquired immunodeficiency syndrome (AIDS) patients who present with bacillary angiomatosis or bacillary peliosis.[7,10] Similarly, neurological signs have also been reported, which may be of concern in military personnel.[14] Several studies have shown that cats and humans can be infected by at least two different genotypes of *B. henselae* based on the 16S rRNA gene analysis, and the prevalence in both cat and human populations varies according to the geographic zone involved.[7,8] It has also been suggested that *B. henselae* type I strains could be more likely to be zoonotic.[15]

T. gondii is a protozoal parasite with a worldwide distribution that can infect virtually all warm-blooded animals, including humans.[16,17] *T. gondii* has a complex lifecycle involving both definitive hosts, in which the parasite reproduces sexually, and intermediate hosts, in which asexual replication occurs.[16] Domestic and wild felids are usually asymptomatic, and they are the definitive hosts of *T. gondii*. They are essential to the parasite's lifecycle, because they are the only host species that can release the resistant oocytes in the environment.[18–20] Cats are primarily infected by ingesting cysts present in the tissues of intermediate hosts, and they may also be infected by ingesting environmental oocysts.[17] The wall of the tissue cyst is degraded by the cat's gastric enzymes; then, the parasite undergoes sexual reproduction, with the unsporulated oocysts shed in the feces 3–7 days post-infection. Oocysts sporulate to become infectious in several

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days. Infected cats can shed upward of 100 million oocysts for up to 20 days.[16] Cats usually shed oocysts one time in their life after a primary infection, and reshedding is rare in the absence of immunosuppression.[17] Because cats usually shed oocysts one time and for a short period and because these oocysts are not infectious until they sporulate in the environment, direct contact with cats does not usually cause infection with *T. gondii*.[16,17] The infective sporulated oocysts can survive in the environment from months to years.[19] Intermediate hosts of *T. gondii*, including humans, are infected through the fecal–oral route on ingestion of oocyst-contaminated soil or water, on ingestion of tissue cysts in undercooked meat, or congenitally.[20] If acute infection occurs during pregnancy, the parasite can also be transmitted transplacentally, causing severe neurologic or ocular disease in the fetus.[16,20] Immunocompetent individuals are usually asymptomatic.[21] When clinically expressed, patients present with mild lymphadenopathy, fever, or malaise, although more severe symptoms, such as encephalitis, myocarditis, hepatitis, and retinochorioditis, have been documented.[17,20] *T. gondii* is also an important opportunistic pathogen among AIDS patients, and it can cause severe encephalitis in this risk group.[17] Human infection with toxoplasmosis is very common. Limited information is available on the prevalence of *T. gondii* antibodies in humans in Iraq. One study in women of childbearing age estimated a prevalence of 47.1%,[22] and another study conducted in three areas (rural, urban, and suburban semirural) in Basra governorate, Southern Iraq, indicated a prevalence ranging from 41.1% to 52.1%.[23]

Stray cat populations generally have a higher prevalence of both *Bartonella* spp. and *T. gondii* infection relative to owned cats.[4,24,25] Given the overpopulation of stray animals on US military bases in Iraq and therefore, heightened exposure of military personnel to cat bites, scratches, and feces, it was necessary to implement an animal control program to prevent transmission of zoonotic diseases. This study aimed to determine the seroprevalence of *B. henselae*, *B. clarridgeiae*, and *T. gondii*, the degree of coinfection in this stray cat population, the prevalence of *Bartonella* bacteremia, and the *Bartonella* species involved.

MATERIALS AND METHODS

Sample collection. A convenience blood sample from 207 stray cats trapped on several US military bases throughout Iraq was collected between February and December of 2008 as part of the US Army Feral Animal Control and Zoonotic Disease Surveillance Program. Before euthanasia, the cats were humanely treated and chemically restrained. A volume of 1.5–2 mL whole blood was collected from the saphenous or jugular veins in (ethylenedinitrilo)tetraacetic acid (EDTA) tubes. Categorical information on age, sex, and location was also provided with the samples when possible. Age was estimated by tooth examination and body weight. These blood samples were frozen and shipped to the University of California (UC) at Davis School of Veterinary Medicine.

