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FULL PAPER

Parasitology

Prevalence of *Toxoplasma gondii* and other intestinal parasites in cats in Tokachi sub-prefecture, Japan

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ABSTRACT. The present study investigated the prevalence of *Toxoplasma gondii* and other intestinal parasites in cats in the Tokachi subprefecture in Japan. A total of 365 household cats were included in the study, and 353 serum and 351 fecal samples were collected and analyzed. *T. gondii* IgG antibodies were detected in the sera of 16.14% of cats based on Latex agglutination test and ELISA. For ELISA, *T. gondii* RH strain tachyzoites lysate and *T. gondii* SAG2 recombinant protein were used as antigens. Low seropositivity was detected in cats younger than one year and older than 11 years; outdoor and hunter cats showed significantly high seropositivities. Neutering either in male or female cats, but not gender, had a considerable effect on seroprevalence. *Toxoplasma gondii* oocysts were detected in one fecal sample. The overall parasitic infestation in cats was 12.5%. Other detected parasites included *Toxocara* species, which showed the highest prevalence of 7.7%, followed by *Isospora* spp. (2%), *Taenia* spp. (1.7%), and *Ancylostoma* spp. (0.9%). *Spirometra* spp. was detected in only one sample. Outdoor cats comprised 50% of all 44 parasite-infested cats. Although *T. gondii* oocysts were detected in only one sample, the relatively high seroprevalence of *T. gondii* indicated that it can pose significant risks to the environment. Our findings highlighted the potential of outdoor cats as a source of *T. gondii* and other parasites.

KEY WORDS: cat, intestinal parasites, prevalence, *Toxoplasma gondii*

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Toxoplasma gondii (*T. gondii*) is an intracellular protozoan parasite that infects virtually all warm-blooded animals. The only known definitive hosts of *T. gondii* are members of the Felidae family [9]. The sexual cycle of *T. gondii* occurs within the feline intestines, after which oocysts are shed in the feces. Although *T. gondii* transmission was traditionally attributed to the domestic cat (*Felis silvestris catus)*, several feline species have been suggested to be definitive hosts [21, 24]. Ingestion of tissue cysts from undercooked meat or consumption of food and water that are contaminated with sporulated oocysts are the major modes of *T. gondii* transmission [38]. Toxoplasmosis is of medical, veterinary, and economic significance [23] and has attracted renewed research interest because of its involvement in the pathogenesis of AIDS and other immunosuppressive diseases [6, 23].

T. gondii has a wide range of intermediate hosts; rats (*Rattus norvegicus*) are considered the most important ones [38, 39]. Domestic cats are obligate carnivores and annually consume billions of birds and small mammals as prey [22]. In addition, rodents that coexist with wild felines were found to have high *T. gondii* infection rates [32]. Feral cats do not have specific owners and are thus not subjected to prophylactic programs and do not receive treatment against parasites. In addition, feral cats have easy access to food resources other than wild prey, such as farms or garbage bins. Thus, feral cats are potential reservoirs for parasites of zoonotic and/or veterinary importance [2, 27].

Household cats are likely to be infected by helminth eggs, which are present in the feces of feral cats and thus could be responsible for increased risk of infection in humans by *Toxocara* spp., *Taenia* spp., *Ancylestoma* spp., *Spirometra* spp., and other parasites [4]. Environmental contamination with oocysts and eggs of feline intestinal parasites continues to pose significant public health hazards. In Japan, contamination with feline intestinal parasites has been widely investigated in studies conducted in different prefectures [11, 14, 30, 40]. The Tokachi area was included in a previous study conducted on domestic cats from 17

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prefectures that covered the north (Hokkaido) and the south (Okinawa) regions of Japan [24]. However, the present study focused only on the Tokachi area and reported recent prevalence rates of *T. gondii* and other intestinal parasites in domestic cats and stray cats. In addition, *T. gondii* serodiagnosis was performed based on multiple tests, including IgG enzyme-linked immunosorbent assay (ELISA) using parasite lysates and recombinant protein, latex agglutination test (LAT), western blotting, and indirect immunofluorescence assay (IFA).

MATERIALS AND METHODS

Study area and sample collection

Sample collection was performed over a period of nine months from October 2013 until June 2014. A total of 365 household cats were sampled from ten different small animal hospitals and clinics in Tokachi sub-prefecture, Japan. Blood and fecal samples and case reports were obtained by veterinarians. The case reports included data on the age, sex, breed, and living styles of the cats, as well as case history, primary complaints, and clinical signs at presentation. Samples and case reports were received and transported by the authors to the Infection and Pathology Research unit at National Research Center for Protozoan Diseases-Obihiro University of Agriculture and Veterinary Medicine. Written consents to use samples were obtained from all cat owners, and experiments were conducted according to the guidelines issued by the Obihiro University of Agriculture and Veterinary Medicine (approval number 26-3). A total of 353 serum samples and 351 fecal samples from the 365 cats were available for analysis. Collected sera were separated, labeled, and stored at −20°C until assayed. Fecal samples were kept at 4°C until analysis.

Microscopic examination

Fecal samples were examined following a conventional flotation method. Before analysis, samples were washed twice with distilled water, sieved with a mesh, filtered through gauze to remove the large particles, and subsequently centrifuged. Samples were suspended in sucrose solution (50% w/v containing 650 m*l* of hot water, 500 g of sucrose, and 6.5 m*l* of water-saturated phenol for preservation; specific gravity, 1.2) and subsequently centrifuged at 2,000 × *g* for 10 min. The upper layer was collected, washed with phosphate buffered saline (PBS) by centrifugation, and transferred to a slide. Slides were examined under a light microscope at 400 × magnification to identify the presence of sporulated or unsporulated *T. gondii* oocysts and other parasite eggs [4, 35].

