The Korean Journal of Parasitology
Vol. 33, No. 1, 45-54, March 1995

Oocyst production and immunogenicity of *Cryptosporidium baileyi* in chickens and mallards

Jae Ku RHEE*, Beung Gui JANG and Bae Keun PARK

Department of Parasitology, School of Veterinary Medicine, Chonbuk National University Chonju 560-756, Korea

Abstract: Two-day-old chickens and mallards were orally inoculated with one of 5 doses varying from 2×10^2 to 2×10^6 of *C. baileyi* oocysts per individual. Generally, the more oocysts inoculated were, the longer the patent periods were, and the more oocysts shedding were. Meanwhile increasing the inoculative dose, the prepatent periods were shortened except that mallards inoculated with 2×10^2 and 2×10^3 oocysts failed to produce the oocysts. The more parasites involving oocysts appeared from the chicken in comparison to the mallard. In the chickens challenged with a single dose of 2×10^6 oocysts, a small number of oocysts were detected from feces on days 4-14 after challenge infection (ACI) in all of carrageenan administered groups and in the control groups inoculated with 2×10^2 and 2×10^3 oocysts. In the mallards, a few oocysts were also recognized on days 5-15 ACI in all of carrageenan treated groups and in the control groups inoculated with 2×10^2, 2×10^3 and 2×10^4 oocysts. Just prior to challenge infection, phagocytic activity of peritoneal macrophages (Mø) and the number of peripheral Mø in both birds were significantly decreased in the carrageenan treated groups as compared to the control groups. Mild challenge infection in both birds denoted that the immunogenicity of *C. baileyi* to the birds was very strong, despite Mø blocker carrageenan administration.

Key words: *Cryptosporidium baileyi*, immunogenicity, macrophage blocker carrageenan, chicken, mallard, oocyst production, susceptibility

INTRODUCTION

Nowadays, two species among *Cryptosporidium* spp. are considered as distinct species in avian hosts. *Cryptosporidium baileyi* Current, Upton and Haynes, 1986, with oval oocysts measuring 6.2×4.6 μm, only produced mild infections confined to the bursa of Fabricius (BF) of less than half of young turkeys inoculated orally with oocysts and did not infect quail, meanwhile most developmental stages of the protozoa occurred mainly in enterocytes of the cloaca and BF of chickens (Current *et al.*, 1986), and before the protozoa was proposed as a valid species by them, *Cryptosporidium* sp. was already observed in the cecum, BF, cloaca, trachea, nasal cavities, infraorbital sinues, larynx and bronchi of chickens (Dhillon *et al.*, 1981; Itakura *et al.*, 1985; Lindsay *et al.*, 1986), although oocysts whose morphology and measurement corresponded with those of the protozoa were found in the stool of an immunodeficient patient and the coccidia were also detected in various organs of autopsy material (Ditrich *et al.*, 1991). *Cryptosporidium meleagridis* Slavin, 1955, with nearly round

• Received Dec. 6 1994, accepted after revision Jan. 19 1995.

• This study was financially supported by a Grant-in-Aid for Korea Science and Engineering Foundation in 1994 (NO. 941-0600-019-2).

* Corresponding author

oocysts measuring 4.5 × 4.0 μm, produced very heavy infections in the lower third of the small intestine of turkeys inoculated with the oocysts (Slavin, 1955).

In coccidia, the number of oocysts shed is affected by dose of oocyst inoculated (Long, 1973), and the peak level of oocysts per gram of feces (OPG) exhibits a definite pattern (e.g., one peak or two peaks) according to species (Mesfin and Bellamy, 1978; Ito *et al.*, 1978). Owing to such characteristics, it is important that oocyst discharge, with susceptibility and immunogenicity, is monitored for investigating the kinetics of infection in the hosts. In addition, it is useful to decide the efficacy potential of a therapeutic drug and to identify species. In the different avian hosts, however, the susceptibility of the hosts, oocyst output and immunogenicity of *C. baileyi* have not been currently defined in detail, and the studies in those respects seem to be fragmentary.

Therefore, the present studies aimed to clarify systematically the susceptibility of the hosts and oocyst production of *C. baileyi* in chickens and mallards for monitoring the kinetics of infection; to confirm if birds become resistant to subsequent oral challenge with oocysts of the same species for investigating the intensity of development of immunity utilizing Mø blocker carrageenan.

