[Jpn. J. Parasitol., Vol. 45, No. 2, 139–143, April, 1996]

Infectivity and Immunogenicity to Japanese Quails (*Coturnix coturnix japonica*) of *Cryptosporidium* sp. Isolated from Chickens in Japan

TAKASHI FUJINO[1)], TOSHIHIRO MATSUI[1)], YOSHIATSU TSUTSUMI[1)], YASUHIDE SAITO[2)], FUMIE KOBAYASHI[1)] AND MORIYASU TSUJI[1)]

[1)]*Department of Tropical Diseases and Parasitology, Kyorin University School of Medicine, Shinkawa 6-20-2, Mitaka City, Tokyo 181, Japan.*
[2)]*Department of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara 229, Japan.*
(Accepted April 5, 1996)

Abstract

Two groups of three quails were inoculated orally with 1.0×10^7 oocysts of *Cryptosporidium* sp. isolated from chickens in Japan. One group of them was treated with dexamethasone phosphate before inoculation. Both groups shed oocysts from day 4 postinoculation (PI). Their maximum OPG values (numbers of oocyst per gram of feces) were in the range of 10^5 and the patency was 7 to 9 days. In a separate experiment, young (one week old) and adult (7 weeks old) Japanese quails were inoculated orally with 3.0×10^6 oocysts. The OPG values of young quails were higher than that of adult ones, although the prepatent period and the patency were the same, suggesting that young quails are more susceptible to this parasite than older ones. When the younger quails were necropsied on days 6 and 7 PI, several asexual and sexual stages were found in the bursa of Fabricius and cecum. Japanese quails of both age groups were challenged with 3.0×10^6 oocysts on day 35 after primary inoculation. Neither young nor adult quails shed any oocysts after the challenge inoculation. These results demonstrate that this parasite has infectivity and immunogenicity to Japanese quails.

Key words: chicken *Cryptosporidium*; infectivity; Japanese quail; immunogenicity; oocyst production.

Introduction

Cryptosporidium meleagridis isolated from turkeys (Slavin, 1955) and *C. baileyi* from chickens (Current *et al.*, 1986) have been known as the chicken *Cryptosporidium*. Current *et al.* (1986) have reported that the oocyst size of *C. baileyi* is larger than that of *C. meleagridis* and that *C. baileyi* can not infect bobwhite quails.

In Japan, *Cryptosporidium* sp. from chickens was first isolated by Itakura *et al.* (1984), who studied the life cycles in the chickens (Itakura *et al.*, 1985). The oocyst size of *Cryptosporidium* sp. was between those of *C. meleagridis* and *C. baileyi* oocysts (Matsui *et al.*, 1992). The infectivity of this parasite to birds except chickens has not yet been clarified. The present study was performed to examine the infectivity and immunogenicity of *Cryptosporidium* sp. to Japanese quails.

Correspondence: Takashi Fujino
藤野隆志[1]，松井利博[1]，堤　可厚[1]，斉藤康秀[2]，小林富美恵[1]，辻　守康[1]（[1]杏林大学医学部熱帯病・寄生虫学教室，[2]麻布大学獣医学部寄生虫学教室）

Materials and Methods

Animals

Japanese quails (*Coturnix coturnix japonica*) were bred in the animal facility in School of Veterinary Medicine, Azabu University, and used at one to 7 weeks of age. Male White Leghorn chickens, 3 weeks old, were purchased from a commercial source (Japan SLC, Inc., Hamamatsu, Japan) and used as infected controls. All the animals were subjected to the fecal examination several times before the experiments in order to confirm the oocyst-free. The oocyst-free birds were raised separately as groups under a coccidia-free condition.

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 multiply oocysts. The oocysts were stored in 2% potassium dichromate solution at 4°C and were used within 4 weeks after isolation.

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 monitored daily by the method described by Matsui *et al.* (1992).

Infectivity

Two groups of three quails (3 weeks old) and three chickens (3 weeks old) used as infected controls were inoculated orally with 1.0×10^7 oocysts. A quail was given 0.3 mg of dexamethasone phosphate (Mitaka Pharmaceutical Co., Ltd., Mitaka, Japan) intraperitoneally for three days before inoculation. Their feces were examined daily for oocyst discharge.

To examine the susceptibility of young and adult Japanese quails to this parasite, five quails (one week old) and three quails (7 weeks old) were inoculated orally with 3.0×10^6 oocysts. For oocyst detection, pooled group feces were examined daily postinoculation (PI). Two of one-week-old Japanese quails were necropsied on days 6 and 7 PI to detect the parasite. A piece of the bursa of Fabricius or cecum from the necropsied quails were stamped or smeared on a slide glass. Then, they were stained with Giemsa solution and examined under a microscope.

Immunogenicity

The infected young and adult quails mentioned above were challenged with 3.0×10^6 oocysts on day 35 after primary inoculation. Their feces were examined daily for oocyst discharge.