T. gondii culture

T. gondii tachyzoites from the RH and ME49 strains were maintained in human foreskin fibroblast (HFF) cells cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, Dorset, U.K.) supplemented with 7.5% heat-inactivated fetal bovine serum (FBS). For purification of tachyzoites, infected cells were washed with cold PBS. Cell pellets were resuspended in medium and passed through a 27-gauge needle and subsequently through a 5.0-μm-pore filter (Millipore, Bedford, MA, U.S.A.). After centrifugation at 2,000 × *g* for 5 min at 4°C, the pellet was resuspended in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.25 mM sodium deoxycholate, 0.1% Triton X-100, and 1% Nonidet P-40). The tachyzoite lysates were recovered by centrifugation at 2,000 × *g* for 5 min.

Recombinant protein production

Recombinant *T. gondii* surface antigen 2 (TgSAG2) and *Neospora caninum* surface antigen 1 (NcSAG1) proteins were generated according to previously described methods [15, 16].

Serological tests

ELISA was performed using either the tachyzoite lysates of the *T. gondii* RH strain or recombinant proteins as previously described [34]. Cat sera were diluted at 1:500 with PBS containing 1% skimmed milk (PBS-SM). After washing, plates were incubated with anti-cat IgG diluted at 1:5,000 (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, U.S.A.). The cutoff point was calculated as the average of seronegative cat samples (n=24) plus three standard deviations. All samples were analyzed at least twice for reproducibility. Latex agglutination test was performed according to the manufacturer's instructions (Toxocheck-MT, Eiken Chemical, Tokyo, Japan). Samples were considered positive when agglutination was observed at a dilution of 1:32 or higher. Western blot analysis was performed using the *T. gondii* (RH strain) lysate as an antigen [34]. Parasite lysate was dissolved in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 140 mM 2-mercaptoethanol, 10% glycerol, and 0.02% bromophenol blue), heated at 95°C for 5 min and separated on a 12% polyacrylamide gel. All separated proteins were electrically transferred onto polyvinylidene fluoride (PVDF) membrane (Immobilon-P Transfer Membrane, Millipore) using western blot apparatus (HorizeBlot Type AE-6677, ATTO Bioscience & Biotechnology, Tokyo, Japan). After blocking for 1 hr in PBS containing 1% skimmed milk (PBS-SM), membranes were probed with the cat serum sample diluted at 1:500 with PBS-SM for one hour. The membranes were washed 3 times for 10 min with PBS containing 0.05% Tween 20 (PBS-T), then probed with horseradish peroxidase (HRP)-conjugated anti-cat IgG diluted at 1:2,000 in PBS-SM for one hour at RT. After washing with PBS-T, the membranes were immersed in the detecting solution (0.1 M Tris pH 8.5, 1.25 mM luminol, 0.2 mM coumaric acid, 0.075% H_2O_2) and exposed on the X ray film. Prestained molecular mass standards (GeneDirex, Biospeed, Las Vegas, NV, U.S.A.) were used for the estimation of molecular weight.

Indirect immunofluorescence assay (IFA) was performed as previously described [34] using glass coverslips containing confluent

Table 1. Results of the serological tests performed on cat serum samples for detection of *T. gondii* antibodies

Total number of cat sera	IgG-ELISA						LAT	IFA	Western blot
	Tz lysate Abs (OD)			SAG2 Abs (OD)					
	0.6–1	1–2	>2	0.098–1	1–2	>2			
353	10	35	10	41	21	2	57	57	57

ELISA, Enzyme-linked immunosorbent assay; LAT, Latex agglutination test; IFA, Indirect immunofluorescence antibody assay; Tz, Tachyzoite; OD, Optical density.

monolayers of HFF cells infected with *T. gondii* ME49 strain with some modifications. Cat serum samples were diluted at 1:100 in 3% BSA/PBS. Cover slips were washed thrice in PBS and subsequently incubated with FITC-conjugated goat anti-cat IgG (Sigma-Aldrich).

Statistical analysis

Results of the serological tests were estimated based on the percentage of agreement, sensitivity and specificity, and the kappa values using the online tool available at http://vassarstats.net/. Analysis of different variables that potentially influenced *T. gondii* prevalence, including gender, neutering, rearing conditions, mouse predation, and age, were analyzed using chi-squared test. Statistical analysis was performed using GraphPad Prism software for Windows. Statistically significant differences were considered at $P<0.05$.

RESULTS

Signalment and history of cat patients

The sample collection period lasted for nine months, which covered all seasons, including autumn (October-November; n=141; 38.6%), winter (Dec-Jan-Feb; n=130; 35.6%), spring (March-April-May; n=84; 23.0%), and summer (June; n=9; 2.5%). A total of 365 cats of different breeds were included in the study. The majority of cats were of mixed breed (n=292; 80.0%). Other breeds included Scottish Fold (n=10; 2.7%), American Shorthair (n=9; 2.4%), Munchkin cat (n=7; 1.9%), Maine coon (n=6; 1.6%), Ragdoll (n=4; 1.1%), Abyssinian cats (n=4; 1.1%), American Curl (n=3; 0.8%), Tonkinese cat (n=3; 0.8%), Russian Blue (n=2; 0.5%), Chinchilla cats (n=2; 0.5%) and a Siamese cat (n=1; 0.3%). The rest of the sample included 22 (6.0%) cats of unknown breed. The cats were categorized into the following age groups: cats younger than 1 year (kitten-hood), (n=107; 29.3%), adult cats aged between 1 and 5 years (n=102; 27.9%), senior cats aged between 6 and 10 years (n=71; 19.5%), and geriatric cats aged over 11 years (n=74; 20.3%). Eleven cats (3.0%) were of unknown age.