Blood culture for *Bartonella* isolation. Whole blood was collected into plastic EDTA tubes and stored at ≤ –20°C until tested. After thawing, the tubes were centrifuged at 5,000 × *g* for 30 minutes at room temperature. The resulting pellet was resuspended in 120 μL M199 inoculation medium.[26] The blood pellet was plated onto heart infusion agar (BBL; Becton Dickinson, Cockeysville, MD) containing 5% fresh rabbit blood, and the plates were incubated at 35°C in 5% CO_2 for 4 weeks. Cultures were examined at least two times per week for bacterial growth. The number of colonies observed was recorded as the number of colony-forming units per milliliter of blood (CFU/mL). A random colony was picked up for each culture-positive sample, and DNA was extracted for identification.

DNA extraction and polymerase chain reaction–restriction fragment length polymorphism. The DNA was extracted as previously described.[27] Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of the *gltA* gene was then performed for all samples.[27] The primers used for the *gltA* gene were BhCS.781p 5′ and BhCS.1137n 5′ as previously described.[28] An approximately 400-bp fragment of the *gltA* gene was amplified and then verified by gel electrophoresis. The amplified product was then digested with restriction endonucleases *TaqI* (Promega, Madison, WI), *Hha*I, *Mse*I, and *Dde*I (New England BioLabs, Ipswich, MA). Banding patterns were compared with standard strains of *Bartonella*, including *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*.

***Bartonella* serology by indirect immunofluorescence antibody test.** An indirect immunofluorescence antibody assay (IFA) was used to detect the presence of antibodies against *B. henselae* and *B. clarridgeiae* in the serum or blood supernatant samples. Whole-cell preparations of *B. henselae* (Strain Houston-1 and strain U-4; UC) and *B. clarridgeiae* (ATCC #51734) were used as antigen according to the method described for testing sera from humans with CSD using Vero cells.[29] Forty microliters antigen were dotted onto each well of 12-well polytetrafluoroethylene-coated slides (Celline Associates, Newfield, NJ), which were incubated overnight. The slides were washed two times in phosphate-buffered saline (PBS). The slides were then air dried, fixed in acetone, air dried again, and stored at –20°C until use. Serum samples were diluted in PBS at 1:64, and 20 μL each dilution were added to the test wells. Positive and negative controls were included on each slide. The slides were then incubated for 30 minutes at 37°C and washed for 5 minutes in PBS. Fluorescein-conjugated goat anti-cat immunoglobulin (whole-molecule immunoglobulin G; Cappel; Organon Teknika Corp., Durham, NC) was diluted at 1:800 in 0.01 M PBS with 0.001% Evan's blue, and the mixture was applied to each well. The slides were incubated for 30 minutes at 37°C and washed again in PBS for 5 minutes before interpretation with a fluorescence microscope (magnification, 400×). The intensity of bacillus-specific fluorescence was scored subjectively from one to four, with a score of two at 1:64 dilution considered positive by two individuals blinded to the culture results.

***T. gondii* serology by latex agglutination test.** A latex agglutination test (LAT) was performed using the microtiter-based Toxotest-MT Kit (V-ST06; Eiken Chemical Co., Tokyo, Japan) to detect *Toxoplasma* antibodies in the cat sera. The serum samples and a positive and negative sera were diluted twofold serially in U-shaped 96-well microtiter plates using dilution buffer (0.2 M amino-2-methyl-1-propanol) and reacted with *Toxoplasma* antigen-adsorbed latex suspension (1% polystyrene latex) overnight at room temperature. Antibody titers were determined by the last dilution number of sera, which agglutinated latex in the middle class dispersion. Based on the manufacturer's recommendation, agglutination at a dilution of 1:32 or higher was regarded as positive.

