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Increase in Size of Oocysts of *Cryptosporidium* sp. from Chicken and *C. muris* during Patency and during Preservation

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Abstract

Mean lengths, widths, and length/width ratios of oocysts of chicken *Cryptosporidium* sp. isolated in Japan and *C. muris* were measured during patency and preservative period. The oocysts of *Cryptosporidium* sp. on days 10, 14 and 20 postinoculation (PI) increased in length by 2–3% from those on day 7, nevertheless, oocyst length/width ratio did not change. The oocysts of *C. muris* from mice on day 30 PI increased in length by 2–4% from those on days 10 and 14 PI, and former oocysts also increased in length/width ratio. The oocysts of *Cryptosporidium* sp. on day 10 and *C. muris* on day 14 were stored in 2% potassium dichromate solution at 4°C. They were measured at 3 and 12 months later and compared with oocysts measured at 7 days after preservation. The twelve-month-preservated oocysts from chickens increased in either length and width by 3% and 5–6% from 7-day- and 3-month-preservated oocysts, respectively. The length/width ratio decreased by 2%. In *C. muris*, twelve-month-preservated oocysts increased in either width or length/width ratio by 4–5%, though the length was not different. These results suggest that the sizes of cryptosporidian oocysts increased by 2–5% during patency and during preservation.

Key words: oocyst size; *Cryptosporidium*; classification.

Introduction

Although many species of *Cryptosporidium* have been named according to the hosts in which the parasite were found, *C. muris* and *C. parvum* in mammals and *C. meleagridis* and *C. baileyi* in birds are accepted as distinct and valid species (Fayer *et al.*, 1990). The former two species are different in their sizes and sites of infection (Upton and Current, 1985; Iseki, 1986), and the latter two species are slightly different in their oocyst size. The morphological structure of sporulated oocysts is important in the classification of coccidian parasites. Lindsay *et al.* (1989) compared the structure of oocysts of *C. meleagridis* with that of *C. baileyi* and reported that both oocyst sizes were larger than those reported originally by Slavin (1955) and Current *et al.* (1986) and the mean lengths and widths of *C. meleagridis* oocysts were significantly smaller than those of *C. baileyi* oocysts. Itakura *et al.* (1984) isolated cryptosporidian oocysts from chickens in poultry farms in Japan, and the oocyst size of this parasite was found to be medium in size between *C. meleagridis* and *C. baileyi* (Matsui *et al.*, 1992). This parasite has not yet been identified at the present time.

Increase of coccidian oocysts in size during patency was described by Duszynski (1971), however, that of cryptosporidian oocysts has not yet been examined. The present study describes the mean lengths, widths, and length/width ratios of oocysts of chicken *Cryptosporidium* isolated in Japan and *C. muris* during patency and during preservation.

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Materials and Methods

Oocysts

Oocysts of *Cryptosporidium* sp. from chicken

(Itakura *et al.*, 1984) were obtained from Dr. Itakura, Faculty of Veterinary Medicine, Hokkaido University. *C. muris* (strain RN 66) isolated from the house rat was supplied by Iseki (1986), Osaka City University Medical School. Both species were subjected to passage in chickens or mice to multiply oocysts, respectively. Oocysts were stored at 4°C in 2% potassium dichromate solution before use.

Experimental animals

The hosts used in this study were 2- to 3-week-old White Leghorn, 3-week-old mice (SPF, ICR), 5-week-old Japanese quails and 3-week-old rats (SPF, Wistar). All rodents and birds were confirmed to be free of natural coccidial infections by repeated fecal examinations prior to experiments and were housed separately as groups in wire-bottom cages and raised under the coccidia-free environment.

Experimental design

To determine the variability of shape and size of the cryptosporidial oocysts during patency, 3 chickens were inoculated with 3.5×10^6 *Cryptosporidium* sp. oocysts/bird and the feces were collected on days 7, 10, 14 and 20 postinoculation (PI). Five mice were inoculated with 2.0×10^6 *C. muris* oocysts/animal and their feces were collected on days 10, 14 and 30 PI. Oocysts were collected from the feces of hosts within 1 hr after shedding and suspended in water. They were stirred for 1 hr, filtered through double wire-screen (50- and 100-mesh), and centrifuged (2000 rpm for 7 min). The precipitates were suspended in a small amount of distilled water, poured into about 10 vols. of 2% potassium dichromate solution in the collecting flask and then stored at 4°C. The oocysts in each flask were concentrated by the sugar flotation method (sp. gr. 1.266) prior to examination and 50 oocysts of each flotation were measured using a microscope with a calibrated ocular Nikon micrometer at 1000×. Differences between lengths and between widths of oocysts at test days were calculated using Student's *t* test.

To determine the variability of oocysts during preservation, the oocysts from feces of the chickens on day 10 and the oocysts from mice on day 14 were measured 3 and 12 months later and compared with the size measured on day 7 after preservation.