The study included the following intact and neutered males and females: intact males (n=84; 23.0%), neutered males (n=90; 24.7%), intact females (n=97; 26.6%), and spayed females (n=87; 23.8%). A total of 218/365 (59.7%) cats were reared inside houses, while 147/365 (40.3%) cats lived both indoors and outdoors. Cats lived in residences located in urban areas 309/365 (84.6%) or farms 56/365 (15.3%). Some cats were stray before being adopted by owners 62/365 (16.9%). History of mouse predation was also recorded; a total of 173/365 (47.4%) cats did not hunt for prey, 30/365 (8.2%) were recorded as predators, and 162/365 (44.4%) were unknown.

Detection of T. gondii -specific antibodies in naturally infected cats

To investigate the presence of *T. gondii* antibodies in cats sampled from the Tokachi area, a total of 353 serum samples were screened using LAT at 1:32 dilution. Positivity was detected at a rate of 16.14% (57/353) in the examined sera. Only positive sera were assayed by serial dilutions. Distribution of the antibodies titers was variable among the tested sera. Agglutination was observed at titers of 1:32 in nine cats (15.8%), 1:64 in nine cats (15.8%), 1:128 in 15 cats (26.3%), 1:256 in 12 cats (21.1%), 1:512 in three cats (5.3%), 1:1,024 in three cats (5.3%), and >1:2,048 in six cats (10.5%).

In addition, all samples were tested in parallel by IgG-ELISA using *T. gondii* tachyzoite lysates and TgSAG2 recombinant proteins as antigens. *N. caninum* (NcSAG1) was used to exclude the possibility of cross-reactivity with *T. gondii*. Results of IgG-ELISA for *T. gondii* and *N. caninum* were determined after subtracting the OD values of GST. IgG-ELISA detected *T. gondii* antibodies in 15.6% (55/353) of sera using RH lysates and 18.1% (64/353) using the TgSAG2 antigen. Results were obtained based on the calculated cutoff values of 0.6 and 0.098 for the RH lysates and TgSAG2, respectively. IgG-ELISA of tachyzoite lysates showed high variability among the samples in terms of OD values, which ranged from 0.6 to 1. For the majority of positive samples, 35 samples had OD values between 1 and 2, while the remaining ten serum samples had OD values greater than 2. For ELISA based on TgSAG2, 41 samples had OD values between 0.098 and 1. On the other hand, 21 samples had OD values between 1 and 2, while the remaining two samples had OD values greater than 2 (Table 1).

To verify the results, we performed western blotting and IFA on all samples that tested positive based on LAT and IgG-ELISA. Representative results for positive and negative samples are shown in Figs. 1 and 2. Both western blotting and IFA results were consistent with those of LAT (57/353). In other samples (7/353) that showed positive reactivity with TgSAG2, no specific bands or reactive staining were detected and were thus considered negative. IgG-ELISA detected three positive *N. caninum* samples using *N.*