Statistical analysis. Results of the LAT test for *T. gondii* antibodies and the indirect IFA tests for antibodies against

B. henselae and *B. clarridgeiae* were used to calculate the prevalence of each pathogen in this feral cat population. The exact Kruskal–Wallis test was used to calculate differences in prevalence by locations. Spearman rank order correlation test was used to determine whether associations existed between the positive test results for the three pathogens. The exact Mann–Whitney test was used to compare prevalence by groups of interest (e.g., age, sex, and location [Baghdad versus elsewhere in Iraq]), and relative prevalence was calculated for groups with significant associations. Two-tailed Fisher exact test was used for cell values of less than five when calculating odds ratios. The level of significance was established at $P < 0.05$. A commercially available software SPSS (Released 2012, IBM SPSS Statistics for Mac OS; IBM; Armonk, NY) was used for all data analyses.

RESULTS

Sera from 207 feral cats roaming on US Army military bases in Iraq were tested for the presence of antibodies against *B. henselae*, *B. clarridgeiae*, and *T. gondii*. Whole blood from these cats was plated onto blood agar for *Bartonella* isolation. Categorical information about sex and age was collected when possible, although these data were incomplete for sex in seven subjects and age in nine subjects. Among this population of feral cats, 36.5% (73/200) were female, and 63.5% (127/200) were male; 90% (188/198) of the cats were adult cats (≥ 6 months of age), and 10% (5.1%) were young cats (< 6 months). Of 207 cats, 125 (60.4%) cats were from Baghdad (Inner Baghdad: 114 [91.2%]; Outer Baghdad: 7 [5.6%]; North Baghdad: 4 [3.2%]), 55 (26.6%) cats were from North Iraq, and 27 (13%) cats from West Iraq.

Among the cats sampled, 15% (31/207) were positive for antibodies to *B. henselae*, including 29 (23.2%) cats among 125 cats from Baghdad and neighboring areas (28 [24.6%] cats from Inner Baghdad and 1 [14.3%] cat from Outer Baghdad), 1 (1.8%) cat from North Iraq, and 1 (3.7%) cat from West Iraq (Table 1). Nine of these seropositive cats were blood culture positive for *B. henselae* type I, including six cats that were seropositive for *B. henselae* only and three cats that were seropositive for both *B. henselae* and *B. clarridgeiae*; all of these cats were from Inner Baghdad (Table 2). No coinfection was detected. The level of *B. henselae* bacteremia ranged from 3 to 1,000 CFU/mL, with five of nine cats having less than 20 CFU/mL. Seroprevalence was significantly different between cats from Baghdad and other parts of Iraq ($P < 0.0001$). Sex information was missing for one seropositive animal. Therefore, female cats represented 36.7% (11/30) of the seropositive animals, and two females were also positive on culture. Males represented 63.3% (19/30) of the seropositive animals, and seven males were also positive on culture; 2 (20%) of 10 young cats and 28 (14.9%) of 188 adult cats were seropositive for *B. henselae*. One of two young cats was also seropositive for *B. clarridgeiae*.

The seroprevalence of *B. clarridgeiae* was 12.6% (26/207) (Table 2). All but one of the *B. clarridgeiae* cats were from Inner Baghdad, and the other *B. clarridgeiae* seropositive cat was from West Iraq. Of the *B. clarridgeiae* seropositive cats, 15 cats were also seropositive for *B. henselae* but blood culture negative, and 3 cats were seropositive for both antigens and culture positive as indicated above. Sex information was missing for one seropositive cat, but there were significantly more seropositive females (60%, 15/25) than males (10/25, $P = 0.038$). Female cats (15/73) were three times more likely than male cats (10/127) to be seropositive for *B. clarridgeiae* (odds ratio [OR] = 3.03; 95% confidence interval [95% CI] = 1.19, 7.99). Of 25 *B. clarridgeiae* seropositive cats, all cats but 1 were adults. However, this finding is likely because of the preponderance of adult cat samples in this study, because no significant association was observed between age and *Bartonella* seropositivity. There was a significant association between seropositivity for both species of *Bartonella* ($N = 17$, $P < 0.001$), because cats that were seropositive for *B. henselae* were also more likely to be seropositive for *B. clarridgeiae* (OR = 22.53; 95% CI = 7.67, 67.3) compared with 14 cats seropositive for *B. henselae* only and 9 cats seropositive for *B. clarridgeiae* only.

