Infectivity of *Cryptosporidium* sp. Isolated from Chickens in Japan to Turkeys, Bobwhite Quails and Several Kinds of Experimental Animals

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Abstract

The infectivity of *Cryptosporidium* sp. isolated from chickens in Japan to turkeys, bobwhite quails and several kinds of experimental animals was examined. Four turkeys (2 and 4 weeks old) were inoculated orally with $10^6 - 10^7$ oocysts. One of them was treated with dexamethasone phosphate before oocyst inoculation. Three of four turkeys including the treated bird shed a few oocysts on day 4, 6 or 8 postinoculation (PI), respectively. Two 5-week-old and five 10-week-old bobwhite quails were inoculated orally with 10^6 oocysts. Two 5-week-old birds and two of five birds (10 weeks old) were treated with dexamethasone phosphate before oocyst inoculation. Five-week-old birds and one treated bird (10 weeks old) shed oocysts from day 4 PI. Their maximal numbers of oocyst per gram of feces were in a range of 10^6 and the patency was 3 or 4 days. Mice (SCID and ICR), rats, guinea pigs and a dog which had received $10^6 - 10^7$ oocysts did not shed any oocyst, though the animals except SCID mice were treated with dexamethasone phosphate before oocyst inoculation.

Key words: chicken Cryptosporidium; infectivity; bobwhite quail; turkey.

Introduction

It is generally known that *Cryptosporidium* meleagridis from turkeys (Slavin, 1955) and *Cryptosporidium baileyi* isolated from chickens (Current *et al.*, 1986) are infectious to chickens. Although *C. baileyi* could not infect mammals (Current *et al.*, 1986; Lindsay *et al.*, 1986; O'Donoghue *et al.*, 1987), Ditrich *et al.* (1991) reported that it was detected in an immunodeficient patient. Thus, *C. baileyi* has been thought to be one of the parasites which cause zoonosis.

In Japan, Itakura *et al.* (1984) isolated *Cryptosporidium* sp. from chickens in poultry farms and the life cycles and infectivity to chickens has been reported (Itakura *et al.*, 1985; Ogimoto *et al.*, 1987; Takano *et al.*, 1992). The parasite has not yet been classified because the oocyst was found to be medium in size between *C. meleagridis* and *C. baileyi* (Matsui *et al.*, 1992, 1996). Previously we reported that this parasite was infectious to Japanese

quails (Fujino *et al.*, 1996), however, the infectivity to the other animals has not yet been investigated. In the present study, I examined the infectivity of *Cryptosporidium* sp. to turkeys, bobwhite quails and several kinds of experimental animals.

Materials and Methods

Animals

Four 2- and 4-week-old turkeys, seven 5- and 10week-old bobwhite quails, five 4-week-old SCID mice, five 4-week-old ICR mice, five 3-week-old SPF Wistar rats, three 3-week-old guinea pigs and one stray dog (1780 g) were used as experimental animals. Six 4- and 8-week-old male White Leghorn chickens were used as infected controls. These birds and mammals except a dog were purchased from a commercial source (Japan SLC, Inc., Hamamatsu, Japan). Feces of all the animals were examined for several times and the oocyst-free animals were raised in separate cages under a coccidia-free condition.

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Oocysts

Oocysts of *Cryptosporidium* sp. were originally supplied by Dr. Itakura, Faculty of Veterinary Medicine, Hokkaido University, and were isolated from the bursa of Fabricius of experimentally infected chickens. The oocysts were subjected to passage in coccidia-free chickens to be multiplied, stored in 2% potassium dichromate solution at 4°C and used within 4 weeks after shedding.

Detection of oocysts

Feces were examined by the sugar flotation method (specific gravity of 1.266) to detect oocysts. When the oocysts were detected, the numbers of oocysts per gram of feces (OPG values) were counted daily by the method described by Matsui *et al.* (1994).

Infectivity

Three 4-week-old turkeys and one 2-week-old turkey and three 4-week-old chickens were inoculated orally with 4.8×10^6 to 1.0×10^7 oocysts. One of three 4-week-old turkeys was given 1.0 mg of dexamethasone phosphate (Mitaka Pharmaceutical Co., Ltd., Mitaka, Japan) intraperitoneally for five days before oocyst inoculation. Chickens were used as infected controls.