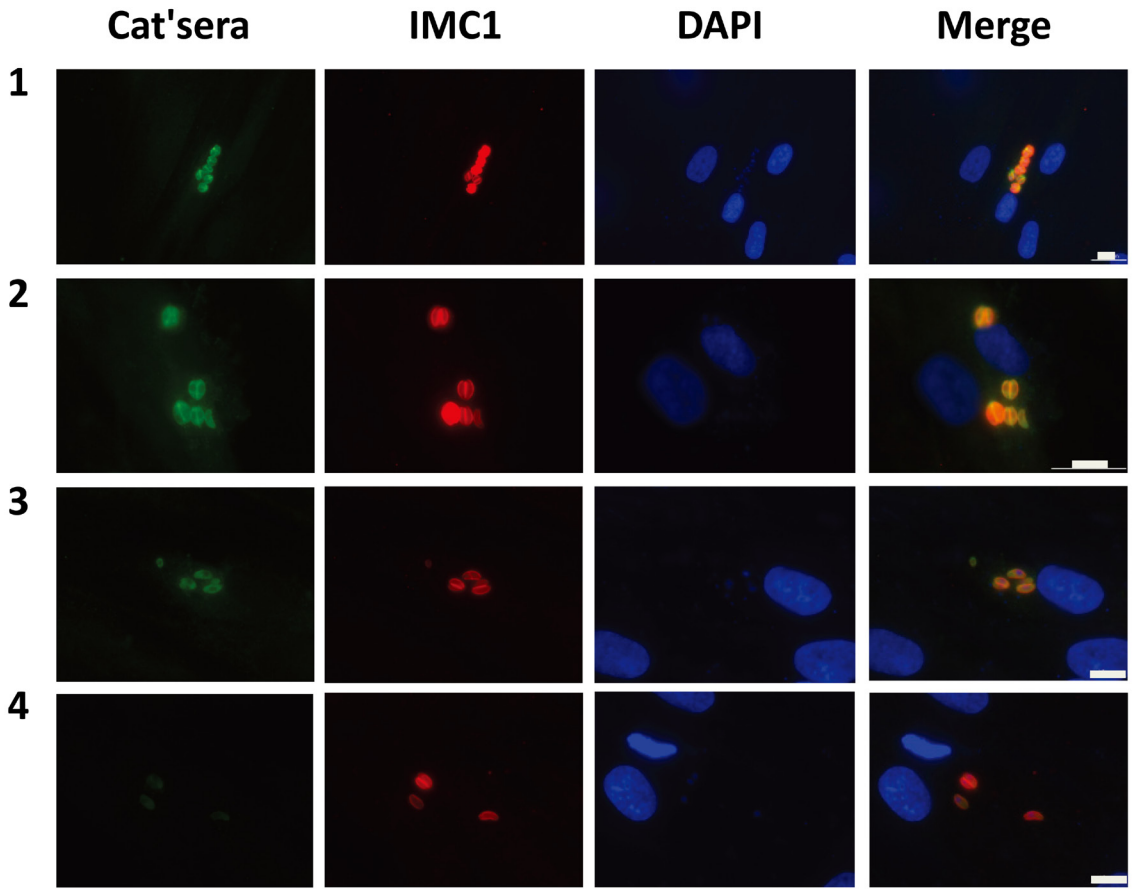

Fig. 1. IFA. Detection of specific antibodies in the tachyzoites of *T. gondii* ME49 strain. Rows 1, 2 and 3 represent seropositive *T. gondii* cat serum samples. LAT dilutions for these samples are × 256, 64 and 128, respectively. Row 4 represents a seronegative sample. Columns represent serum samples (left panel, green). Anti-*T. gondii* IMC1 antibody was used for counterstaining, and DAPI was used for nucleus staining. The right panel represents the merge. White scale bars represent 10 μm.

caninum SAG1 as antigen at the calculated cutoff value of 0.11. Of these, only one sample tested positive for *T. gondii*. Results of ELISA using two different antigens were used to calculate the specificity and sensitivity and the kappa value to evaluate the ability of ELISA to detect *T. gondii* antibodies in cat sera in comparison with the LAT, which was used as the reference test [34].

Factors that affected the seroprevalence of T. gondii in cats

In the present study, cat samples were classified into different groups according to age, gender, rearing conditions, and predatory behavior. Seropositivity for each group was expressed as percentage (Table 2). Based on age distribution, cats aged between 1 and 5 years (26.3%) showed the highest *T. gondii* seropositivity, followed by cats aged between 6 and 10 years (17.6%). On the other hand, low prevalences were recorded in cats older than 11 years and less than 1 year (Table 2). Females showed the highest seropositivities in either the whole sample (23.4%) or neutered (16.3%) cats relative to the intact (19.0) and castrated (8.0) males; however, results were not statistically significant, suggesting the weak effect of gender on *T. gondii* seroprevalence. However, neutering showed a significant effect on seropositivity, since intact (males + females) cats had higher seropositivities relative to neutered ones (Table 2). In terms of rearing circumstances and living styles, cats that lived outdoors or had access to outside and thus have higher risk of infection (30%) relative to indoor cats (7.5%). In

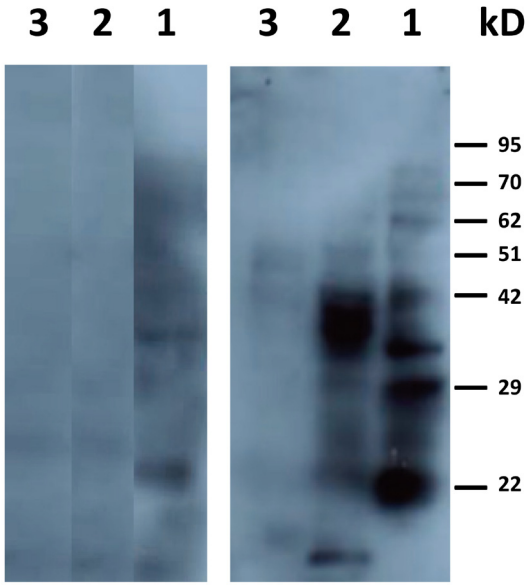

Fig. 2. Western blotting. Lanes 1, 2 and 3 are lysates of the RH strain and ME49 strain of *Toxoplasma gondii* and *Neospora caninum*, respectively. Right and left panels represent positive and negative cat serum samples, respectively.

Table 2. Classification of 353 cat samples according to factors affecting *T. gondii* seropositivity. Seropositive samples, their percentages, and relevant statistics are indicated

A) Age (years)	<1	1–5	6–10	>11	Unknown	*P*-Value
Total	102	99	68	73	11	
Positive (%)	8 (7.8)	26 (26.3)	12 (17.6)	6 (8.2)	6 (54.5)	<0.0001[a)]
B) Gender	Male	Female	Unknown			
Total	167	180	6			
Positive (%)	22 (13.2)	36 (20)	0 (0.0)			0.09
C) Neuter status	Intact	Neuter				
Total	173	174				
Positive (%)	37 (21.4)	21 (12)				0.02
D) Raising condition	Inside	Inside/Outside				
Total	213	140				
Positive (%)	16 (7.5)	42 (30.0)				<0.0001
E) Mice predation	Yes	No	Unknown			
Total	30	176	147			
Positive (%)	20 (66.7)	4 (2.3)	34 (23.1)			<0.0001

a) Cats less than 1 year and over 11 years old were compared with those aged 1–10 years.