MATERIALS AND METHODS

Cryptosporidium isolate and preparation of inoculum

Cryptosporidium baileyi used in the present study was the medium type previously isolated from the domestic chicken, *Gallus gallus*, and passaged in 2-day-old specific pathogen free (SPF) chickens (Rhee *et al.*, 1991a & b). Oocysts were concentrated from the feces of infected SPF chickens by the Sheather's sugar flotation method, washed with distilled water, resuspended in 2.5% aqueous solution of potassium dichromate, and stored at 4°C for less than 2 months. Prior to oral inoculation to the experimental animals, oocysts were washed by centrifugation in distilled water, and counted with the aid of a Fuchus-Rosenthal hemocytometer.

Patterns of oocyst discharge

The 2 × 10^6 of oocysts were diluted with distilled water in a ten-fold titration system. Two-day-old SPF chickens and mallards (*Anas platyrhynchos*) were inoculated orally with a single dose of 2 × 10^2, 2 × 10^3, 2 × 10^4, 2 × 10^5 or 2 × 10^6 of each respective *C. baileyi* oocysts per individual (20 birds for each dosage level per group; 200 birds total). Each of another ten age-matched SPF chickens and mallards served as uninoculated controls. Each group of birds was separately housed in a wire-floored cage, placed on a tray containing a 5-mm depth of water to keep the feces wet.

Daily fecal examinations for oocysts were carried out by the Sheather's sugar flotation method. When oocyst numbers were too low to count, 2 ml of the supernatant was recentrifuged with distilled water 2 times, the residuum was smeared on a glass slide, and was stained with Kinyoun's modified acid-fast staining method, in order to detect accurately even a small number of oocysts, as described previously (Rhee *et al.*, 1991a). The number of oocysts discharged per day (OPD) for each bird was calculated.

Exploring endogenous stages in various organs

Each of 5 birds inoculated with 2 × 10^6 dosage group in both species was sacrificed on day 12 postinoculation (PI), when they discharged the peak number of oocysts in their feces. The lungs, trachea, duodenum, jejunum, ileum, cecum, cloaca and BF of the birds were removed, fixed with 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with PAS by the routine procedures for monitoring the presence of endogenous stages of *C. baileyi*.

Challenge infection

A primary infection in chickens terminates 32 day (21 day in mallards) after inoculation with the disappearance of oocysts in their feces, and then each dosage group was divided into 2 groups to investigate the strength of immunogenicity of the protozoa. One group was intraperitoneally injected with carrageenan and the other group was injected with saline as

a control. While, each of ten additional age-matched SPF birds also served as intrinsic controls. Carrageenan type II (dissolved 20 mg/ml saline; Sigma Chemical Co., St Louis, MO, USA) was administered to the chickens intraperitoneally at 40 mg and 10 mg/30 g body weight on days 35 and 36 after a primary infection with a dose of each respective oocysts on day 0. While carrageenan was administered into the mallards also on days 24 and 25 after a primary infection. All birds were challenged with a single dose of 2×10^6 oocysts at 6 hr after the second carrageenan injection. Daily fecal examinations for oocysts were also carried out as described previously.

The numbers of T-cells from splenocytes and peripheral Mø, and phagocytic activity of peritoneal Mø were checked in 5 birds from each group at 6 hr after the second carrageenan injection.

Counting of peripheral Mø

Blood was collected via the brachial vein. The total number of leucocytes/mm^3 was counted after staining with 0.1% toluidine blue solution. A differential cell count was performed on > 1,000 cells in smears stained with Giemsa. The absolute number of peripheral Mø/mm^3 was calculated by the following formula:

$$\text{Number of Mø} = \frac{\text{Total number of leucocytes} \times \text{Number of Mø}}{> 1{,}000}$$

Phagocytic activity of peritoneal Mø

It was carried out as suggested by Metcalf *et al.* (1986). Obtaining peritoneal Mø, 10 ml RPMI 1640 was injected intraperitoneally and massaged for 2-3 min. Peritoneal suspensions in RPMI 1640 were placed in 100-mm glass Petri dishes and incubated for 1 hr at 41°C in a 5% CO_2 incubator. Nonadherent cells were decanted and adherent cells were obtained by extensive washing with cold RPMI 1640. The 1 ml of Mø suspension (1×10^7/ml) with 0.1 ml of inactivated *Candida albicans* (1×10^9/ml) was carried in 35-mm plastic Petri dishes placed a cover glass and was incubated for 3 hr at 37°C in a 5% CO_2 incubator. To remove nonadherent cells the suspension was washed with warm RPMI 1640, and adherent cells were rapidly dried, fixed with methanol and stained with Giemsa for 15 min. The number of phagocytic Mø was examined under microscopy and 200 cells were counted. Finally phagocytic Mø were calculated as follows:

$$\text{Per cent of phagocytic Mø} = \frac{\text{Phagocytic Mø}}{\text{Cell counted}} \times 100$$

