NOTE *Parasitology*

Serological survey of *Encephalitozoon cuniculi* infection in cats in Japan

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ABSTRACT. Antibodies to *Encephalitozoon cuniculi* (*E. cuniculi*) were examined by enzyme-linked immunosorbent assay using *E. cuniculi* PTP2 recombinant protein from serum samples that had been collected from a total of 295 cats in Japan. Of these samples, 6.1% (18/295) had antibodies against *E. cuniculi*, which included 6.3% (6/96) of the male cats and 6.0% (12/199) of the female cats. The incidence was slightly higher in feral cats (8.3%, 11/132) compared to domesticated cats (4.3%, 7/163). This suggests the possibility that the cats of our country have become a reservoir of *E. cuniculi*. This study is the first to demonstrate the prevalence of *E. cuniculi* infection in cats in Japan.
KEY WORDS: cat, ELISA, *Encephalitozoon cuniculi*, serological survey

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Encephalitozoon cuniculi (*E. cuniculi*) is an obligate intracellular microsporidian parasite and infects a wide range of mammalian hosts, such as rabbits, rodents, dogs and humans [10, 11, 15]. *E. cuniculi* is an opportunistic pathogen in human patients with acquired immunodeficiency syndrome and other immunocompromised people [3, 8]. Infected animals are arguably the reservoirs of human encephalitozoonosis [3]. Encephalitozoonosis is commonly observed in rabbits, and therefore, clinical assessment and diagnosis is well established in this species [8]. Although infections due to *E. cuniculi* are not common in cats, *E. cuniculi* infections have been reported to occur in cats abroad [2, 8, 14]. One author reported that the potential role of cats in the transmission of the infectious agent cannot be excluded [14]. In Japan, a seroepidemiological survey of *E. cuniculi* infection in domesticated dogs and domesticated rabbits has been performed [7, 16], but the prevalence of *E. cuniculi* infections in cats has not been reported. *E. cuniculi* in subclinically infected cats may represent a reservoir and potential risk for immunocompromised patients [11, 12]. The purpose of this study was to determine the seroprevalence of *E. cuniculi* in cats in Japan to serve as baseline epidemiologic data and a potential source of microsporidial infection in Japan. In addition, we compared the seropositive rate of domesticated cats with feral cats.

Furthermore, we examined four infectious diseases including *Toxoplasma gondii* (*T. gondii*), Feline coronavirus (FCoV), feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) that are major diseases of the cat and compared the seroprevalence in feral cats and domesticated cats.

Serum samples were collected from 295 cats that were brought in for surgical neutering to a private animal hospital in the Nagano Prefecture in Japan from September 2011 to September 2012. On examination, there were no clinical signs. The living environment of the examined cats was investigated by questioning the owners, and cats were classified into two groups: feral cats (i.e., no owner, lives outdoors) or domesticated cats (i.e., lives indoors and sometimes outdoors). The age was unidentified because not managed breeding environment. Serum samples were stored at −30°C prior to serological examination. Animal experimentation protocols (e.g., epidemiology investigation) were approved by the president of Kitasato University in accordance with the judgment of the Institutional Animal Care and Use Committee of Kitasato University (Towada, Japan; approval no. 15-064).

Testing for antibodies against *E. cuniculi* was performed using an enzyme-linked immunosorbent assay (ELISA) with glutathione *S*-transferase fusion polar tube protein 2 as the antigen [16]. Individual cat serum samples diluted to 1:400 and horseradish peroxidase-conjugated goat anti-feline immunoglobulin G antibody (MP Biomedicals, Santa Ana, CA, U.S.A.) diluted to 1:10,000 were used as the first and second antibodies, respectively.

Testing for antibodies against *T. gondii* was performed using a commercial latex agglutination test kit (Toxotest, Eiken Chemical Co., Ltd., Tokyo, Japan) in accordance with the manufacturer's instructions. Tests for antibodies against FCoV were performed using a previously published ELISA protocol with feline infectious peritonitis virus solubilization antigen as the antigen [17]. Tests for FeLV antigen and FIV antibody were performed using a commercial assay kit (SNAP FIV/FeLV Combo; IDEXX Laboratories, West-

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brook, ME, U.S.A.) in accordance with the product manual.