Table 3. Prevalence of intestinal parasites in the feces of 351 examined cats

Parasites	Positive (%)	Age (years)					Clinical signs				
		<1	1–2	2–3	>3	Unknown	No	GIT	Urinary	FVR	Others
Toxocara spp.	27 (7.7)	15	6	2	1	3	19	5	1	1	1
Ancylostoma spp.	3 (0.9)	0	3	0	0	0	3	0	0	0	0
Taenia spp.	6 (1.7)	1	1	1	1	1	4	1	0	0	0
Spirometra spp.	1 (0.3)	0	0	0	1	0	0	0	1	0	0
Toxoplasma gondii	1 (0.3)	0	0	0	1	0	0	1	0	0	0
Isospora spp.	7 (2.0)	4	0	1	2	0	4	1	1	1	0

No, No obvious signs; GIT, Gastrointestinal tract malfunction; Urinary, Urinary malfunction; FVR, Feline viral rhinotracheitis; Others, other symptoms.

addition, cats that were reared outside and preyed on mice or reared inside but have a history of predation had significantly higher toxoplasma antibodies (Table 2).

Parasitic infestations in cat feces

Based on microscopic analysis, *T. gondii* oocysts in cat feces (352 samples) were successfully detected in only one sample (Table 3). Sporulated and non-sporulated *T. gondii* oocysts had diameters within the range of 9–15 μm. The overall parasitic infestation detected in cat feces was 12.5% (44/351). The most abundant parasites were *Toxocara* spp., with a prevalence of 7.7% (27/351), followed by *Isospora* spp. at 2% (7/351), *Taenia* spp. at 1.7% (6/351), and *Ancylostoma* spp. at 0.9% (3/351). *Spirometra* spp. was detected in only one sample (0.3%). The cats presented variable clinical signs (Table 3). Toxocariasis and isosporosis were detected mainly in kittens less than one year old. All cats that were infested with *Ancylostoma* spp. were 1–3 years old. *Taenia* spp. infestation showed no correlation with age. Among the 44 cats with parasitic diseases, those with access to the outdoor environment or reared in the countryside constituted 50% (22/44) and 34% (15/44) of the total sample, respectively. Furthermore, 18.2% (8/44) of parasitically infested cats had history of mouse predation, whereas the remaining 81.8% (36/44) of cats had no predatory information available.

DISCUSSION

Felidae is a family of predatory carnivorous mammals whose predatory behavior is the primary cause of infection by a wide range of parasites, which require various preys as intermediate hosts [1, 18]. Felines are the only definitive hosts for *T. gondii* and are therefore the most important hosts in the epidemiology of toxoplasmosis. The sexual cycle of *T. gondii* occurs solely in the cat intestines. Upon infection, cats excrete unsporulated *T. gondii* oocysts in their feces, which subsequently undergo sporulation within 14 days in the external environment and become highly resistant to extreme conditions [10]. Sporulated oocysts readily infect a wide range of hosts, including humans. Domesticated cats are popular as pets and thus live in close proximity to humans and other household animals. In the present study, we investigated the prevalences of *T. gondii* and other intestinal parasites in domesticated cats at Tokachi subprefecture in Japan.

Table 4. Specificity and sensitivity of ELISA for the detection of *T. gondii*-specific antibodies in cat sera compared with LAT as a reference test

Parameter	ELISA	
	Tachyzoite lysate	SAG2
Sensitivity (%)	94	91
Specificity (%)	99	95
Concordance (%)	98	95
Kappa value	0.94	0.83

Herein, the overall seroprevalence of *T. gondii* in cats was determined to be 16.14% (based on IgG antibodies) in 353 examined sera, a value that is comparable to that reported in the Chiba Prefecture (13.4%) [14]. Other studies conducted on domestic cats ranging from the north (Hokkaido) to the south (Okinawa) prefectures in Japan recorded a low seroprevalence of 5.4% (78/1,447) [25]. Similarly, another study reported a low seropositivity of 6% (48/800) throughout Japan based on LAT [28]. Kimbita *et al.* reported a higher seropositivity of 20.7% (40/193) compared to our currently reported value [20]. The seroprevalence of *T. gondii* has been widely investigated worldwide, including Spain [26] and China [42]. Reports from different countries indicated highly variable prevalences, which was dependent on the cat population, testing methods, and environmental contamination.

The present study focused on the factors affecting *T. gondii* seroprevalence at the Tokachi subprefecture. Cats aged between 1 and 5 years showed the highest seropositivity, followed by cats aged between 6 and 10 years. Previous studies indicated that latent toxoplasmosis is common among domestic cats, corresponding to a high prevalence of up to 74% in adult cats [8, 12, 38]. By contrast, we estimated lower *T. gondii* seropositivities in older cats aged over 11 years and in kittens aged less than 1 year when compared to other age groups. The low antibody titers in aged cats can be attributed to the preference of *T. gondii* to undergo sexual reproduction in feline species. On the other hand, *T. gondii* more commonly undergoes asexual reproduction through bradyzoite cyst formation when infecting other species, including humans. In cats, *T. gondii* cysts can be either eliminated by the immune system or are not efficiently formed in tissue. Consequently, *T. gondii* seropositivity in cats decreases with age; however, further investigation is required to verify these initial findings. In a study based on 800 serum samples of domesticated cats from different parts of Japan, authors reported no *T. gondii* seropositivity in cats aged 12–16 years [28]. On the other hand, the low seropositivity detected in cats younger than one year of age was attributed to less developed hunting behavior or reduced hunting opportunities in young kittens.