Seven bobwhite quails (two of 5-week-old birds and five of 10-week-old birds) and three chickens (8 weeks old) were inoculated orally with 5.0×10^6 oocysts. Four birds (two of 5-week-old birds and two of 10-week-old birds) of them each were given 1.0 mg of dexamethasone phosphate intraperitoneally for five days before inoculation.

Five SCID mice, 5 ICR mice, 5 rats, 3 guinea pigs and a dog were inoculated orally with 3.9×10^6 or 1.0×10^7 oocysts. Each one of mammals except SCID mice was given 0.5 or 1.0 mg of dexamethasone phosphate intraperitoneally for five days before inoculation.

All animals were examined daily for oocyst discharge in their feces.

Results

The patterns of oocyst shedding in experimentally infected turkeys and infected control chickens are shown in Fig. 1. Three of four turkeys including dexamethasone phosphate-treated one shed oocysts on days 4, 6 or 8 PI, respectively. One turkey (2 weeks old) showed 10^3 OPG values but in two turkeys (treated and nontreated) the oocysts were detected only by sugar flotation method. The chickens shed oocysts from day 5 PI and showed 10^6 OPG values from day 10 PI.

Some of the treated bobwhite quails with dexamethasone phosphate (one of 5-week-old birds and one of 10-week-old birds) shed oocysts from 4 days PI (Fig. 2). Their maximal OPG values were in a range of 10⁶, and the patency was 3 or 4 days. One treated bird and 3 non-treated bobwhite quails did not shed any oocyst. The control chickens shed oocysts from day 5 PI and showed 10⁶ OPG values from day 8 PI.

SCID mice, ICR mice, rats, guinea pigs and a dog did not shed any oocyst.



Fig. 1 Mean OPG values in turkeys inoculated with *Cryptosporidium* sp. oocysts from chickens. Turkey C (4 weeks old) was treated with dexamethasone phosphate (1.0 mg/day) for five days before oocyst inoculation. Turkeys A (2 weeks old) and B (4 weeks old) were not treated.

igodot: Oocysts were detected only by sugar flotation method.



Fig. 2 Mean OPG values in bobwhite quails inoculated with *Cryptosporidium* sp. oocysts from chickens. Bobwhite quails A (5 weeks old) and B (10 weeks old) were treated with dexamethasone phosphate (1.0 mg/ day) for five days before oocyst inoculation.

Discussion

On the infectivity of chicken cryptosporidia, C. meleagridis was infectious to turkeys (Slavin, 1955; Woodmansee et al., 1988), chickens (Woodmansee et al., 1988; Lindsay et al., 1989a) and domestic ducks (Lindsay and Blagburn, 1990). The other animals have not yet been examined. C. haileyi was infectious to chickens (Current et al., 1986; Lindsay et al., 1986; Blagburn et al., 1987), turkeys (Current et al., 1986; Lindsay et al., 1986; Lindsay et al., 1987), domestic ducks (Current et al., 1986; Lindsay et al., 1989b), geeses (Current et al., 1986), muscovy ducks (Lindsay et al., 1986), pheasants, guinea fowls and chuckar partridges (Lindsay and Blagburn, 1990), but could not infect bobwhite quails (Current et al., 1986; Lindsay et al., 1986) and mammals (Current et al., 1986; Lindsay et al., 1986; Palkovic and Marousek, 1989; Lindsay and Blagburn, 1990).

In the present experiments, *Cryptosporidium* sp. isolated in Japan was found to infect turkeys and

bobwhite quails but not to infect several kinds of rodents and a dog. Therefore, it is supposed that this parasite might be infectious to several birds of family Phasianidae but not to mammals including humans. Current et al. (1986) reported that the turkeys infected with C. baileyi shed a few oocysts for 6 and 7 days after oocyst inoculation. The number of shedding oocysts of Cryptosporidium sp. was also small and the patency was short in the turkeys. In the bobwhite quails inoculated with Cryptosporidium sp., birds treated with dexamethasone phosphate shed oocysts. However, nontreated bobwhite quails did not shed any oocyst after oocyst inoculation as C. baileyi reported by Current et al. (1986). In addition, dexamethasone phosphate had an effect on the oocyst production in the bobwhite quails but not in the infected turkeys.

The difference in the classification of coccidia was not found among the present species and those two chicken *Cryptosporidia* from the results obtained in the present experiments. Therefore, it is necessary to accumulate more detailed observation in order to identify the present species.