Number of T-cells from splenocytes

The spleens were minced in phosphate buffered saline (PBS) and passed through wire-mesh (#100) to obtain free cell suspensions. These suspensions overlaid on histopaque were centrifuged at 400 g for 20 min to remove erythrocytes (RBC). An additional centrifugation for 10 min with Gey's solution was manipulated to remove RBC, and the residuum was washed by centrifugation with PBS at 400 g for 10 min to prepare the splenocytes.

The rabbit anti-chicken thymocyte serum (RACTS) and the rabbit anti-mallard thymocyte serum (RAMTS), primary antibodies, were prepared in accordance with the method used by Chandra *et al.* (1980). The chicken or mallard thymus was minced in PBS (pH 7.2) and passed through wire-mesh (#60) to obtain free cell suspensions. A 5 ml aliquot of cell suspension (1×10^9/ml thymocytes) was homogenized with Freund's incomplete adjuvant (1/1, v/v) and was injected 2 times into 2 rabbits intramuscularly at 10-days interval. A booster injection was performed on day 15 after the second injection. Blood was obtained from the rabbits on 10 day after the last injection. Heat-inactivated serum was adsorbed 3 times with chicken or mallard RBC. The adsorbed serum was negative for RBC, but positive for thymocytes (at 1:64), and the titer was determined by agglutination test.

T-lymphocytes from splenocytes were identified by modified avidin-biotin-peroxidase complex method (ABC technic) as suggested by Hsu *et al.* (1981). Briefly, an 1 ml aliquot of cell suspension (1×10^7/ml) of splenocytes was treated with 0.3% H_2O_2 in methanol for 30 min, washed twice with tris-buffered saline (TBS, 0.05 M, pH 7.6), followed by 3% normal goat serum for 20 min to block the endogenous

peroxidase activity and to reduce the nonspecific background staining, and washed once with TBS. After that, the cells were kept overnight at 4°C with primary antibody diluted appropriately, washed 3 times with TBS, treated with biotin-labeled goat anti-rabbit IgG antibody (Sigma Chemical Co.) diluted 1:200 for 1 hr, washed three times with TBS, followed by avidin-biotin-peroxidase complex (Vector Laboratories Inc.) for 1 hr, and washed three times with TBS.

Fresh avidin-biotin-peroxidase complex was made by incubating 10 μg/ml avidin and 2.5 μg/ml biotin-peroxidase in TBS for 30 min at room temperature before use. The final reaction was achieved by incubating the cell suspensions with substrate mixture (3-3'-diaminobenzidine tetrahydrochloride, DAB; Sigma Chemical Co.) for 5 min and these reactants were washed once with TBS.

Cell suspensions were examined by microscopy and 200 cells were counted. Cells completely encircled by a red brown-ring of staining were counted as positive. Finally, immunoperoxidase positive cells were calculated as follows:

$$\text{Per cent of ABC positive cells} = \frac{\text{Positive number of ABC}}{\text{Cells counted}} \times 100$$

All experiments in the present study carried out of three repetition.

RESULTS

Oocyst discharge in chickens

In general, a small number of oocysts were first recognized in the feces obtained from all chickens on days 3-4 PI. The OPD for the chickens increased with the lapse of days, reached a peak (6×10^5 to 4×10^6 oocysts) on days 9-11 PI, decreased rapidly thereafter, and became negative on days 31-33 PI. The coccidium had a prepatent period (time from inoculation until the appearance of endogenously produced oocysts in the feces) of 2-3 days PI and a patent period (time of oocysts occurrence in feces) of 3-32 days PI. Generally, these effects were inoculative dose-dependent fashion (Table 1).

Oocyst discharge in mallards

Although oocysts were not detected in the feces obtained from the two lower dose groups inoculated with 2×10^2 and 2×10^3 oocysts, the other higher dose groups inoculated with 2×10^4, 2×10^5 and 2×10^6 oocysts appeared to shed a small to moderate number of oocysts in the feces. The prepatent period was 3-4 days PI, the patent period being 4-21 days PI. The peak level of oocyst output was 2×10^5 to 4×10^5 oocysts on days 9-10 PI in the two higher dose groups inoculated with 2×10^5 and 2×10^6 oocysts. Thereafter, oocyst counts declined rapidly until 14-21 days PI, and then oocysts were undetectable in all infected groups. The relationship between the number of oocyst inoculated and pattern of oocyst shedding was similar to that observed in chickens (Table 2).