Based on the case histories and the obtained data, stray cats or cats that were allowed to roam freely outdoors or those reared at farms (30%) had greater chances to chase and hunt for prey and consequently showed higher seropositivities than cats that were kept indoors (7.5%). Nogami *et al*. [28] also found higher seropositivity in outdoor cats (11.1%) than indoor cats (4%) in a serological survey performed on 800 cat samples. Maruyama *et al*. [25] observed a significant difference between the seropositivities of outdoor (15.0%) and indoor cats (4.6%). In addition, antibodies against *T. gondii* were detected in 25.7% of stray adult cats in the western region of Japan [29]. By contrast, a study conducted in Tehran reported significantly higher infection rate in stray (90%) than household cats (36%) [13]. Furthermore, IgG-specific antibodies against *T. gondii* in sera were detected in 29.2% of stray cats in Colima Mexico based on indirect ELISA [33].

The physiological demands of female cats were previously suggested to be associated with strong predatory behavior and increased incidence of toxoplasmosis [3, 5, 37]. On the other hand, results showed that gender did not significantly affect predatory behavior and toxoplasmosis. However, neutering was associated with significantly lower *T. gondii* seropositivity, which can be attributed to inhibited hunting instincts [31]. In the present study, LAT and ELISA were used for serological detection of *T. gondii* antibodies in cat sera. To verify the results, positive samples were further analyzed using IFA and western blotting. To highlight the diagnostic ability of ELISA for detection of *T. gondii* antibodies, results were compared with those of LAT as the reference test (Table 4). ELISA achieved 94.91% sensitivity and 99.95% specificity. The kappa values were 0.94 and 0.83 based on tachyzoite lysates and recombinant proteins, respectively, thereby demonstrating strong agreement between the two methods.

Shedding of *T. gondii* oocysts is known to occur once in the cat's lifetime within a considerably short period ranging from 1 to 3 weeks [10, 19]. Therefore, detection of *T. gondii* oocysts in cat feces is relatively difficult. In addition, oocyst shedding in cats can be influenced by several factors, such as immune status, age, and breed [7]. In the present study, *T. gondii* oocysts were detected microscopically and isolated from the feces of one cat (0.3%) that suffered from diarrhea. In a previous study, the reported prevalence of oocysts shedding in Japanese stray cats was less than 2% [17]. Consistent with the findings of a study conducted in the Chiba Prefecture (1998–1999), only 0.3% of 326 cats were found to excrete oocysts [14]. Similarly, in another study performed on adult stray cats, oocysts were detected in only one sample (0.3%), although a high seropositivity (25.7%, 86/335) was observed [29]. The above studies were conducted to estimate the oocyst shedding by cats in Japan. However, few studies have evaluated environmental contamination by oocysts.

The overall intestinal parasitic infestation in this study was 12.5%. Observed parasites included *Toxocara* spp., which showed the highest prevalence of 7.7% (27/351), followed by *Isospora* spp. at 2% (7/351). Feline intestinal parasites had been previously investigated in different regions in Japan [11, 14, 30, 40, 41]. For example, the overall prevalence of intestinal parasites in cats from public animal shelters in Saitama prefecture was 43.1%, which is higher than the value obtained from our current findings.

The most prevalent parasite was found to be *Toxocara cati*, 21.8% [40].

Here, *Isospora* spp. was detected in only 2% of cat feces, which represents a lower value than that reported in another study where authors found 19.0% and 14.3% prevalence of *Isospora revolta* and *Isospora felis*, respectively [14]. However, *Spirometra* was detected in only one case (0.3%); these results were similar to the findings of a previous study that detected 0.8% prevalence of *Spirometra erinaceieuropaei* [14]. Furthermore, we detected low prevalence of *Ancylostoma* spp. at 0.9% (3/351), while another study reported a high prevalence of 13.2% [40]. Different results were found for *Taenia* spp.; in the present study, *Taenia* spp. had a prevalence of 1.7% (6/351), while other authors detected only 0.2%. Toxocariasis was determined to be common in kittens aged below 1 year (15/27) and between 1 and 2 years (6/27); the incidence of infection was found to decrease with age. Furthermore, we found no association between the infection and specific clinical manifestations in most cases (19/27), and only few cats (5/27) presented GIT symptoms. The three cats infested with *Ancylostoma* spp. were aged 1–2 years and did not present any clinical signs. For *Taenia* spp., age did not show a significant effect on *Taenia* spp. infection. Only one adult cat aged over 3 years was found to have *Spirometra* spp. infection (Table 3). Lifestyle and predation history were also found to exert a strong influence on the infection rate of different parasites. Among 12% cats suffered from parasitism, outdoor cats were 50%, and hunters were 18%.

In the present study, the detection of *Toxocara*, *Taenia*, *Ancylostoma* and *Spirometra* spp. in cat feces indicated the importance of obtaining current data on the parasite distributions in companion animals. Household cats that have access to the outdoor environment can acquire helminths by cysts and eggs that are present in the feces of feral cats, which in turn contaminate the external environment and pose significant risk to human health [27, 36].

In conclusion, this study provided an updated report of the prevalence of *T. gondii* and other intestinal parasites in cats in the Tokachi region. The low prevalence of *T. gondii* oocysts suggested that most of the household cats in the Tokachi area are managed well by owners and veterinarians. However, stray and rural cats are potential reservoirs for parasites that can pose veterinary and public health concerns.

