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Two Species of *Cryptosporidium* Naturally Infecting House Rats, *Rattus norvegicus*

MOTOHIRO ISEKI

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Introduction

Protozoan parasites of genus *Cryptosporidium* are known to cause diarrheal illness in various animals and immunocompromised humans (Angus, 1983; Tzipori, 1983; Current *et al.*, 1983). Cryptosporidial infections occur worldwide and have been reported in a variety of vertebrates including mammals, birds, reptiles and fish.

Tyzzer described two species of *Cryptosporidium*; *C. muris* from the gastric glands of laboratory mice (Tyzzer, 1907, 1910) and *C. parvum* from the small intestine of laboratory mice (Tyzzer, 1912). Oocysts of the former species, measuring 7 × 5 μm, are distinctly larger than those of the latter, measuring up to 4.5 μm. Although variously named or unnamed species of *Cryptosporidium* have been reported since the first description by Tyzzer, the oocysts in most cases have been described as being about 5 μm in diameter. Recently, Upton and Current (1985) reported a large form and a small form of oocyst of *Cryptosporidium* from naturally infected calves, which they considered should be regarded as those of *C. muris* and *C. parvum*, respectively.

In this paper, the results of a survey on the prevalence of *Cryptosporidium* among house rats in Osaka are presented. The morphologic and biologic characteristics of two types of *Cryptosporidium*, a larger type and a smaller type, obtained from naturally infected rats are also described.

Department of Medical Zoology, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan.

Materials and Methods

Fecal samples were collected from 64 house rats, 61 *Rattus norvegicus* (♂ 26, ♀ 35; 26–328 g in body weight) and 3 *R. rattus* (♂ 1, ♀ 2; 30–100 g), captured at various places in 7 districts of Osaka City in February 1986. Fecal materials were obtained from the cecum and colon of each rat by necropsy, and preserved in a 2.5% (*w/v*) solution of $K_2Cr_2O_7$ for several days prior to further examination. Fecal examinations were carried out by the sugar centrifugal-flotation method using Sheather's sugar solution (sp. gr. 1.2).

When oocysts of *Cryptosporidium* were detected, the floated materials were transferred to a test tube containing water and washed twice with water by centrifugations. The sediment containing oocysts was resuspended in 2.5% $K_2Cr_2O_7$ solution and stored at 4°C for up to 2 weeks prior to further examination.

As two types of oocysts of *Cryptosporidium* were obtained from 9 rats, the following experiments were carried out:

In order to examine the infectivity, each of 9 concentrated fecal samples containing oocysts of *Cryptosporidium* was inoculated *per os* to SPF laboratory rats (Slc:SD strain, 3-week-old males). Each of 5 non-treated control rats and

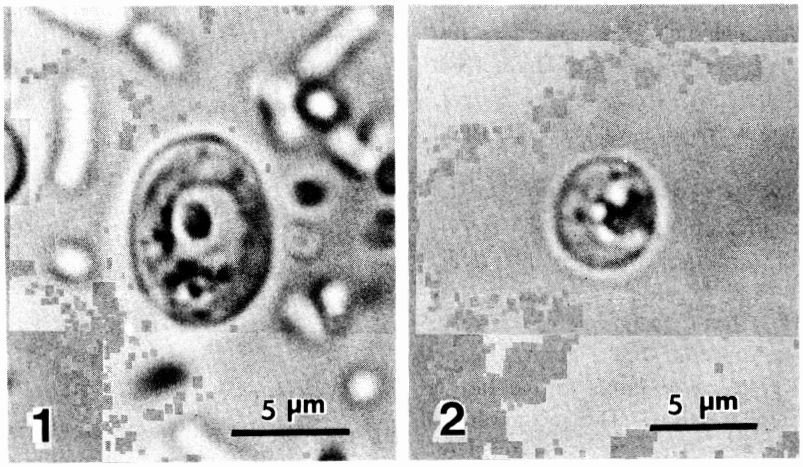
9 inoculated rats was separately kept in a wirecage placed on a clean stainless steel tray containing a 5-mm depth of water in order to keep the feces wet. Daily fecal examinations were carried out for each rat by the sugar flotation method. One strain of each of the two types of *Cryptosporidium* was selected from the 9 experimentally infected SPF rats, and their oocysts were subinoculated into clean SPF rats for further examination. Giemsa-stained mucosal stamp smears of the stomach, small intestine (each of upper, middle and lower parts), cecum and colon of the sub-inoculated rats were prepared and the endogenous developmental stages of both types of *Cryptosporidum* were examined.

Oocysts of both the small and the large types obtained from experimentally infected rats were measured using a microscope with a calibrated ocular micrometer at 1,500×. The measurements were made for specimens prepared in three different ways: (1) fresh oocysts in water, (2) oocysts in fecal smears pre-fixed with fumes of 2% OsO_4 followed by methanol fixation and stained with Giemsa's solution (Tyzzer, 1910), and (3) oocysts in the stomach or intestinal smears stained by the same procedure as in (2). Fifty oocysts were used for each measurement.