While, none of uninoculated controls discharged any oocyst in the feces. And, each of six 2-day-old SPF chickens was orally inoculated with 5×10^5 oocysts originating from the mallards. A large number of *C. baileyi* oocysts were observed in fecal flotations of chickens examined on days 9 and 11 PI.

Meanwhile, chickens and mallards orally inoculated with the protozoa exhibited no clinical signs or gross lesions.

Oocyst discharge in challenge infection

In the control chickens, the two lower dose groups inoculated primarily with 2×10^2 and 2×10^3 oocysts excreted an extremely small number of oocysts in the feces on days 4-13 ACI. However, oocysts were not recovered from the feces of the other higher dose groups inoculated primarily with 2×10^4, 2×10^5 and 2×10^6 oocysts at any time. On the other hand, in all chickens injected with carrageenan we detected a small number of oocysts on days 4-14 ACI depending on dose of oocyst inoculated primarily (Table 5). While in the mallards, a few oocysts were observed from the three lower dose control groups inoculated primarily with 2×10^2, 2×10^3 and 2×10^4 oocysts and all carrageenan treated groups (Table 6). At that time, intrinsic controls inoculated with 2×10^6 oocysts discharged an enormous number of oocysts.

Table 1. Mean daily oocyst production[a] in chickens inoculated with *Cryptosporidium baileyi*

Days after inoculation	Number of oocysts by inoculative dose				
	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6
2	–	–	–	–	–
3	–	–	–	+	+
4	+	++	++	5	5
5	+	++	15	20	30
6	++	7	20	25	35
7	17	28	32	40	158
8	19	31	38	45	165
9	61	58	158	257	354
10	61	59	122	299	80
11	96	60	52	175	98
12	50	25	25	178	68
13	33	25	14	35	20
14	23	13	10	22	12
15	19	9	9	9	5
16	8	5	++	6	++
17	5	++	++	++	++
18	++	++	++	+	+
19	++	++	++	+	+
20	++	++	++	+	+
21	++	+	++	+	+
22	++	+	++	+	+
23	+	+	+	+	+
30	+	+	+	+	+
31	+	+	–	+	+
32	–	–	–	+	+
33	–	–	–	–	–

[a]The number ($\times$ 10^4) of oocysts detected by Sheather's sugar floatation method from per head. –: Negative, +: $< 2 \times 10^4$ oocysts, ++: $5 < 10^4$ oocysts.

Endogenous development

Endogenous stages of *C. baileyi* were present in the cloaca and BF gathered from chickens on day 12 PI. Infections were very heavy, in fact there was a virtual monolayer of parasites in the microvillus region of these organs in some cases. Developmental stages did not appear in lungs, trachea, small intestine and large intestine. Similarly, a small number of intracellular stages were also confined to the cloaca and BF of mallards.

Number and activity of Mø and number of T-cells

As shown in Tables 3 and 4, the number of Mø in peripheral blood and the phagocytic activity of peritoneal Mø were significantly decreased in the carrageenan-treated chickens and mallards as compared with the control groups ($P < 0.05$). Meanwhile, the proportion of T-lymphocytes from splenocytes was slightly also decreased in the carrageenan-treated chickens and mallards (Tables 3 and 4).

DISCUSSION

Oocyst production of *Cryptosporidium* spp. in avian hosts was reported in outline by several investigators (Blagburn *et al.*, 1987; Rhee *et al.*, 1991b; Matsui *et al.*, 1992), but a large part of phenomenon pertaining to oocyst output of the coccidia still remained unclear.

In the present study no oocysts were present in the feces obtained from the two lower dose groups of mallards because the number of

Table 2. Mean daily oocyst production in mallards inoculated with *Cryptosporidium baileyi*

Days after inoculation	Number of oocysts by inoculative dose				
	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6
3	–	–	–	–	–
4	–	–	–	+	+
5	–	–	+	+	++
6	–	–	+	++	5
7	–	–	+	12	23
8	–	–	+	13	22
9	–	–	+	17	40
10	–	–	+	20	38
11	–	–	+	11	34
12	–	–	+	8	12
13	–	–	+	5	19
14	–	–	+	+	8
15	–	–	–	+	++
16	–	–	–	+	+
17	–	–	–	+	+
18	–	–	–	+	+
19	–	–	–	–	+
20	–	–	–	–	+
21	–	–	–	–	+
22	–	–	–	–	–

Applied correspondingly the table 1 note to the table 2.