Results

Infectivity

The patterns of oocyst shedding in experimentally infected Japanese quails and chickens served as infected controls are shown in Fig. 1. Oocyst excretion was observed in two groups of quails from day 4 after oocyst inoculation. Their maximum OPG values were in a range of 10^5, and the patency was 7 to 9 days. The group treated with dexamethasone phosphate shed oocysts in a pattern similar to that of the nontreated group. The chickens shed oocysts from day 5 PI and showed 10^6 OPG values from day 10.

Both the young (one week old) and adult (7 weeks old) quails shed oocysts from day 4 PI (Fig. 2). The OPG values of young age group were 10^5 to 10^6 and those of adult group were 10^4 to 10^5. The control chickens shed oocysts from day 5 PI and showed 10^5 to 10^6 OPG values from day 7.

Merozoites, schizonts, gametocytes and zygotes were found in the bursa of Fabricius and cecum of infected Japanese quails necropsied on days 6 and 7 PI (Fig. 3).

Immunogenicity

Neither young nor adult Japanese quails shed any oocysts after the challenge inoculation, but the control quails and chickens infected primarily at the same time shed oocysts from day 4 and 5 PI and OPG values of them were 10^4 and 10^6, respectively (Fig. 2).

Discussion

The morphological structure of sporulated oocysts and infectivity to hosts are important in the classification of coccidian parasites including *Cryptosporidium*. Lindsay *et al.* (1989) reported that the mean lengths and widths of *C. meleagridis* oocysts from turkeys were significantly smaller than those of *C. baileyi* oocysts from chickens. Lindsay and Blagburn (1990) reported that *C. meleagridis* was infectious to turkeys, chickens and domestic ducks, and that *C. baileyi* had infectivity to turkeys, geese, domestic ducks, muscovy ducks, pheasants, guinea fowls and chuckar partridges. They also reported that *C. baileyi* could not infect bobwhite quails.

In the present study, this parasite has infectivity to Japanese quails. However, the OPG values of the

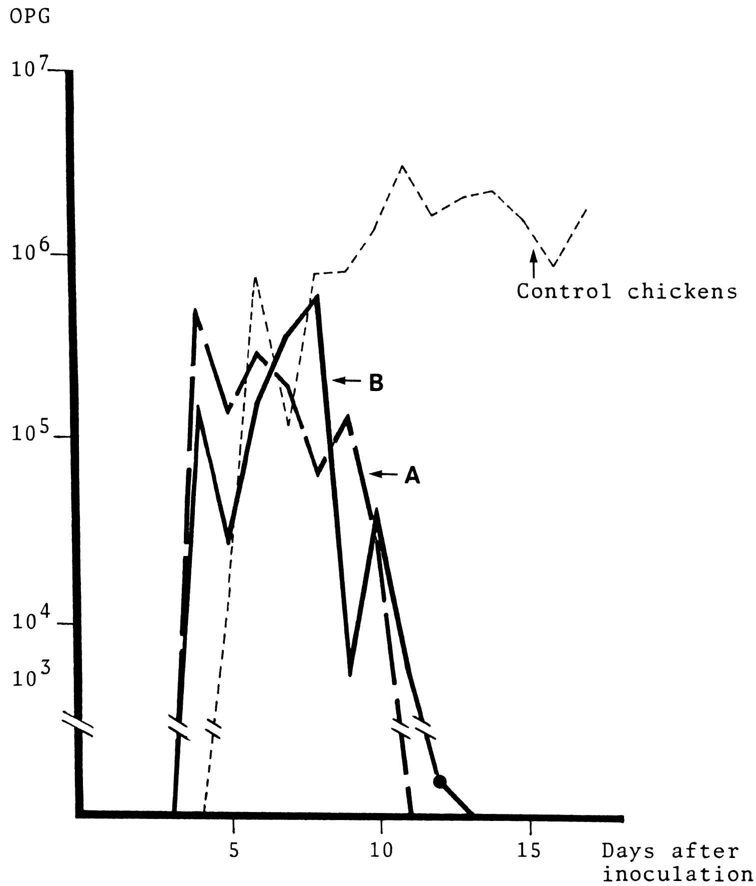

Fig. 1 Mean OPG values in Japanese quails inoculated with 1.0×10^7 oocysts of *Cryptosporidium* sp. (3 birds/group).
A: Japanese quails treated with dexamethasone phosphate (0.3 mg/day) for three days before oocyst inoculation.
B: Japanese quails not treated with dexamethasone phosphate.
●: Oocysts detected by sugar flotation method.

quails were one to 2 orders of magnitude lower and the patency was shorter than those of control chickens. These results suggest that Japanese quails may not be a suitable host for this parasite. In addition, dexamethasone phosphate did not show any effect on the oocyst production in Japanese quail.

One-week-old quails showed one order of magnitude higher OPG values than 7-week-old quails. These results support the contention of others that young animals show higher susceptibility to *Cryptosporidium* than adult animals (Sherwood *et al.*, 1982; Tzipori *et al.*, 1983; Lindsay *et al.*, 1988).