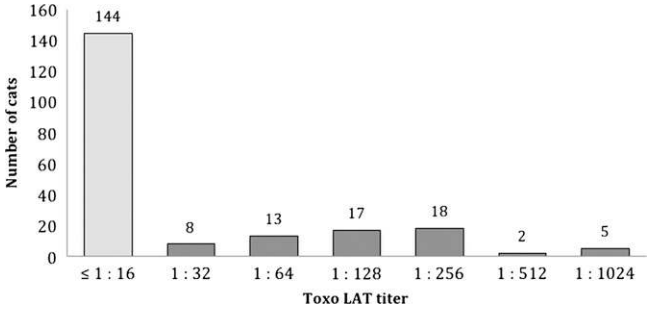

FIGURE 1. Prevalence of antibodies to *T. gondii* using an LAT by titer in 207 stray cats in Iraq.

Antibodies against *T. gondii* were detected in 30.4% (63/207) of the cats, ranging from 1:16 to 1:1,024 (Figure 1). Cats from North Iraq had a higher seroprevalence (40%, 22/55) than cats from West Iraq (29.6%, 8/27) and cats from Baghdad (26.4%, 33/125), but the difference was not statistically significant. Of the seropositive animals, 66.6% (42/63) were male cats, but no significant association was detected between sex and *Toxoplasma* seropositivity. Almost all of the seropositive animals were adults (98.4%); however, no significant association was found between age and seropositivity. Overall, 13 (6.3%) of the cats were seropositive for *T. gondii* and *Bartonella*, including 5 cats seropositive for *T. gondii* and both *Bartonella* species, 4 cats seropositive for *T. gondii* and *B. henselae*, and 4 cats seropositive for *T. gondii* and *B. clarridgeiae*; however, no significant association was identified between *T. gondii* and *Bartonella* seropositivity (Tables 1 and 2).

DISCUSSION

Feral/stray cats were trapped during an animal control program in the Green Zone of Baghdad and on several US military bases throughout Iraq to reduce stray overpopulation and limit the transmission of zoonotic diseases. Overall,

TABLE 1

Bartonella spp. and *T. gondii* seroprevalence in feral cats in Iraq ($N = 207$)

	B. henselae	*B. clarridgeiae*	*T. gondii*
Positive	31 (15%)	26 (12.6%)	63 (30.4%)
Baghdad ($N = 125$)	29	25	33
West Iraq ($N = 27$)	1	1	8
North Iraq ($N = 55$)	1	0	22
Negative	176	181	144

TABLE 2
Characteristics of nine *B. henselae* type I culture-positive cats

ID number	Age	Sex	Location	CFU/mL	Serology		
					B. henselae	*B. clarrdigeiae*	*T. gondii*
A820	Adult	Male	Baghdad	1,000	+	–	0
A859	Adult	Female	Baghdad	80	+	–	0
IA1360	Adult	Female	Baghdad	110	+	+	0
IA1344	Adult	Male	Baghdad	4	+	–	0
A924	Adult	Male	Baghdad	3	+	–	0
A898	Adult	Male	Baghdad	40	+	+	0
A825	Adult	Male	Baghdad	4	+	+	0
IA1460	Adult	Male	Baghdad	3	+	–	64
IA1109	Adult	Male	Baghdad	5	+	–	0