Table 3. Effect of carrageenan on the numbers of Mø in peripheral blood and T-cells in splenocytes and phagocytic activity of peritoneal Mø in chickens

Dose of oocysts	Treatment	Number of Mø ($\times 10^2$/mm^3)	Phagocytic activity of Mø (%)	T-cells in splenocytes (%)
2×10^3	Saline	6.6 ± 1.7	47.5 ± 10.2	55.9 ± 4.8
	Carrageenan	2.9 ± 2.1[a)]	29.0 ± 5.6*	49.8 ± 6.4
2×10^5	Saline	4.8 ± 2.2	44.5 ± 9.2	54.5 ± 2.8
	Carrageenan	2.0 ± 1.1[a)]	22.9 ± 6.1*	49.9 ± 6.9

[a)] $P < 0.05$ (SAS t-test) compared with the values for saline injected groups. Each value represents the mean of five determinations with the standard deviations per group.

oocysts inoculated was small, while in the other higher dose groups we found a small to moderate number of oocysts in the feces. A moderate or a large number of oocysts were observed in the feces from all chickens.

Matsui *et al.* (1992) indicated that a *Cryptosporidium* sp. isolated from Japanese chicken origin had a relationship between dose of oocysts inoculated and the OPG value and a relatively strong immunogenicity to the chickens. Blagburn *et al.* (1987) noted that oocyst output of *C. baileyi* (AU-B1) obtained from bursal scraping was similar regardless of route or level of inoculation in broiler chickens.

In chickens and mallards, generally, a pattern of oocyst shedding was inoculative dose-dependent fashion, as described previously in coccidia. It is consistent with the results of Matsui *et al.* (1992), but not with those of Blagburn *et al.* (1987). And, the rise and fall of OPD gave rise to one peak, as was found in *Eimeria* infection.

Table 4. Effect of carrageenan on the numbers of Mø in peripheral blood and T-cells in splenocytes and phagocytic activity of peritoneal Mø in mallards

Dose of oocyst	Treatment	Number of Mø ($\times 10^2$/mm^3)	Phagocytic activity of Mø (%)	T-cells in splenocytes (%)
2×10^3	Saline	5.8 ± 1.2	41.4 ± 7.8	60.1 ± 3.8
	Carrageenan	1.8 ± 1.1*	22.2 ± 8.9*	56.0 ± 1.4
2×10^5	Saline	4.6 ± 0.8	43.9 ± 7.1	59.7 ± 2.1
	Carrageenan	2.9 ± 1.8*	22.1 ± 6.1*	54.0 ± 1.4

Applied correspondingly the table 3 note to the table 4.

Table 5. Mean daily oocyst output in chickens after primary and challenge inoculations with *Cryptosporidium baileyi*

(Modified acid-fast staining method)

Days after inoculation		Dose of *Cryptosporidium baileyi* oocysts									
Primary	Challenge	2×10^2		2×10^3		2×10^4		2×10^5		2×10^6	
		I	II	I	II	I	II	I	II	I	II
39	3	–	–	–	–	–	–	–	–	–	–
40	4	+	+	+	–	–	–	–	–	–	–
41	5	+	+	+	+	+	–	–	–	–	–
42	6	+	+	+	+	+	–	+	–	–	–
43	7	+	+	+	+	+	–	+	–	+	–
44	8	+	+	+	+	+	–	+	–	+	–
45	9	+	+	+	+	+	–	+	–	+	–
46	10	+	+	+	+	+	–	+	–	+	–
47	11	+	+	+	+	+	–	+	–	+	–
48	12	+	+	+	+	+	–	+	–	+	–
49	13	+	+	+	+	–	–	–	–	–	–
50	14	+	–	–	–	–	–	–	–	–	–
51	15	–	–	–	–	–	–	–	–	–	–

The group I was administered intraperitoneally with carrageenan at 40 mg and 10 mg/30 g body weight on days 35 and 36 after a primary inoculation with *C. baileyi* oocysts on day 0 and the group II was administered with 0.9% saline as a control. +: Detectable oocysts, –: No detectable oocysts.