Three Japanese quails were inoculated with 3.5×10^6 *Cryptosporidium* sp. oocysts and 3 rats were received 2.0×10^6 *C. muris* oocysts in order to determine the variability of oocysts from different hosts. The oocysts from Japanese quails on day 10 PI and rats on day 14 PI were measured and compared with those from chickens on day 10 PI and mice on day 14 PI, respectively.

Results

Variability in oocyst size of Cryptosporidium *during patency*

The fecal samples collected from chickens at test days showed that oocysts increased in length from day 7 to days 10, 14 and 20 PI (Table 1). The oocysts on day 7 and 14 PI measured 5.4–6.5 μm (mean 6.00 μm) and 5.6–7.0 μm (mean 6.18 μm), respectively. This difference was considered significant ($P<0.001$). Despite the increase in length of the oocysts during patency, there was no change in the length/width ratios (shape-index). The *C. muris* oocysts from mice increased in length from the 14th (mean 7.79 μm) to the 30th (mean 7.98 μm) day PI (Table 2), and this difference was significant ($P<0.05$). The length/width ratio of oocysts increased from the 10th day to the 30th day ($P<0.01$).

Variability in oocyst size of Cryptosporidium *during preservation*

Twelve-month-preservative oocysts (6.32 by 4.87 μm) from chickens increased ($P<0.001$) in length and width from 3-month-preservated oocysts (6.12 by 4.61 μm), and the length/width ratio decreased by 2% (Table 3). In *C. muris*, the width of twelve-month-preservated oocysts (5.81 μm) increased ($P<0.001$) from that of 3-month-preservated oocysts (5.57 μm), and the length/width ratio decreased by 4%, though the size of length was not different (Table 4).

Variability in oocyst size of Cryptosporidium *from different hosts*

The oocysts from Japanese quails did not differ significantly in length or width from those of chickens. Similarly, the sizes of *C. muris* oocysts from mice were not different from those of rats (Table 5).

Table 1 Mean size (in μm) of oocyst length and width of *Cryptosporidium* sp. from chicken in Japan on days 7, 10, 14 and 20 PI

Days PI	Length Mean±S.D. (Range)	Width Mean±S.D. (Range)	L/W ratio Mean±S.D. (Range)
7	6.00±0.28 (5.4–6.5)	4.58±0.24 (4.2–5.1)	1.31±0.09 (1.12–1.49)
10	6.13±0.26 (5.6±6.7) *	4.64±0.20 (4.2–5.0)	1.32±0.07 (1.13–1.46)
14	6.18±0.28 (5.6–7.0) †	4.62±0.18 (4.3–5.1)	1.34±0.06 (1.22–1.52)
20	6.12±0.23 (5.7–6.6) *	4.63±0.17 (4.3–5.1)	1.32±0.07 (1.19–1.44)

*$P<0.05$ †$P<0.001$

Table 2 Mean size (in μm) of oocyst length and width of *C. muris* on days 10, 14 and 30 PI

Days PI	Length Mean±S.D. (Range)	Width Mean±S.D. (Range)	L/W ratio Mean±S.D. (Range)
10	7.66±0.35 (6.9–8.3)	5.58±0.25 (5.1–6.2)	1.38±0.08 (1.20–1.54)
14	7.79±0.46 (6.8–8.8)	5.54±0.27 (5.0–6.1)	1.41±0.10 (1.25–1.62)
30	7.98±0.38 (7.0–8.7) ‡ (vs. day 10), * (vs. day 14)	5.62±0.33 (5.0–6.4)	1.42±0.11 (1.21–1.70) † (vs. day 10)

*$P<0.05$ †$P<0.01$ ‡$P<0.001$

Discussion

In the present experiments, the oocysts of *Cryptosporidium* sp. from chickens and *C. muris* from mice increased in only length by 3–4% (0.18–0.32 μm) during patency. The size of oocysts (6.18 × 4.62 μm) on day 14 PI of *Cryptosporidium* sp. from chicken was similar to that of *C. baileyi* (6.2 × 4.6 μm) reported originally by Current *et al.* (1986), though the oocysts on day 7 PI (6.00 × 4.58 μm) was medium in size between *C. meleagridis* (5.2 × 4.6 μm) and *C. baileyi* (6.6 × 5.0 μm) reported by Lindsay *et al.* (1989). Moreover, the length and width of *Cryptosporidium* sp. oocyst from chickens and the width of *C. muris* oocyst increased after twelve month preservation. Therefore, it has been confirmed that cryptosporidial oocysts discharged at early times were about 0.1–0.3 μm smaller than those observed at later times during patency and that oocyst size has increased during twelve month pres-

Table 3 Mean size (in μm) of oocyst length and width of *Cryptosporidium* sp. from chicken in Japan during preservation

