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# Molecular prevalence of *Cryptosporidium* species among household cats and pet shop kittens in Japan

**Yoichi Ito[1,2], Naoyuki Itoh[1], Yuko Iijima[1] and Yuya Kimura[1]**

## Abstract

*Objectives* To address the lack of up-to-date published data, the present study evaluates the PCR-based prevalence of *Cryptosporidium* species infection and molecular characteristics of isolates among household cats and pet shop kittens in Japan.

*Methods* A total of 357 and 329 fresh faecal samples were collected from household cats and pet shop kittens, respectively, with or without clinical signs of infection. A nested PCR assay targeting the 18S rRNA gene was employed for the detection of *Cryptosporidium* species. After specific DNA fragments (approximately 826 base pairs) were confirmed, the amplicons were sequenced to determine species.

*Results* Seven (2.0%) household cats and one (0.3%) pet shop kitten tested positive for the presence of *Cryptosporidium* species. In household cats, there was a significant difference in prevalence between cats aged <1 year (4.6%) and those aged ⩾1 year (0.4%). No significantly different prevalence was observed with regard to faecal condition in either household cats or pet shop kittens. A total of eight *Cryptosporidium* species isolates, seven from household cats and one from a pet shop kitten, were identified as *Cryptosporidium felis*.

*Conclusions and relevance* The present study demonstrates the risk of zoonotic transmission of *Cryptosporidium* species from household cats and pet shop kittens to humans is low in Japan.

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## Introduction

*Cryptosporidium* species are one of the most common intestinal protozoan parasites in cats and can induce gastrointestinal obstruction.[1] Moreover, *Cryptosporidium* species harboured in cats have the potential for zoonotic transmission, which can result in potentially severe gastrointestinal disease.[1] Epidemiological data on *Cryptosporidium* species infection among household cats and pet shop kittens are important for preventing the transmission of this parasite from cats to their owners and carers.

In Japan, however, only one report has been published regarding the PCR-based prevalence of *Cryptosporidium* species infection in household cats,[2] and there are no reports on pet shop kittens. Herein, we report on the recent PCR-based prevalence and molecular characteristics of *Cryptosporidium* species among household cats and pet shop kittens in Japan.

## Materials and methods

Between May 2013 and June 2015, a total of 357 and 329 fresh faecal samples were randomly collected on a single occasion from household cats (aged 1 month to 23 years, 178 males and 179 females) and pet shop kittens (aged 1–3 months, 132 males and 197 females), respectively, with or without a history of illness. The household cats were presented to 10 veterinary clinics

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located in different regions (Hokkaido/Tohoku: five clinics; Kanto: two clinics; Kinki: one clinic; Kyushu/Okinawa: two clinics). The pet shop kittens were maintained in four pet shops (A–D) located in two different regions (Tohoku: two shops [A and B]; Kanto: two shops [C and D]). All faecal samples were donated by the owners or managers, who granted permission to include their cats in the examination. The samples were collected immediately after natural defecation and were stored at 4°C prior to DNA extraction (within 3 days). The oocysts of *Cryptosporidium* species were isolated using a sucrose gradient concentration method with a specific gravity of 1.26, and DNA extraction from isolated oocysts was performed using a QIAamp DNA Mini Kit (QIAGEN). The DNA samples were stored at −20°C prior to analysis.

A nested PCR assay targeting the 18S rRNA gene was employed for the detection of *Cryptosporidium* species.[3] All secondary PCR products were identified by electrophoresis on 1.5% agarose gels. Specific DNA fragments (approximately 826 base pairs) were confirmed after ethidium bromide staining under ultraviolet light. After amplicons were sequenced by a commercial laboratory (Takara Bio), sequence alignment and compilation were performed using the MEGA 6.06 (www.megasoftware.net) program. The DNA sequences were compared with GenBank references of *Cryptosporidium* species by BLAST searches (http://www.ncbi.nlm.nih.gov/).

The data were compared according to age group (<1 year vs ⩾1 year), origin of cats (private owners vs pet shop vs rescue), living condition (indoor vs outdoor), faecal condition (normal vs soft vs diarrhoea) and region (Hokkaido/Tohoku vs Kanto vs Kinki vs Kyushu/Okinawa) in household cats. The data of pet shop kittens were compared in terms of faecal condition and shop (A vs B vs C vs D). For statistical analysis of the data, Fisher's exact probability test was employed, with values of $P < 0.05$ considered significant.