9 (4.3%) of 207 tested cats were *B. henselae* bacteremic; 31 (15%) cats were seropositive for this bacterium, and 26 (12.6%) cats were seropositive for *B. clarridgeiae*, including 18 (69.2%) cats seropositive for both antigens. Studies have shown that *Bartonella* prevalence varies widely between cat populations, with low prevalence in cold climates (0% in Norway) and very high prevalence in warm and humid climates (68% in the Philippines).[8,10] All *Bartonella* bacteremic cats as well as most of the seropositive cats were from Baghdad. This finding was significantly different from the two other Iraqi regions. The prevalence of bacteremic cats was quite low compared with stray cat populations in other parts of the world.[8] Furthermore, it was surprising that no cats from Western or Northern Iraq were *Bartonella* bacteremic, and very few were seropositive. Unfortunately, no information was available on the exact geographical location, including altitude and climatic conditions. If sampling was biased to arid areas or higher altitudes, such conditions could explain the low prevalence, which was shown in other mountainous zones (Switzerland and Colorado).[10] The low to moderate seroprevalence of *Bartonella* spp. in Baghdad was not expected considering the likely high density of feral cats in this region, which has been shown to increase to prevalence of fleas. However, the very warm and dry environment of Baghdad may not be suitable for flea proliferation.[5,30] Unfortunately, the level of flea infestation in the tested cats was not recorded. There was no significant association between *B. henselae* and sex. However, a significant association between *B. clarridgeiae* and sex was observed, with female cats being three times more likely to be seropositive than males. There is no obvious explanation for this finding. The convenience sampling yielded a relatively low number of female cats (36.5%), which may have introduced another sampling bias. Additional studies with a larger sample size and random sampling would be needed to further investigate this finding. Although no significant association was found between *Bartonella* seropositivity and age, it is difficult to make a strong conclusion regarding this association as a risk factor, because only 10 (5.1%) young cats were sampled using a cross-sectional study design. A significant association between infection with *B. henselae* and *B. clarridgeiae* was reported, because cats infected with the former had 22.5 greater odds of being seropositive for the latter. This finding seems logical, because both species of *Bartonella* are transmitted by cat fleas.[25] Many of these cats cohabited on the same military bases, and therefore, it is possible that crowding could have exposed them to fleas infected with both species of bacteria. However, it is also known that cross-reactivity can occur between *B. henselae* and *B. clarridgeiae*. Therefore, it is difficult to determine if seropositivity detected for both *Bartonella* species was a result of actual coinfection or cross-reactivity.[9] Because the study design was cross-sectional, seropositivity for both species of *Bartonella* could also represent sequential infections over time rather than true coinfection.

Toxoplasmosis is a very common zoonotic disease worldwide.[17] Our study reported a seroprevalence of 30.4% in this stray population of cats from Iraq. A high prevalence was expected considering that these cats were feral and therefore, had opportunities to prey on intermediate hosts of *T. gondii*, such as rodents, or become infected through contaminated soil or water.[4] Other recent LAT-based serosurveys for *T. gondii* antibodies among cats in Middle Eastern and Western Asian countries reported slightly higher prevalence, such as 40% in Iran and 60% in Pakistan.[31,32] However, it is difficult to compare seroprevalence studies because of variations in sample size, age of cats sampled, lifestyle of the cats, and cutoff titers used for serological tests.[19,23] No associations were found between *T .gondii* seropositivity and age or sex. Previous studies have shown higher seroprevalence in adult versus young cats because of increased exposure to the pathogen over time.[25,33] This result was not observed in the present study but could be attributed to the small number of young cats. Although a high prevalence of seropositive cats was observed, it is not possible to know whether these cats were actively shedding viable parasites. Shedding occurs 3–7 days after *T. gondii* cyst ingestion and continues for approximately 2 weeks thereafter; however, some cats may not develop immunoglobulin G titers for 4–6 weeks.[16,25,34] Therefore, some animals shedding oocysts may not have been detected by the LAT. Most likely, the seropositive animals represented cats that had latent infections or previous exposure and not necessarily active clinical disease.[16] Because many of the seropositive cats had most likely already shed oocysts, and because of the resistant nature of these environmental oocysts, the high seroprevalence of *T. gondii* suggests that the military bases located in the study area experienced a high degree of environmental contamination with oocysts.[19] No statistically significant association was observed between *T. gondii* and *Bartonella* seropositivity, a finding that is consistent with previous serosurveys conducted on feral cat populations in other countries.[19,33,35]

To our knowledge, these findings represent the first report of *Bartonella* spp. and *T. gondii* seroprevalence in cats from Iraq. Because no research has been done on the pet cat population in this country, it is difficult to say how the feral cat seroprevalence compares with seroprevalence of the local pet cat population. The findings of this study do show a moderate

to high seroprevalence of both zoonotic pathogens among stray/feral cats on US military bases in Iraq. In the future, it would be of value to determine the seroprevalence for antibodies to *T. gondii* and *Bartonella* spp. among Iraqi people and US military personnel pre- and post-deployment in Iraq to better assess the risk of disease transmission between this feral animal population and humans in close proximity.

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