Previously, some investigators revealed that *C. baileyi* in 2 or 7-day-old chickens had patent periods of 4-24 days PI (Current *et al.*, 1986), 5-25 days PI (Blagburn *et al.*,1987) and 5-24 days PI (Rhee *et al.*, 1991b). In this experiment, the prepatent and patent periods between chicken (2 to 3 days and 27 to 30 days) and mallard (3 to 4 days and 10 to 18 days) were different in some degree, and the number of oocysts shed in the chicken was more abundant than that in the mallard.

Current *et al.* (1986) intimated that no oocysts were found in the feces obtained from quail and 7- and 10-day-old turkeys inoculated with oocysts of *C. baileyi*. Conversely, ducks inoculated at one day of age and geese inoculated at two days of age with oocysts of *C. baileyi* had endogenous stages in the BF only. Lindsay *et al.* (1989) indicated that ducks were more resistant to experimentally induced respiratory cryptosporidiosis caused by *C. baileyi* than were chickens and turkeys. Current *et al.* (1986) reported that although developmental stages observed 2-8 hr PI were confined to the ileum and large intestine, no parasites were observed in those organs of the

Table 6. Mean daily oocyst output in mallards after primary and challenge inoculations with *Cryptosporidium baileyi*

(Modified acid-fast staining method)

Days after inoculation		Dose of *Cryptosporidium baileyi* oocysts									
Primary	Challenge	2×10^2		2×10^3		2×10^4		2×10^5		2×10^6	
		I	II	I	II	I	II	I	II	I	II
29	4	–	–	–	–	–	–	–	–	–	–
30	5	+	+	+	–	+	+	–	–	–	–
31	6	+	+	+	+	+	+	–	–	–	–
32	7	+	+	+	+	+	+	–	–	–	–
33	8	+	+	+	+	+	+	+	–	+	–
34	9	+	+	+	+	+	+	+	–	+	–
35	10	+	+	+	+	+	–	+	–	+	–
36	11	+	+	+	+	+	–	+	–	+	–
37	12	+	+	+	+	–	–	+	–	+	–
38	13	+	+	+	+	–	–	–	–	–	–
39	14	+	+	+	+	–	–	–	–	–	–
40	15	+	–	+	–	–	–	–	–	–	–
41	16	–	–	–	–	–	–	–	–	–	–

It is applied correspondingly the table 5 note to the table 6 with the exception of that the group I was administered with carrageenan on days 24 and 25 after a primary inoculation.

chickens necropsied on days 4-18 PI of *C. baileyi* oocysts and numerous stages were, however, present in the BF and cloaca. Whereas, numerous stages were localized only in the BF of ducks. These features are identical to those of the present study in the chickens, but not in the mallards. The literature reported previously and this experimental result indicate that chickens are more susceptible to infection than are mallards.

Oral and intracloacal inoculations of *Cryptosporidium* sp. oocysts for chickens resulted in development of the protozoa in the BF and cloaca, but scarcely in the respiratory tract (Lindsay and Blagburn, 1986; Lindsay *et al.*, 1986). Whereas, in case of intratracheal inoculation for chickens and ducks, developmental stages of *Cryptosporidium* sp. were parasitized in trachea (Lindsay *et al.*, 1986 & 1989). The distribution of developmental stages of *C. baileyi* in the present study was confined to the cloaca and BF among various organs in both birds on 12 day PI. This could be due to difference of inoculation route.

In the present study, the rapid clearance on day 32 (chicken) PI and day 21 (mallard) PI of *C. baileyi* oocysts from their feces, respectively, even in the higher dose groups, suggests the development of acquired immunity. Additionally, the above mentioned results of Current *et al.* (1986), Blagburn *et al.* (1987) and Rhee *et al.* (1991b) strongly support such a suggestion.

In 2-week-old broiler chickens inoculated orally with 1×10^6 oocysts of *C. baileyi*, no developmental stages were, ten days following the challenge inoculation, observed in mucosal scrapings of the BF and cloaca of the chickens challenged with oral inoculation of 2×10^6 oocysts on five days after all birds stopped shedding oocysts (Current and Snyder, 1988). In 4-week-old chickens challenged with 3.5×10^6 oocysts of *Cryptosporidium* sp. after primary inoculation with 3.5×10^2 and 3.5×10^3 oocysts, $1 \times 10^{4-5}$ or a small number of oocysts were shed for 1-25 days ACI and a single day on 10 day ACI, respectively, and the other groups inoculated primarily with higher doses did not shed any oocysts (Matsui *et al.*, 1992). The present study showed that the challenged chickens discharged an extremely small to a small number of oocysts in the feces for 4-14 days ACI from the two lower dose

control groups and all of carrageenan administered groups. In the mallards, a few oocysts were also observed for 5-15 days ACI in all carrageenan treated groups and the three lower dose control groups. The results of the present study was similar to those observed in suffered chickens challenged with oocysts of *C. baileyi* (Current and Snyder, 1988) or *Cryptosporidium* sp. (Matsui *et al.*, 1992).