## Results

Of the 357 household cats, seven (2.0%) were positive for *Cryptosporidium* species. According to age group, there was a significant difference in the prevalence between cats aged <1year and those aged ⩾1 year (Table 1). No significant differences were observed with regard to origin, living condition and faecal condition. Although prevalence in the Kinki region was significantly higher than that in the Tohoku/Hokkaido region, the protozoa were detected in all regions. Of the 329 pet shop kittens, only one individual (0.3%) was positive for *Cryptosporidium* species presence. There was no significant difference in prevalence among faecal condition and pet shops.

An analysis of the 18S rRNA gene fragments indicated that all eight PCR-positive samples showed 99–100% similarity to the sequences of *Cryptosporidium felis* (accession numbers: KJ194110.1, KM977642.1 and AF112575.1).

## Discussion

The present study suggests that the prevalence of *Cryptosporidium* species infection is low in both household cats and pet shop kittens in Japan. The molecular prevalence of *Cryptosporidium* species infection has recently been recorded as 7.1% in Australia,[4] 0% in Italy,[5] 12.7% in Japan[2] and 4.6% in the Netherlands[6] in household cats, and 0% in China[7] and 3.4% in Australia[4] in pet shop kittens. The wide ranging distribution of prevalence observed in previous studies regarding household cats was likely dependent on the differences in the investigated population. We agree with studies that suggest a higher prevalence of *Cryptosporidium* species infection in young cats because the present study shows a significantly higher prevalence in cats aged <1 year.[2,8] It is believed that the high prevalence in young cats is owing to their immunological immaturity,[8] although the immunological defence system against *Cryptosporidium* species infection is currently unknown.[9]

Pet shop cats are at higher risk for infection because of the easy transmission between animals due to high contact density,[10,11] and the difficulty of controlling contamination by environmentally resistant oocysts.[12,13] In contrast with these views, young populations of pet shop kittens did not exhibit a high prevalence in the present investigation. Since 2/7 detected infections in households were in young cats that originated from pet shops, it is difficult to believe that pet shops in Japan are free from *Cryptosporidium* species contamination. However, infection was found in only one pet shop kitten, implying that infection rates in pet shop kittens are likely low in Japan.

However, the details of the other five positive cats in households include one adult (2 years old) outdoor cat and four young (<1 year old) rescued indoor cats. It is assumed that the adult cat was infected with *Cryptosporidium* species in the field. The four young indoor cats were also likely infected by environmental contamination in the field as they were possibly living outdoors until their rescue. Previous reports have suggested a high prevalence of *Cryptosporidium* species infection in stray and shelter cats.[4]

There was also a significant difference in the prevalence among regions. The Kinki region had a higher prevalence than the Tohoku/Hokkaido region, but this result did not indicate that the Kinki region was the dominant region for *Cryptosporidium* species infection. The major factor influencing the higher prevalence in the Kinki region was certainly the very high percentage of young (<1 year old) cats (88.2%). Nevertheless, the results of the present study suggest that *Cryptosporidium* species infection is low but has widely infected household cats in Japan as the infected cats were detected in all regions.

**Table 1** Molecular prevalence of *Cryptosporidium* species among household cats and pet shop kittens in Japan

| Category | Examined (n) | Positive (n) | Prevalence (%) | *P* value |
|---|---|---|---|---|
| Household cats | 357 | 7 | 2.0 | – |
| Age (year) | | | | |
| <1 | 128 | 6 | 4.6 | <0.05 |
| ⩾1 | 229 | 1 | 0.4 | – |
| Origin | | | | |
| Private owners | 83 | 0 | 0 | – |
| Pet shop | 75 | 2 | 2.7 | NS |
| Rescue | 199 | 5 | 2.5 | NS |
| Living condition | | | | |
| Indoor | 271 | 6 | 2.2 | NS |
| Outdoor | 86 | 1 | 1.2 | – |
| Faecal condition | | | | |
| Normal | 316 | 6 | 1.9 | NS |
| Soft | 26 | 1 | 3.8 | NS |
| Diarrhoea | 14 | 0 | 0 | – |
| Region | | | | |
| Hokkaido/Tohoku | 191 | 1 | 0.5 | – |
| Kanto | 117 | 2 | 1.7 | NS |
| Kinnki | 17 | 2 | 11.8 | <0.01 |
| Kyushu/Okinawa | 32 | 2 | 6.3 | NS |
| Pet shop kittens | 329 | 1 | 0.3 | – |
| Faecal condition | | | | |
| Normal | 275 | 0 | 0 | – |
| Soft | 43 | 1 | 2.3 | NS |
| Diarrhoea | 11 | 0 | 0 | NS |
| Pet shop | | | | |
| A | 25 | 0 | 0 | – |
| B | 116 | 0 | 0 | NS |
| C | 7 | 0 | 0 | NS |
| D | 178 | 1 | 0.6 | NS |