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References

- 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.
- Blagburn, B. L., Lindsay, D. S., Giambrone, J. J., Sundermann, C. A. and Hoerr, F. J. (1987): Experimental cryptosporidiosis in broiler chickens. Poult. Sci., 66, 442–449.
- Ditrich, O., Palkovic, L., Sterba, J., Prokopic, J., Loudova, J. and Giboda, M. (1991): The first finding of *Cryptosporidium baileyi* in man. Parasitol. Res., 77, 44– 47.
- Fujino, T., Matsui, T., Tsutsumi, Y., Saito, Y., Kobayashi, F. and Tsuji, M. (1996): Infectivity and immunogenicity to Japanese quails (*Coturnix coturnix japonica*) of *Cryptosporidium* sp. isolated from chickens in Japan.

Jpn. J. Parasitol., 45, 139-143.

- Itakura, C., Goryo, M. and Umemura, T. (1984): Cryptosporidial infection in chickens. Avian Pathol., 13, 487–499.
- Itakura, C., Nakamura, H., Umemura, T. and Goryo, M. (1985): Ultrastructure of cryptosporidial life cycle in chicken host cells. Avian Pathol., 14, 237–249.
- Lindsay, D. S. and Blagburn, B. L. (1990): Cryptosporidiosis in birds. In Cryptosporidiosis of Man and Animals, Dubey, J. P., Speer, C. A. and Fayer, R. (eds.), CRC Press, Boca Raton, Florida, 133–148.
- Lindsay, D. S., Blagburn, B. L. and Hoerr, F. J. (1987): Experimentally induced infections in turkeys with *Cryptosporidium baileyi* isolated from chickens. Am. J. Vet. Res., 48, 104–108.
- Lindsay, D. S., Blagburn, B. L. and Sundermann, C. A. (1986): Host specificity of *Cryptosporidium* sp. isolated from chickens. J. Parasitol., 72, 565–568.
- Lindsay, D. S., Blagburn, B. L. and Sundermann, C. A. (1989a): Morphometric comparison of the oocysts of *Cryptosporidium meleagridis* and *Cryptosporidium haileyi* from birds. Proc. Helminthol. Soc. Wash., 56, 91–92.
- Lindsay, D. S., Blagburn, B. L., Sundermann, C. A. and Hoerr, F. J. (1989b): Experimental infections in domestic ducks with *Cryptosporidium baileyi* isolated from chickens. Avian. Diseases., 33, 69–73.
- 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.

- Matsui, T., Fujino, T., Kobayashi, F., Morii, T. and Tsuji, M. (1994): Oocyst production and immunogenicity of *Cryptosporidium muris* in the experimental mice. Jpn. J. Parasitol., 43, 199–204.
- 14) Matsui, T., Fujino, T., Kobayashi, F., Tsutsumi, Y. and Tsuji, M. (1996): Increase in size of oocysts of *Cryptosporidium* sp. from chicken and *C. muris* during patency and during preservation. Jpn. J. Parasitol., 45, 134–138.
- Ogimoto, K., Inamoto, T., Soga, T. and Itakura, C. (1987): Experimental infection of chickens with *Cryptosporidium*. Zbl. Bakt. Hyg. A., 264, 343–347.
- 16) O'Donoghue, P. J., Tham, V. L., de Saram, W. G., Paull, K. L. and McDermott, S. (1987): *Cryptosporidium* infections in birds and mammals and attempted crosstransmission studies. Vet. Parasitol., 26, 1–11.
- 17) Palkovic, L. and Marousek, V. (1989): The pathogenicity of *Cryptosporidium parvum* Tyzzer, 1912 and *C. baileyi* Current, Upton et Haynes, 1986 for chickens. Folia. Parasitologica., 36, 209–217.
- Slavin, D. (1955): Cryptosporidium meleagridis (sp. nov.). J. Comp. Pathol., 65, 262–266.
- 19) Takano, H., Inamoto, T., Ogimoto, K. and Nakai, Y. (1992): Developmental process of *Cryptosporidium* in the intestine and bursa of fabricius of chickens. J. Vet. Med. Sci., 54, 289–292.
- 20) Woodmansee, D. B., Pavlasek, I., Pohlenz, J. F. L. and Moon, H. W. (1988): Subclinical cryptosporidiosis of turkeys in Iowa. J. Parasitol., 74, 898–900.

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