Macrophages are considered to be the final effectors of cell-mediated immunity against a variety of bacteria and fungi (Tatsukawa *et al.*, 1979). There are also several reports that show contributions of Mø to protective immunity against helminth and protozoan parasites. Infection of mice with *Nematospiroides dubius* induces resistance to *Babesia microti*. According to Mzembe *et al.* (1984), this resistance was dependent on the presence of the spleen and viable Mø, and splenectomy or the injection of carrageenan or silica abrogated the protective effect against *B. microti* induced by infection with *N. dubius* in mice. *Hymenolepis nana* is highly immunogenic, and mice receiving a single oral inoculation of eggs of the worm become completely resistance to reinfection by inhibiting larval growth in the intestinal villi. When immunized mice from primary infection were treated with carrageenan just before challenge infection with eggs of *H. nana*, they failed to exhibit sterile immunity to the egg challenge, with evidence of a decrease in the number of peripheral Mø and the rate of carbon clearance. These results strongly suggest that Mø have an important role in protective immunity to *H. nana* in mice (Asano *et al.*, 1992).

In immunized mice treated with carrageenan just before challenge infection with *H. nana* eggs, challenge infection is established (Asano *et al.*, 1992), but the present study failed to develop numerous developmental stages and to shed a great number of oocysts of *C. baileyi* in both birds, even though the number of Mø in peripheral blood and the phagocytic activity of peritoneal Mø were significantly decreased in the carrageenan-treated chickens and mallards as compared with the control groups ($P < 0.05$).

Considering the experimental results, it is probable that the mallards are more resistant to *C. baileyi* than the chickens. Mild challenge infection in both birds denotes that the strength of immunogenicity of *C. baileyi* to the birds is very strong despite Mø blocker carrageenan administration.

It is expected, in future, that species is identified by judging the strength of immunogenicity through cross-challenge transmission experiment of different strains of *Cryptosporidium* spp. isolated from various sources. In addition, potential of exploring vaccine using inactivated protozoa is anticipated.

REFERENCES

Asano K, Muramatsu K, Ito A, Okamoto K (1992) Macrophages in protective immunity to *Hymenolepis nana* in mice. *Immunol Cell Biol* **70:** 417-420.

Blagburn BL, Lindsay DS, Giambrone JJ, Sundermann CA (1987) Experimental cryptosporidiosis in broiler chickens. *Poultry Sci* **66:** 442-449.

Chandra P, Chanana AD, Joel DD (1980) Distribution of T and B lymphocytes in blood and lymphoid tissues of fetal and adult sheep. *Am J Vet Res* **41:** 2092-2094.

Current WL, Upton SJ, Haynes TB (1986) The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. *J Protozool* **33**(2): 289-296.

Current WL, Snyder DB (1988) Development of and serologic evaluation of acquired immunity to *Cryptosporidium baileyi* by broiler chickens. *Poultry Sci* **67:** 720-729.

Dhillon AS, Thacker HL, Dietzel AV, Winterfield RW (1981) Respiratory cryptosporidiosis in broiler chickens. *Avian Dis* **25:** 747-751.

Ditrich O, Palkovic L, Sterba J, Prokopic J, Loudova J, Giboda M (1991) The first finding of *Cryptosporidium baileyi* in man. *Parasitol Res* **77**(1): 44-47

Hsu SM, Raine L, Fanger H (1981) A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* **75:** 734-738.

Itakura C, Nakamura H, Umemura T, Goryo M (1985) Ultrastructure of cryptosporidial life cycle in chicken host cells. *Avian Pathol* **14:**

237-249.

Ito S, Tsunoda K, Shimura K (1978) Life cycle of the large type of *Isospora bigemina* of the cat. *Nat Inst Anim Hlth Quart* **18:** 69-82.

Lindsay DS, Blagburn BL (1986) *Cryptosporidium* sp. infections in chickens, produced by intracloacal inoculation of oocysts. *J Parasitol* **72** (4): 615-616.

Lindsay DS, Blagburn BL, Sundermann CA, Hoerr FJ, Ernest JA (1986) Experimental *Cryptosporidium* infections in chickens: Oocyst structure and tissue specificity. *Am J Vet Res* **47:** 876-879.