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REFERENCES

1. Afonso, E., Thulliez, P., Pontier, D. and Gilot-Fromont, E. 2007. Toxoplasmosis in prey species and consequences for prevalence in feral cats: not all prey species are equal. *Parasitology* **134**: 1963–1971. [Medline] [CrossRef]
2. Baneth, G., Thamsborg, S.M., Otranto, D., Guillot, J., Blaga, R., Deplazes, P. and Solano-Gallego, L. 2015. Major parasitic zoonoses associated with dogs and cats in Europe. *J. Comp. Pathol.* **155**: S54–74.
3. Bateson, P. 2000. Behavioral development in the cat. pp. 9–22. *In*: The Domestic Cat: The Biology of Its Behavior, 2nd ed. (Turner, D. and Bateson, P. eds), Cambridge University Press, Cambridge.
4. Bowman, D. D., Hendrix, C. M., Lindsay, D. S. and Barr, S. C. 2002. Feline Clinical Parasitology. 1st ed., Iowa State University Press, Ames.
5. Bradshaw, J. W. 2006. The evolutionary basis for the feeding behavior of domestic dogs (Canis familiaris) and cats (Felis catus). *J. Nutr.* **136** Suppl: 1927S–1931S. [Medline] [CrossRef]
6. Domingos, A., Ito, L. S., Coelho, E., Lúcio, J. M., Matida, L. H. and Ramos, A. N. Jr. 2013. Seroprevalence of *Toxoplasma gondii* IgG antibody in HIV/AIDS-infected individuals in Maputo, Mozambique. *Rev. Saude Publica* **47**: 890–896. [Medline] [CrossRef]
7. Dubey, J. P. 1998. Advances in the life cycle of *Toxoplasma gondii*. *Int. J. Parasitol.* **28**: 1019–1024. [Medline] [CrossRef]
8. Dubey, J. P. 2010. Toxoplasmosis of Animals and Humans, 2nd ed. CRC Press, Boca Raton.
9. Dubey, J. P. and Beattie, C. P. 1988. Toxoplasmosis of Animals and Man. CRC Press Inc., Boca Raton.
10. Frenkel, J. K. and Dubey, J. P. 1973. Effects of freezing on the viability of toxoplasma oocysts. *J. Parasitol.* **59**: 587–588. [Medline] [CrossRef]
11. Fujinami, F., Tanaka, H. and Ohshima, S. 1983. [Prevalence of protozoans helminths among cats purchased for experimental use in the Kanto Area]. *Jikken Dobutsu* **32**: 133–137 (in Japanese). [Medline]
12. Furuya, H., Nogami, S., Inoue, I., Takahashi, D., Mizusawa, K. and Tohyama, K. 1993. Seroepidemiological survey on *Toxoplasma gondii* infection in kittens. *Kisechugaku Zasshi* **42**: 388–391.
13. Haddadzadeh, H. R., Khazraiinia, P., Aslani, M., Rezaeian, M., Jamshidi, S., Taheri, M. and Bahonar, A. 2006. Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Tehran. *Vet. Parasitol.* **138**: 211–216. [Medline] [CrossRef]
14. Hata, H., Aosai, F., Norose, K., Kobayashi, M., Mun, H. S., Mei, C., Ito, I., Isegawa, N., Ishikawa, M., Mori, S. and Yano, A., 2000. Prevalence of *Toxoplasma gondii* and other intestinal parasites in cats in Chiba Prefecture, Japan. *Jpn. J. Trop. Med. Hyg.* **28**: 365–368. [CrossRef]
15. Huang, X., Xuan, X., Kimbita, E. N., Battur, B., Miyazawa, T., Fukumoto, S., Mishima, M., Makala, L. H., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Mikami, T. and Igarashi, I. 2002. Development and evaluation of an enzyme-linked immunosorbent assay with recombinant SAG2 for diagnosis of *Toxoplasma gondii* infection in cats. *J. Parasitol.* **88**: 804–807. [Medline] [CrossRef]
16. Ibrahim, H. M., Huang, P., Salem, T. A., Talaat, R. M., Nasr, M. I., Xuan, X. and Nishikawa, Y. 2009. Short report: prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in northern Egypt. *Am. J. Trop. Med. Hyg.* **80**: 263–267. [Medline]
17. Ito, S., Tsunoda, K., Nishikawa, H. and Matsui, T. 1974. Small type of Isospora bigemina: isolation from naturally infected cats and relations with toxoplasma oocyst. *Natl. Inst. Anim. Health Q. (Tokyo)* **14**: 137–144 (Tokyo). [Medline]
18. Jackson, M. H. and Hutchison, W. M. 1989. The prevalence and source of Toxoplasma infection in the environment. *Adv. Parasitol.* **28**: 55–105. [Medline] [CrossRef]
19. Jeffrey, L. J. and Dubey, J. P. 2012. Foodborne Toxoplasmosis. *Food safety. CID* **55**: 845–851. [CrossRef]
20. Kimbita, E. N., Xuan, X., Huang, X., Miyazawa, T., Fukumoto, S., Mishima, M., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Suzuki, N., Mikami, T. and Igarashi, I. 2001. Serodiagnosis of *Toxoplasma gondii* infection in cats by enzyme-linked immunosorbent assay using recombinant SAG1. *Vet. Parasitol.* **102**: 35–44. [Medline] [CrossRef]