NS = not significant

There was no correlation between infection and faecal condition in either household cats or pet shop kittens. It has previously been demonstrated that most cats infected with *Cryptosporidium* species are normal in immunocompetent cases.[1]

All eight *Cryptosporidium* species isolates in the present study were identified as *C felis*, which is believed to be a cat-adapted *Cryptosporidium* species.[1] Although the predominant species for human cryptosporidiosis are *Cryptosporidium hominis* and *Cryptosporidium parvum*,[13] immunocompromised humans are rarely at risk of infection with *C felis*.[1,13] Nevertheless, considering the low prevalence in this study, it is likely that the risk of zoonotic transmission of *Cryptosporidium* species from household cats and pet shop kittens to humans is low in Japan.

## Conclusions

The prevalence of *Cryptosporidium* species infection is low in both household cats and pet shop kittens in Japan. In addition, the risk of zoonotic transmission of *Cryptosporidium* species from household cats and pet shop kittens to humans is also likely low in Japan, since all *Cryptosporidium* species isolates were identified as *C felis*.

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## References

1 Lucio-Foster A, Griffiths JK, Cama VA, et al. **Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats**. *Trends Parasitol* 2010; 26: 174–179.

2 Yoshiuchi R, Matsubayashi M, Kimata I, et al. **Survey and molecular characterization of *Cryptosporidium* and *Giardia* spp. in owned companion animal, dogs and cats, in Japan**. *Vet Parasitol* 2010; 174: 313–316.

3 Xiao L, Morgan UM, Limor J, et al. **Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species**. *App Environ Microbiol* 1999; 65: 3386–3391.

4 Yang R, Ying JLJ, Monis P, et al. **Molecular characterisation of *Cryptosporidium* and *Giardia* in cats (*Felis catus*) in Western Australia**. *Exp Parasitol* 2015; 155: 13–18.
5 Paoletti B, Otranto D, Weigl S, et al. **Prevalence and genetic characterization of *Giardia* and *Cryptosporidium* in cats from Italy**. *Res Vet Sci* 2011; 91: 397–399.
6 Overgaauw PAM, van Zutphen L, Hoek D, et al. **Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands**. *Vet Parasitol* 2009; 163: 115–122.
7 Li W, Li Y, Song M, et al. **Prevalence and genetic characteristics of *Cryptosporidium, Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China**. *Vet Parasitol* 2015; 208: 125–134.
8 Rambozzi L, Menzano A, Mannelli A, et al. **Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors**. *J Feline Med Surg* 2007; 9: 392–396.
9 McDonald V. **Cryptosporidiosis: host immune responses and the prospects for effective immunotherapies**. *Expert Rev Anti Infect Ther* 2011; 9: 1077–1086.
10 Kirkpatrick CE and Dubey JP. **Enteric coccidial infections. *Isospora, Sarcocystis, Cryptosporidium, Besnoitia,* and *Hammondia*.** *Vet Clin North Am Small Anim Pract* 1987; 17: 1405–1420.
11 McGlade TR, Robertson ID, Elliot AD, et al. **Gastrointestinal parasites of domestic cats in Perth, Western Australia**. *Vet Parasitol* 2003; 117: 251–262.
12 Adam RD. **Biology of *Giardia lamblia***. *Clin Microbiol Rev* 2001; 14: 447–475.
13 Bouzid M, Hunter PR, Chalmers RM, et al. ***Cryptosporidium* pathogenicity And virulence**. *Clin Microbiol Rev* 2013; 26: 115–134.