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Effects of *Cryptosporidium baileyi* infection on the bursa of Fabricius in chickens

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Abstract: In order to clarify the effect of cryptosporidiosis on immune response, histopathological changes associated with experimentally occurring bursal cryptosporidiosis in chickens were chronologically observed as the first step. A total of 150 2-day-old chickens was each inoculated orally with a single dose of 5×10^5 *Cryptosporidium baileyi* oocysts. The chickens showed a normal profile of oocyst shedding in droppings. The bursa indices throughout the experimental period indicated negligible reactions. Numerous cryptosporidia occurred in the microvillous border of bursal epithelium between days 4 and 16 postinoculation (PI). Appearance of the most mast cells was followed by a dramatic loss of the protozoa in the bursa of Fabricius (BF). The distribution of the coccidium coincided with heterophil infiltration in the epithelium and adjacent lamina propria. The histopathological lesion was marked diffuse chronic superficial purulent bursitis with heterophil infiltration in the epithelium and adjacent lamina propria and mucosal epithelial hyperplasia. These results suggest that the bursitis may induce immunosuppressive effect.

Key words: *Cryptosporidium baileyi*, histopathological findings, mast cell, chicken

INTRODUCTION

In chickens, *Cryptosporidium baileyi* is a primary pathogen that can produce respiratory and/or intestinal disease resulting in morbidity and mortality. The parasite is frequent establishment of infections in the mucosal epithelium of a wide variety of organs. For example, the protozoa can infect the cloaca, the BF, the upper and lower respiratory tracts, and the eye lids.

BF is a sac-like dorsal diverticulum of the proctodeum that is unique to birds. Since the discovery by Glick in 1954 on the role of BF in antibody formation and the establishment of delineation of the immune system (Glick *et al.*, 1956), BF has generally been regarded as a central and a peripheral lymphoid organs. It is more than probable that both functions of BF are not independent of each other (Toivanen *et al.*, 1987). In chicken, primary B cell lymphopoiesis occurs in a discrete lympho-epithelial organ, BF. B cells differentiate into plasma cells from lymphoid stem cells.

Since BF is a principal target organ in *Cryptosporidium*-infected chickens, functional impairment of this organ may occur in infected chickens. However, histopathologic and chronologic appearances of BF from chickens experimentally or naturally infected with *Cryptosporidium* sp. have not been elaborately described excepting fragmentary delineations of only 2 cases (Guy *et al.*, 1988; Goodwin and Brown, 1989). Moreover, the significance of

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Cryptosporidium-parasitized mucosal epithelial cells in BF is not well defined. Especially, the kinetics of mast cell response in BF has not investigated as far as the authors are aware.

The objective in the present experiment was to clarify the effect of *C. baileyi* infection on the histopathological findings in BF as a series of studies to determine the immunosuppressive effect of cryptosporidiosis on immune response against the other pathogens in chickens.

MATERIALS AND METHODS

C. baileyi oocysts used for the present study were the same origin of the previous work (Rhee *et al.*, 1995). A total of 150 2-day-old SPF chickens (Dekalb-Warren, Sex-Sal-Link, male) was each inoculated orally with a single dose of 5×10^5 *C. baileyi* oocysts. Meanwhile, a total of 150 uninfected age-matched chickens served as a control for the experiment. Following inoculation, feeding of chickens and examination of fecal samples were subjected to the methods previously described by Rhee *et al.* (1995). Enumeration for the number of oocysts discharged per day for each chick was carried out daily (Rhee *et al.*, 1995). For examinations, 5 chickens were victimized each stage at 4-8 days intervals excepting every day for initial stages.

Each chick was weighed, and the weight of its BF determined. From these data, a bursa index for each stage was calculated as following:

$$\text{Bursa index} = \frac{\text{Bursa weight/Body weight of experimental animals}}{\text{Bursa weight/Body weight of controls}}$$

A bursa index of < 1.0 thus indicated no response or atrophy, more than 1.0 being considered an enlargement of the bursa.

BFs obtained from each bird were fixed in 10% neutral buffered formalin, and submitted to the histology service for routine histologic processing, paraffin-embedding, and hematoxylin and eosin staining for the observation of the histopathological findings. Additionally, to observe the kinetics of mast cell response BFs were fixed in Carnoy s solution for 2-6 hrs depending on size, and the sectioned samples were stained with 0.5% toluidine blue (pH 0.5) for 5 minutes, as suggested by Tronchin *et al.* (1979).

The experiments were repeated three times resulting in similar results.