Matsui *et al.* (1992) reported that chickens, which had shed 10^5 oocysts for 10 days in primary inoculation, did not shed any oocyst after the challenge inoculation. In the present study, Japanese quails also did not shed any oocysts after the challenge inoculation. The result suggest that Japanese quails acquired the strong protective immunity as well as chickens.

Japanese quails belong to the same family (Phasianidae) as bobwhite quails. Thus, the possibility of infection of this parasite to bobwhite quails was obtained from the present experiments. To

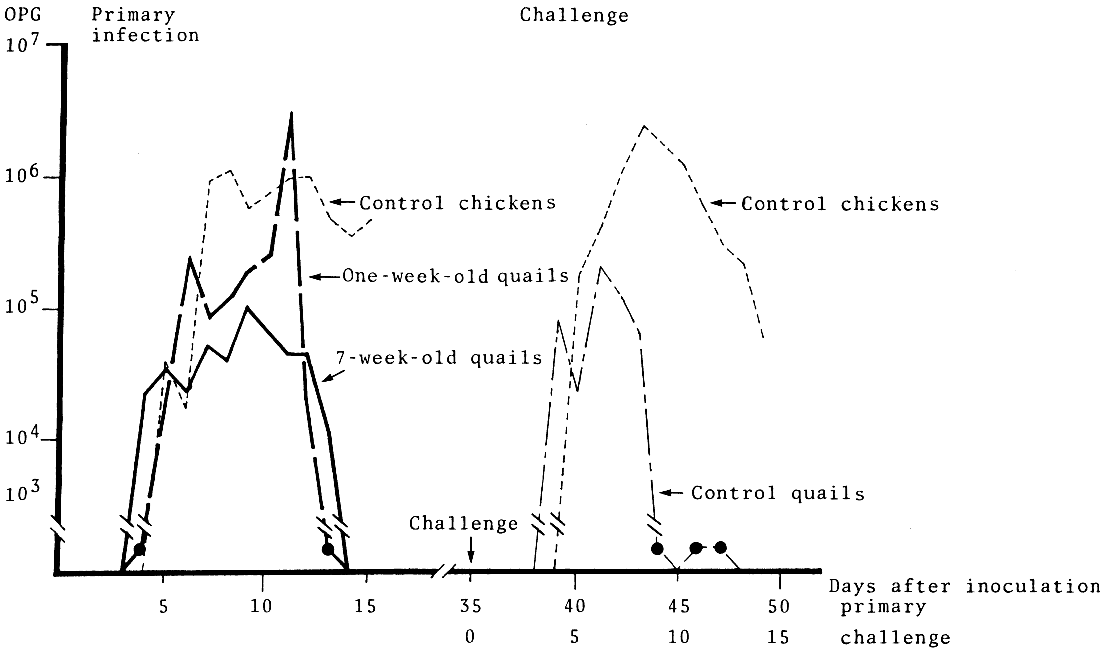

Fig. 2 Mean daily oocyst output in Japanese quails (one and 7 weeks old) inoculated with 3.0×10^6 oocysts of *Cryptosporidium* sp. after primary and challenge inoculations. (3 birds/group).
Challenge: On day 35 after primary inoculation.
●: Oocysts detected by sugar flotation method.

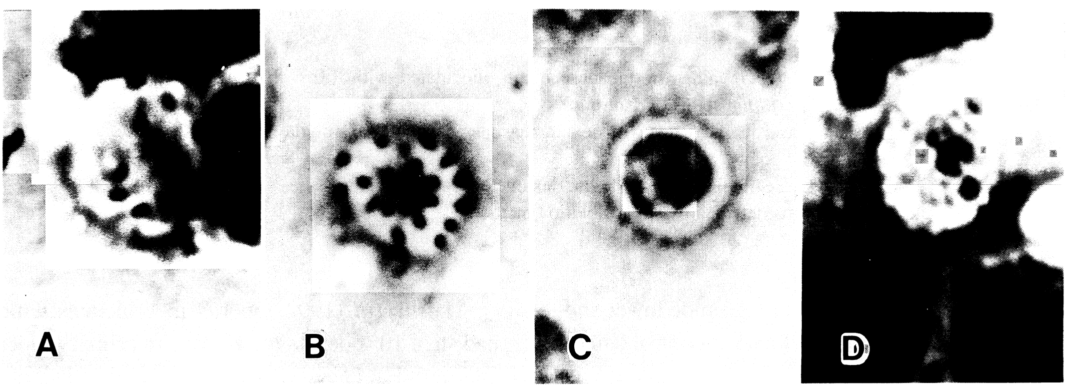

Fig. 3 Endogenous stages of *Cryptosporidium* sp. in Giemsa-stained smears of the bursa of Fabricius of Japanese quail on day 7 after inoculation.
A: A mature schizont.
B: A young microgametocyte.
C: A macrogamete.
D: A zygote.

clarify the species of this *Cryptosporidium*, further studies on the infectivity of this parasite to different avian hosts including bobwhite quails should be required.

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