Results

Oocysts of *Cryptosporidium* were detected in 9 (14.8%) out of 61 *R. norvegicus* individuals, but none of the 3 *R. rattus* individuals were infected. Among the 9 infected rats (♂ 4, ♀ 5), collected from 4 districts, the smallest individual had a body weight of 47 g and the largest one weighed 245 g.

Two forms of oocysts of *Cryptosporidium* were obtained (Figs. 1 and 2). The small form of oocyst was detected in the feces of 6 rats (♂ 2, ♀ 4), and the large form of oocyst was detected in the feces of 3 rats (♂ 2, ♀ 1). Oocysts of both the small and the large types showed infectivity in the SPF laboratory rats. All of the 9 inoculated SPF rats discharged oocysts in their feces, while no oocysts were detected in the feces of the 5 control rats. The SPF rats inoculated with the small form of oocyst discharged the same type of oocyst in the feces, beginning 8 days after the inoculation and continuing for 13 days. On the other hand, the SPF rats inoculated with the large form of oocyst also discharged the same type of oocyst from day 13 through day 42 after the inoculation. In other words, its prepatent and patent periods were 12 days and 30 days, respectively.



Figs. 1 and 2 Photomicrographs of fresh oocysts of the large type (Fig. 1) and the small type (Fig. 2) of *Cryptosporidium* isolated from the house rat, *Rattus norvegicus*.

Examinations of Giemsa-stained mucosal stamp smears of the gastrointestinal tracts of experimentally infected rats revealed that the entire development of the large type of *Cryptosporidium* occurred in the stomach, while the small type of *Cryptosporidium* developed in the small intestine.

Oocysts of the small form and the large form measured 5.3 x 4.8 (5.0–6.0 x 4.0–5.5) μm and 8.4 x 6.3 (7.5–9.8 x 5.5–7.0) μm, respectively. There were few differences in the sizes of oocysts among above-mentioned three different specimens for measurements. The oocysts in smears prefixed with OsO_4 fumes maintained their intact shape and were satisfactorily stained with Giemsa's solution.

The rats experimentally infected with the large or the small types of *Cryptosporidium* did not show any clinical symptoms such as diarrhea, low appetite, weakness or weight loss.

Discussion

In the last 5 years it has been revealed that human cryptosporidiosis occurs on a worldwide basis (Jokipii *et al.*, 1983; Tzipori, 1983; Hϕjlyng *et al.*, 1984; Hunt *et al.*, 1984; Shahid *et al.*, 1985; CDC, 1984). Although, up to the end of the 1970s, *Cryptosporidium* was reported to be not only host- but also site-specific (Tyzzer, 1910, 1912; Vetterling *et al.*, 1971; Iseki, 1979), recent transmission experiments have indicated that *Cryptosporidium* from some species of animals can infect a variety of other animals including man (Moon and Bemrick, 1981; Tzipori *et al.*, 1980; 1981; Reese *et al.*, 1982). Therefore, it is important to know the incidence of cryptosporidial infections among domestic, pet and wild animals closely associated with humans.

As far as the author is aware, this is the first report dealing with the natural infection of *Cryptosporidum* in wild rats. The present investigation showed that *Cryptosporidium* is widespread even in rats living in city areas. If cryptosporidial oocysts discharged in the feces of house rats are infective to humans, we should pay more attention to rats as a source of human cryptosporidiosis.

Tyzzer (1907, 1910 and 1912) described two species of *Cryptosporidium*; *C. muris* from the gastric glands of laboratory mice and Japanese waltzing mice, and *C. parvum* from the small intestine of laboratory mice. He performed transmission experiments using both species, and concluded that *C. muris* inhabited always the stomach, and never the intestine, while *C. parvum* occurred only in the small intestine. Oocysts of *C. muris*, measuring 7 x 5 μm, were distinctly larger than those of *C. parvum*, measuring up to 4.5 μm. Since 1912, variously named or unnamed species of *Cryptosporidium* and many cases of infection in animals including man have been reported. However, on the basis of cross-transmission experiments using isolates from calves, Tzipori *et al.* (1980) suggested that *Cryptosporidium* should be regarded as a single-species genus. Moreover, Levine (1984) considered *C. parvum* a synonym of *C. muris* and concluded that all species infecting mammals should be treated as *C. muris*.

Recently, Upton and Current (1985) reported a large form and a small form of oocyst of *Cryptosporidium* from naturally infected calves. On the basis of the oocyst morphology they considered that the smaller form, 5.0 x 4.5 (4.5–5.4 x 4.2–5.0) μm, and the larger form, 7.4 x 5.6 (6.6–7.9 x 5.3–6.5) μm, should be regarded as *C. parvum* and *C. muris*, respectively. The present author also obtained two forms of oocysts of *Cryptosporidium* from wild rats. Oocysts of the large form, 8.4 x 6.3 (7.5–9.8 x 5.5–7.0) μm, were larger than those of *C. muris* described by Tyzzer (1907, 1910) or Upton and Current (1985). In order to confirm this fact, the author performed measurements of oocysts prepared by three different methods as mentioned above. In particular, the Giemsa staining method was the same as that given in Tyzzer's original description. Tyzzer's method, using OsO_4 fumes prior to methanol fixation of smears, produced satisfactory results, i.e., neither shrinkage nor

morphologic changes occurred in the oocysts and the sporozoites in oocysts were stained clearly by Giemsa's solution. As there were few differences in the sizes of the oocysts from these three preparations, the author concludes that the oocysts of the large form obtained from rats are distinctly larger than those of *C. muris* reported previously.