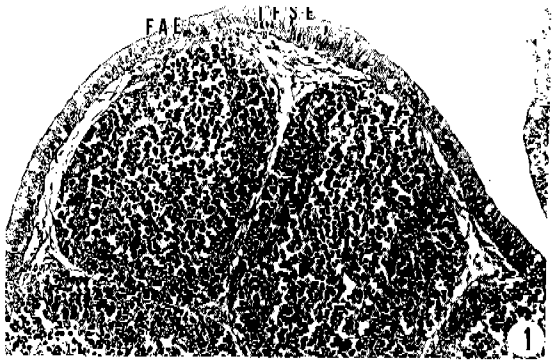
RESULTS

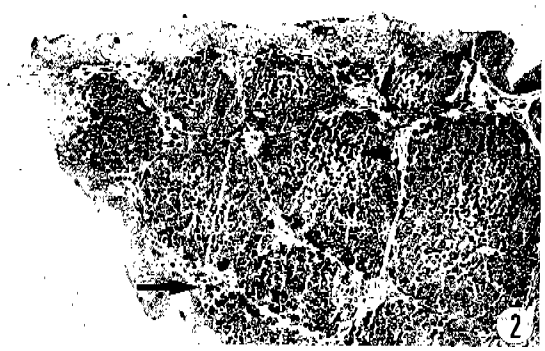
In general, mast cells occurred mainly in the interfollicular stroma, lymphofollicular and trabecular regions in sequence, but a few of them appeared in the epithelium of BF in the experimental group chickens (Fig. 2). The numbers of mast cells rose to a peak at the 3rd and 8th days PI, and then began to decline. Meanwhile, fluctuations in the numbers of mast cells in contorl group chickens were similar to those of experimental

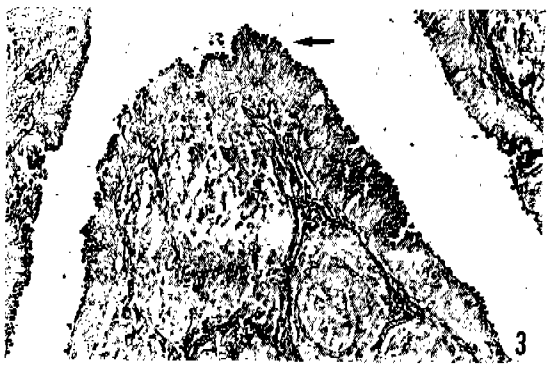
Fig. 1. Light photomicrograph of a section of the BF from an uninfected 12-day-old chicken. The section showing epithelium composed of follicle-associated epithelium (FAE) and interfollicular surface epithelium (IFSE), and follicles including numerous lymphocytes. **Fig. 2-8.** Sequence of changes in the BF of chickens inoculated with *C. baileyi*. **2.** Large number of strongly stained mast cells are found principally in interfollicular stroma (arrow) and lymphoid follicles (arrow head) (day 4). **3.** Infection is very heavy, in fact there is a virtual monolayer of cryptosporidia (arrow) in the microvillous region. Irregular and shallow erosions of the epithelium are generated by invasion of cryptosporidia (day 8). **4.** The epithelium and adjacent lamina propria infiltrated diffusely and heavily with heterophils. A core (arrow) of exudate composed of predominantly heterophils in bursal lumen (day 16). **5.** Mild diminution of lymphocyte, follicular atrophy and enlargement of the lamina propria are present. The lamina propria is minimally infiltrated by inflammatory cells (day 20). **6.** Atrophy of follicles and depletion of lymphocytes within follicles are visible, parasites are still numerous, and more heterophils are present, a characteristic feature of parasitized epithelium in BF (day 24). **7.** Cryptosporidia disappeared, and the epithelium is markedly thickened compared with control BF (day 44). **8.** Hyperplastic response of bursal epithelium can be seen, and the other findings are similar to those of control BF (day 56). Figures are of sections stained with hematoxylin and eosin except for Fig. 2 (toluidine blue) and Fig. 3 (PAS), and magnifications on of all the figures are × 420 exclusive of Fig. 5 (× 210).

group chickens exclusive of decreased numbers of the cells for each stage (Table 1). Additionally, those of intrinsic control chickens were 148.941 – 19.45.

As shown in Table 2, a few cryptosporidia life-cycle stages of variable-sized ovoid structure were first detected in the microvillous border of bursal epithelium on







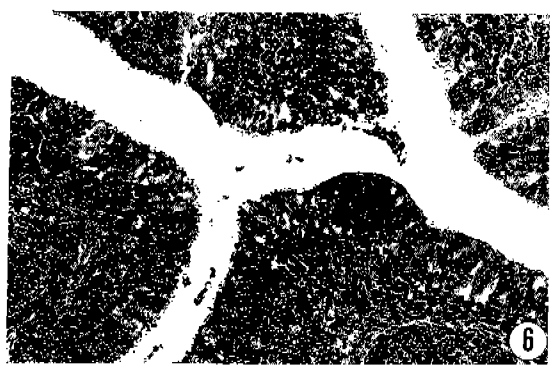


Table 1. The numbers of mast cells in bursa of Fabricius and bursa indices on chickens inoculated with *Cryptosporidium baileyi*