Preservation	Length Mean±S.D. (Range)	Width Mean±S.D. (Range)	L/W ratio Mean±S.D. (Range)
7 days	6.13±0.26 (5.6–6.7)	4.64±0.20 (4.2–5.0)	1.32±0.07 (1.13–1.46)
3 months	6.12±0.24 (5.7–6.7)	4.61±0.23 (4.1–5.1)	1.33±0.07 (1.18–1.49)
12 months	6.32±0.28 † † (5.6–6.9)	4.87±0.22 † † (4.4–5.5)	1.30±0.07 * (1.16–1.48)

Oocysts were collected from chicken on day 10 PI.
$*P<0.05$ $\dagger P<0.001$

Table 4 Mean size (in μm) of oocyst length and width of *C. muris* during preservation

Preservation	Length Mean±S.D. (Range)	Width Mean±S.D. (Range)	L/W ratio Mean±S.D. (Range)
7 days	7.79±0.46 (6.8–8.8)	5.54±0.27 (5.0–6.1)	1.41±0.10 (1.25–1.62)
3 months	7.72±0.47 (6.8–8.7)	5.57±0.25 (5.1–6.2)	1.39±0.08 (1.21–1.52)
12 months	7.76±0.41 (7.1–8.9)	5.81±0.27 ‡ ‡ (5.4–6.4)	1.34±0.09 ‡ † (1.16–1.53)

Oocysts were collected from mice on day 14 PI.
$\dagger P<0.01$ $\ddagger P<0.001$

ervation. Duszynski (1971) examined the oocyst size of *Eimeria separata* from infected rats during patency and reported that the oocysts increased in length and width by approximately 40% from the beginning to the end of the patent period. The increase of oocyst sizes of both *Cryptosporidium* were smaller than those of *E. separata*.

On the other hands, oocyst size of the same species was slightly different by reporters. Namely, oocysts of *C. baileyi* reported by Current *et al.* (1986) were 0.4 μm smaller than those of *C. baileyi* described by Lindsay *et al.* (1989). On *C. muris*, oocysts reported by Iseki (1986) were larger (0.4 μm in length and 0.7 μm in width) than those examined in the present experiments. These difference (0.4–0.7 μm) are bigger than the increases (0.1–0.3 μm) in sizes during patency. This finding indicates that oocyst sizes of *Cryptosporidium* are slightly different by processors and/or instruments for the measurements and by collected days during patency. Therefore, it is considered that the classification for *Cryptosporidium* could not be identified in case of

Table 5 Mean size (in μm) of oocyst length and width of *Cryptosporidium* sp. from chickens and Japanese quails and of *C. muris* from mice and rats

Host	Days PI	Length Mean±S.D. (Range)	Width Mean±S.D. (Range)	L/W ratio Mean±S.D. (Range)
Cryptosporidium sp. from chicken				
Chickens	10	6.13±0.26 (5.6–6.7)	4.64±0.20 (4.2–5.0)	1.32±0.07 (1.13–1.46)
Japanese quails	10	6.07±0.25 (5.7–6.6)	4.71±0.26 (4.2–5.3)	1.29±0.08 (1.16–1.55)
C. muris				
Mice	14	7.79±0.46 (6.8–8.8)	5.54±0.27 (5.0–6.1)	1.41±0.10 (1.25–1.62)
Rats	14	7.80±0.44 (6.9–8.8)	5.52±0.29 (5.1–6.1)	1.41±0.08 (1.20–1.59)

difference in size of oocysts within 1 μm.

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References

1) Current, W. L., Upton, S. J. and Haynes, T. B. (1986): The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. Protozool., 33, 289–296.
2) Duszynski, D. W. (1971): Increase in size of *Eimeria separata* oocysts during patency. J. Parasitol., 57, 948–952.
3) Fayer, R., Speer, C. A. and Dubey, J. P. (1990): General biology of *Cryptosporidium*. Cryptosporidiosis of man and animals (ed. J. P. Dubey, C. A. Speer, R. Fayer), CRC Press, Florida, 1–29.
4) Iseki, M. (1986): Two species of *Cryptosporidium* naturally infecting house rats, *Rattus norvegicus*. Jpn. J. Parasitol., 35, 521–526.
5) Itakura, C., Goryo, M. and Umemura, T. (1984): Cryptosporidial infection in chickens. Avian Pathol., 13, 487–499.
6) Lindsay, D. S., Blagburn, B. L. and Sunderman, C. A. (1989): Morphometric comparison of the oocysts of *Cryptosporidium meleagridis* and *Cryptosporidium baileyi* from birds. Proc. Helminthol. Soc. Wash., 56, 91–92.
7) Matsui, T., Morii, T., Fujino, T., Tadeja, S. L. and Itakura, C. (1992): Oocyst production and immunogenicity of *Cryptosporidium* sp. in chickens. Jpn. J. Parasitol., 41, 24–29.
8) Slavin, D. (1955): *Cryptosporidium meleagridis* (sp. Nov.). J. Comp. Path., 65, 262–266.
9) Upton, S. J. and Current, W. L. (1985): The species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) infecting mammals. J. Parasitol., 71, 625–629.