By transmisison experiments using SPF laboratory rats, it was revealed that the large type of *Cryptosporidium* from wild rats was infective to laboratory rats and that the parasite inhabited only gastric glands throughout its entire development. No endogenous developmental stages, except for mature oocysts passing through the intestine, were found in smears of the upper, middle, and lower parts of the small intestine, cecum, and colon of experimentally infected rats, even though most gastric glands were filled with extremely large numbers of the parasites.

Oocysts of the small form, 5.3 x 4.8 (5.0–6.0 x 4.0–5.5) μm, obtained from rats were also larger than those of *C. parvum* described by Tyzzer (1912) or by Upton and Current (1985). Previously, the present author described *C. felis* from the domestic cat, and reported that the oocyst measured 5 x 4.5 μm (Iseki, 1979). The entire development of the small type occurred only in the small intestine of the experimentally infected rats in the present study.

The site specificity of *C. muris* and *C. parvum* has already been reported by Tyzzer (1910 and 1912). Also, in the case of this study, it was revealed that both the large and the small types of *Cryptosporidium* were site-specific.

No clinical changes were observed in the rats experimentally infected with oocysts of the small type or the large type of *Cryptosporidium,* even though they discharged a large number of oocysts in their feces during the patent periods. Therefore, the pathogenicity of both types of *Cryptosporidium* for rats seems to be low.

The morphologic and biologic characteristics of both forms, except for the host species and the size of oocysts, show that the large type and the small type of cryptosporidia isolated from rats resemble to *C. muris* and *C. parvum,* respectively. However, Tyzzer (1910) reported that *C. muris* did not infect rats in his transmission trial. At this time, the present author considers that further experimental information should be obtained in order to determine whether or not both forms isolated from rats are species distinct from *C. muris* and *C. parvum,* respectively.

Summary

Nine (14.8%) out of 61 *Rattus norvegicus* individuals, captured in Osaka, were found to be infected with *Cryptosporidium.* Two forms of oocysts of *Cryptosporidium* were isolated from their feces. Oocysts of the small form, measuring 5.3 x 4.8 (5.0–6.0 x 4.0–5.5) μm, were detected in 6 (9.8%) out of the 61 rats. Oocysts of the large form, 8.4 x 6.3 (7.5–9.8 x 5.5–7.0) μm, were detected in 3 (4.9%) of the 61 rats. Oocysts of both the small and the large forms were fully sporulated in the fresh feces and were infective to SPF laboratory rats. The entire development of the large type of *Cryptosporidium* occurred in the gastric glands of the experimentally infected SPF rats, whereas the small type of *Cryptosporidium* parasitized the small intestine of the rats. No clinical symptoms such as diarrhea, low appetite, weakness or weight loss were observed in rats experimentally infected with oocysts of the large or the small type of *Cryptosporidium.*

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ドブネズミから分離された2種の *Cryptosporidium* について

井関基弘
（大阪市立大学医学部医動物学教室）

大阪市内の7区で1986年2月に捕獲されたネズミ64頭（ドブネズミ61頭，クマネズミ3頭）について，クリプトスポリジウムの感染状況を調査し，分離されたオーシストはSPFラットに感染させて，その形態および生物学的性状を観察した．

検査に供した糞便材料は麻酔死させたネズミの盲腸および大腸から採取し，比重1.2のショ糖液による遠心沈澱浮遊法によってオーシストを検索した．

ドブネズミ61頭のうち9頭（14.8%）の糞便から2種類のクリプトスポリジウムのオーシストが検出された．9頭の感染ネズミは大阪市内の4区で捕獲されたものであり，本原虫は市内の広域のネズミに感染していることが判明した．ネズミにおける本原虫の自然感染例の報告は今回が初めてである．

分離されたオーシストには大型と小型の2種類があり，両種ともSPFラットに感染性を有した．大型種のオーシストは8.4×6.3（7.5－9.8×5.5－7.0）μmで，3頭（4.9%）から検出され，小型種は5.3×4.8（5.0－6.0×4.0－5.5）μm で，6頭（9.8%）から検出された．感染実験では大型種は腺胃にのみ，小型種は小腸にのみ寄生し，両種の寄生部位に臓器特異性の差異が認められた．

形態および生物学的性状は，大型種は *C.muris* Tyzzer 1907 に，小型種は *C.parvum* Tyzzer 1912 にそれぞれ似ているが，それらと同一種であるか否かについてはさらに詳細な検討が必要である．両種によって感染したSPFラットは全て多数のオーシストを排出したが，下痢や食欲低下，体重減少などの臨床症状は認められなかった．