Lindsay DS, Blagburn BL, Sundermann CA, Hoerr FJ (1989) Experimental infections in domestic ducks with *Cryptosporidium baileyi* isolated from chickens. *Avian Dis* **33:** 69-73.

Long PL (1973) Pathology and pathogenicity of coccidial infections. The coccidia. (ed. Hammond DM with Long PL) p253-294 University Park Press, Baltimore, Butterworths, London.

Matsui T, Morii T, Fujino T, Tadeja SL, Itakura C (1992) Oocyst production and immunogenicity of *Cryptosporidium* sp. in chickens. *Jpn J Parasitol* **41**(1): 24-29.

Mesfin GM, Bellamy JEC (1978) The life cycle of *Eimeria falciformis* var. *pragensis* (Sporozoa: Coccidia) in the mouse, *Mus musculus*. *J Parasitol* **64:** 696-705.

Metcalf JA, Gallin JI, Nauseef WM, Root RK (1986) Laboratory Manual of Neutrophil Function. 1st ed p20, 90-91 Raven Press, New York.

Mzembe SAT, Lloyd S, Soulsby EJL (1984) Macrophage mediated resistance to *Babesia microti* in *Nematospiroides dubius*-infected mice. *Z Parasitenkd* **70:** 753-761.

Rhee JK, Seu YS, Park BK (1991a) Isolation and identification of *Cryptosporidium* from various animals in Korea I. Prevalence of *Cryptosporidium* in various animals. *Korean J Parasitol* **29**(2): 139-148.

Rhee JK, Seu YS, Park BK (1991b) Isolation and identification of *Cryptosporidium* from various animals in Korea III. Identification of *Cryptosporidium baileyi* from Korean chicken. *Korean J Parasitol* **29**(4): 315-324.

Slavin D (1955) *Cryptosporidium meleagridis* (sp. nov.). *J Comp Pathol* **65:** 262-266.

Tatsukawa K, Mitsuyama M, Takeya K, Nomoto K (1979) Differing contribution of polymorphonuclear cells and macrophages to protection of mice against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. *J General Microbiol* **115:** 161-166.

=초록=

병아리 및 청둥오리에 있어서 닭와포자충의 오오시스트 배설상황과 면역원성

이재구, 장병귀, 박배근

전북대학교 수의과대학 기생충학교실

한국산 *Cryptosporidium baileyi*를 2일령의 각 청둥오리와 병아리에 2×10^6개의 오오시스트와 그 10배단계 희석계열을 2×10^2까지 5단계를 만들어 경구접종시켜 오오시스트 배설상황과 여러 장기내 발육기의 분포상황을 조사하고나서 초감염내과 조류에 처음에 40 mg/30 g 그 24시간 후에 10 mg/30 g의 carrageenan을 복강내 투여하고 그 6시간 후에 다시 2×10^6개의 오오시스트를 재차접종하여 면역원성을 조사하였다. 오오시스트 투여량이 많을수록 patent period가 길었고(27-30일간, 병아리: 10-18일간 청둥오리), 오오시스트 배설량도 많았으나(극기에 있어서 6×10^5-3.5×10^6, 병아리: 2×10^5-4×10^5, 청둥오리) prepatent period는 짧았다(2-3일간, 병아리: 3-4일간, 청둥오리). 한편, 청둥오리에서는 2×10^2 및 2×10^3의 오오시스트 접종군에서는 오오시스트가 전혀 검출되지 않았다. 오오시스트 경구접종 후 12일에 총배설강과 파브리시우스낭에서 다수의 발육기를 검출할 수 있었는 데 병아리가 청둥오리보다 현저하였다. Carrageenan 투여군은 모두, 대조군은 병아리에서 2×10^2 및 2×10^3, 그리고 청둥오리에서 이 밖에 2×10^4의 오오시스트 투여군에서도 재차 접종후 4일에서 14일 및 5일에서 15일까지 분변으로부터 오오시스트가 다소 검출되었다. 병아리 및 청둥오리에 있어서 재차접종 직전의 말초혈액내 대식세포수 및 복강내 대식세포 활성은 대조군에 비하여 carrageenan 투여군이 현저하게 낮았다($P < 0.05$). 병아리와 청둥오리는 Mø blocker인 carrageenan을 투여하였음에도 불구하고 이 원충의 재차감염이 미약하였다는 것은 이 원충의 면역원성이 극히 강력하였다는 것을 뜻한다.

(기생충학잡지 33(1): 45-54, 1995년 3월)