The overall seropositive rate to *E. cuniculi* was 6.1% (18/295), which included 6.3% (6/96) of the male cats and 6.0% (12/199) of the female cats; the incidence in feral cats (8.3%, 11/132) was slightly higher than in domesticated cats (4.3%, 7/163) (Table 1). This was not statistically significant. Moreover, 20.0% (59/295) were seropositive to *T. gondii*, 23.7% (70/295) to FCoV, 2.4% (7/295) to FeLV and 10.5% (31/295) to FIV. *Toxoplasma gondii* and FIV seropositive rates were 31.8% and 16.7%, respectively, in feral cats and 10.4% and 5.5%, respectively, in domesticated cats. The FIV seropositivity was significantly higher in males (15.6%) than in females (8.0%) (Table 2). The association between *E. cuniculi* positive samples and the positive samples of four major infectious diseases was assessed, however, this was not significant

E. cuniculi infects a wide range of mammalian hosts, including humans [3]. In animals, the main target organs are the central nervous system and the kidney, which can result in a granulomatous encephalitis and nephritis [12]. The life cycle of *E. cuniculi* is simple and direct and, like other microsporidia, involves a proliferative merogonic stage, followed by a sporogonic stage resulting in rupture of the host cell and release of small (1.5 × 2.5 μm), environmentally resistant, infective spores [12]. Spores can survive for many months in humid environments [9]. However, the natural mechanisms of *E. cuniculi* transmission are not fully understood. It is believed that the disease is spread horizontally in breeding with larger numbers of animals by the fecal-oral route, but above all, along the oro-urinal pathway [4]. Vertical, transplacental transmission of the infection may also play a key role in the epidemiology and pathogenesis, especially in carnivores and rodents [6, 13]. However, little is known about the occurrence of *E. cuniculi* in wildlife [12].

In cats, clinical disease is reportedly rare [12]. Infections with *E. cuniculi* occur in subclinically infected cats and may represent a reservoir and potential risk for immunocompromised patients and animals [7, 8].

In our study, serum samples of 295 cats were examined for the presence of antibodies against *E. cuniculi*. Though there were no clinical signs on general inspection, we found 6.1% of cats in Japan that had *E. cuniculi* antibodies (Table 1). A seroprevalence of 24% (17/72) of cats has been reported in Eastern Slovakia [4], and a recent study in Virginia, U.S.A., found a seroprevalence of 6.5% (15/232) [5]. These differences may reflect the different habitats where samples were obtained and the level of exposure to other sources of infection [12]. Furthermore, it has been reported that there are no sex differences in the prevalence of *E. cuniculi* [1], and the results of our study agree.

Table 1. Seroprevalence of *E. cuniculi* infections in tested serum samples and stratified by living environment and sex

	Total	Living environment		Sex	
		Feral	Domesticated	Male	Female
Samples	295	132	163	96	199
Positive samples	18	11	7	6	12
Positive rate	6.1%	8.3%	4.3%	6.3%	6.0%

In our study, *E. cuniculi* seropositivity was not significantly different in domesticated cats compared to feral cats. This result may reflect the habitat of cats from which samples were obtained and that samples were not obtained from cats that resided completely indoor. On the other hand, it is reported that wildlife species have the potential to be significant reservoirs of infection for both domesticated animals and humans [12]. Furthermore, previous studies have sequenced a mouse strain in cats, indicating that the mouse may be a reservoir for infection in cats [1]. In a previous study, seroprevalence investigations in Japan revealed *E. cuniculi* infection in feral rodents [18]. In addition, we found 8.3% of feral cats in Japan that had *E. cuniculi* antibodies. In the cats of Japan, the mouse can be regarded as one of the important source of *E. cuniculi* infection.

In conclusion, this is the first report of *E. cuniculi* infection in cats in Japan. This suggests the possibility that the cats of our country have become a reservoir of *E. cuniculi*. This result indicates the importance of performing screening examinations of animals with the aim of reducing or halting of the spread of this disease. To further elucidate the route of infection, further studies are needed including the distribution of *E. cuniculi* infection in cats in Japan, other potential hosts or to discover the mouse strain of infection in cats by sequencing.

CONFLICT OF INTEREST. The authors declare no conflicts of interest directly relevant to the content of this article.

Table 2. Seroprevalence of *Toxoplasma gondii* (*T. gondii*), Feline coronavirus (FCoV), feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections in tested serum samples, and stratified by living environment and sex

	Positive samples (positive rate)	Living environment		Sex	
		Feral	Domesticated	Male	Female
T. gondii	59/295 (20%)	42/132 (31.8%)	17/163 (10.4%)	20/96 (20.8%)	39/199 (19.6%)
FCoV	70/295 (23.7%)	28/132 (21.2%)	42/163 (25.8%)	22/96 (23%)	48/199 (24.1%)
FeLV	7/295 (2.4%)	3/132 (2.3%)	4/163 (2.5%)	4/96 (4.2%)	3/199 (1.5%)
FIV	31/295 (10.5%)	22/132 (16.7%)	9/163 (5.5%)	15/96 (15.6%)	16/199 (8.0%)

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