21. Lappin, M. R., Jacobsen, E. R., Kollias, G. V., Powell, C. C. and Stover, J. 1991. Comparison of serologic assays for the diagnosis of toxoplasmosis in nondomestic felids. *J. Zoo Wildl. Med.* **22**: 169–174.
22. Loss, S. R., Will, T. and Marra, P. P. 2013. The impact of free-ranging domestic cats on wildlife of the United States. *Nat. Commun.* **4**: 1396. [Medline] [CrossRef]
23. Luft, B. J. and Remington, J. S. 1986. Toxoplasmosis in the central nervous system. *Curr. Clin. Top. Infect. Dis.* **6**: 316–357.
24. Lukesová, D. and Literák, I. 1998. Shedding of *Toxoplasma gondii* oocysts by Felidae in zoos in the Czech Republic. *Vet. Parasitol.* **74**: 1–7. [Medline] [CrossRef]
25. Maruyama, S., Kabeya, H., Nakao, R., Tanaka, S., Sakai, T., Xuan, X., Katsube, Y. and Mikami, T. 2003. Seroprevalence of *Bartonella henselae, Toxoplasma gondii*, FIV and FeLV infections in domestic cats in Japan. *Microbiol. Immunol.* **47**: 147–153. [Medline] [CrossRef]
26. Miró, G., Montoya, A., Jiménez, S., Frisuelos, C., Mateo, M. and Fuentes, I. 2004. Prevalence of antibodies to *Toxoplasma gondii* and intestinal parasites in stray, farm and household cats in Spain. *Vet. Parasitol.* **126**: 249–255. [Medline] [CrossRef]
27. Nichol, S., Ball, S. J. and Snow, K. R. 1981. Prevalence of intestinal parasites in feral cats in some urban areas of England. *Vet. Parasitol.* **9**: 107–110. [Medline] [CrossRef]
28. Nogami, S., Moritomo, T., Kamata, H., Tamura, Y., Sakai, T., Nakagaki, K. and Motoyoshi, S. 1998. Seroprevalence against *Toxoplasma gondii* in domiciled cats in Japan. *J. Vet. Med. Sci.* **60**: 1001–1004. [Medline] [CrossRef]
29. Oikawa, H., Omata, Y., Kanda, M., Mikazuki, K., Yano, K. and Nakabayashi, T. 1990. Survey on Toxoplasma infection in stray cats in western area of Japan during a two-year period. *Kisechugaku Zasshi* **39**: 462–467.
30. Reeves, W. K., Yore, K., Paul, R. and Lloyd, L. 2013. Veterinary diseases review for mailand Japan, 2000–2010. *U.S. Army Med. Dep. J.* **Jan–Mar**: 38–44.
31. Reichler, I. M. 2010. [Pros and cons of gonadectomy in female and male cats]. *Schweiz. Arch. Tierheilkd.* **152**: 273–278. [Medline] [CrossRef]
32. Rendón-Franco, E., Xicoténcatl-García, L., Rico-Torres, C. P., Muñoz-García, C. I., Caso-Aguilar, A., Suzán, G., Correa, D. and Caballero-Ortega, H. 2014. Toxoplasmosis seroprevalence in wild small rodents, potentially preys of ocelots in north-eastern Mexico. *Parasite* **21**: 57. [Medline] [CrossRef]
33. Rico-Torres, C. P., Del Viento-Camacho, A., Caballero-Ortega, H., Besné-Mérida, A., Luna-Pastén, H., Correa, D. and Palma-García, J. M. 2015. First isolation of *Toxoplasma gondii* from cats of Colima, Mexico: tissue distribution and genetic characterization. *Vet. Parasitol.* **209**: 125–128. [Medline] [CrossRef]
34. Salman, D., Oohashi, E., Mohamed, A. E., Abd El-Mottelib, A.R., Okada, T. and Igarashi, M. 2014. Seroprevalences of *Toxoplasma gondii* and *Neospora caninum* in pet rabbits in Japan. *J. Vet. Med. Sci.* **76**: 855–862. [Medline] [CrossRef]
35. Schares, G., Pantchev, N., Barutzki, D., Heydorn, A. O., Bauer, C. and Conraths, F. J. 2005. Oocysts of *Neospora caninum, Hammondia heydorni, Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. *Int. J. Parasitol.* **35**: 1525–1537. [Medline] [CrossRef]
36. Schuster, R. K., Thomas, K., Sivakumar, S. and O'Donovan, D. 2009. The parasite fauna of stray domestic cats (Felis catus) in Dubai, United Arab Emirates. *Parasitol. Res.* **105**: 125–134. [Medline] [CrossRef]
37. Stasiak, M. 2002. The development of food preferences in cats: the new direction. *Nutr. Neurosci.* **5**: 221–228. [Medline] [CrossRef]
38. Tenter, A. M., Heckeroth, A. R. and Weiss, L. M. 2000. *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol.* **30**: 1217–1258. [Medline] [CrossRef]
39. Webster, J. P. and Macdonald, D. W. 1995. Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. *Parasitology* **111**: 247–255. [Medline] [CrossRef]
40. Yamamoto, N., Kon, M., Saito, T., Maeno, N., Koyama, M., Sunaoshi, K., Yamaguchi, M., Morishima, Y. and Kawanaka, M. 2009. [Prevalence of intestinal canine and feline parasites in Saitama Prefecture, Japan]. *Kansenshogaku Zasshi* **83**: 223–228 (in Japanese). [Medline] [CrossRef]
41. Yasuda, N., Ezaki, K., Akuzawa, M., Izawa, M., Doi, T., Sakaguchi, N. and Tatara, M. 1994. Helminth survey of wildcats in Japan. *J. Vet. Med. Sci.* **56**: 1069–1073. [Medline] [CrossRef]
42. Zhang, H., Zhou, D. H., Zhou, P., Lun, Z. R., Chen, X. G., Lin, R. Q., Yuan, Z. G. and Zhu, X. Q. 2009. Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Guangzhou, China. *Zoonoses Public Health* **56**: 502–505. [Medline] [CrossRef]