Days after inoculation	Mast cells[a)]		Bursa indices
	Control	Experimental	
1	146.692 ± 17.27	242.923 ± 26.47	0.9701146
2	177.360 ± 17.70	222.538 ± 28.33	0.8284729
3	223.160 ± 24.11	341.153 ± 24.57	0.7326471
4	170.330 ± 17.25	291.411 ± 23.50	0.6891303
8	92.857 ± 5.39	274.882 ± 20.68	0.7245878
12	34.600 ± 6.62	113.133 ± 6.63	0.7181450
16	29.714 ± 4.53	130.250 ± 8.86	0.7281351
20	14.285 ± 3.53	100.066 ± 7.38	0.7109721
24	11.583 ± 2.02	51.250 ± 4.11	0.7063870
28	14.750 ± 3.69	60.916 ± 6.28	0.6907301
32	22.750 ± 3.40	36.500 ± 3.98	0.9150741
36	16.333 ± 2.69	51.000 ± 2.00	0.8965565
40	13.000 ± 1.41	14.000 ± 1.82	0.5204320
44	14.666 ± 1.15	24.000 ± 4.28	0.9745854
48	12.000 ± 1.73	15.333 ± 3.52	0.6824764
52	9.400 ± 2.30	14.833 ± 3.81	0.8534266
56	10.240 ± 3.20	13.253 ± 4.28	1.2455042
60	10.200 ± 3.96	12.375 ± 1.99	0.9115603
68	11.250 ± 3.95	13.080 ± 4.12	0.7326620
76	8.500 ± 2.38	9.750 ± 0.95	0.8924200
84	5.750 ± 0.95	6.200 ± 1.64	0.9024379
92	4.000 ± 0.70	4.200 ± 0.83	0.5681887
100	5.600 ± 1.51	5.600 ± 0.89	1.0425579

[a)]Determinations are expressed as the mean ± SD of three times repetitions per allocation (the number of mast cells per cross section of the broadest part in the bursa of Fabricius).

microvillous border of bursal epithelium on day 3 PI. The most parasites occurred between days 4 and 16 PI (Fig. 3). No cryptosporidia were observed from the 28th day PI downward. Following inoculation, the chickens were likely to disclose normal oocyst shedding profiles in droppings similar to those of previous study (Rhee *et al.*, 1995). In this case the prepatent period was 2 days and peak oocyst production occurred between 7 and 10 days with a patent period of 29 days. While, the oocysts did not show up fecal sample from control group chickens during this period. Emergence of the parasite was accompanied by heterophil infiltration in the epithelium and adjacent lamina propria. Infiltration of heterophils was first observed on day 4 PI. It increased to high numbers between days 12 and 16 PI (Fig. 4) and disappeared from the 28th day PI downward.

No significant changes were noted in the bursal lymphoid follicles, although a mild to moderate depletion of lymphocytes and atrophy of follicles attributed to proliferation of lamina propria were observed during the infection as shown in Fig. 5. On day 24 PI, a great deal of characteristic feature of *Cryptosporidium*-infected BF was visualized. Above mentioned findings were observed between days 4 and 32 PI. Since the 32nd day PI it revealed hypertrophy and hyperplasia of the epithelial cells, which resulted in a moderate to marked thickening of the epithelium. The nuclei of affected cells were typically enlarged and vesicular. The hyperplastic alternations were pronounced from the 44th to 68th days PI (Fig. 7 and 8). It is interesting that hyperplasia of the epithelial cells and thickening of the epithelium were not repaired normally until day 100 PI (Table 2).

Table 2. Histopathological changes of bursa of Fabricius in chickens inoculated with *Cryptosporidium baileyi*

Microscopical findings	Days after inoculation																						
	0	2	3	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	68	76	84	92	100
Cryptosporidia in epithelium	—	—	+	++++	++++	++++	+++	++	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Heterophil infiltration in epithelium	—	—	—	+	++	+	+++	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Heterophil infiltration in lamina propria	—	—	—	+	++	+	++	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Depletion of lymphocyte in follicle	—	—	—	*	*	*	*	*	*	*	*	—	—	—	—	—	—	—	—	—	—	—	—
Atrophy of follicle	—	—	—	*	*	*	*	*	*	*	*	—	—	—	—	—	—	—	—	—	—	—	—
Hyperplasia of epithelial cell	—	—	—	—	—	—	—	—	—	—	*	*	**	***	***	***	***	***	***	*	*	*	*
Thickening of epithelium	—	—	—	—	—	—	—	—	—	—	*	*	*	*	*	*	*	*	*	*	*	*	*
Oocyst shedding	—	—	+	++	+++	++++	+++	++	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—

The numbers of cryptosporidia and heterophils in tissues, and of oocysts in droppings were scored:—; not exist, +; a few, ++; moderate, +++; numerous, ++++; very numerous. Tissue changes were scored:—; no response, *; minimal (mild) changes, **; moderate changes, ***; severe changes.

were not observed in control chickens throughout the experimental time period (Fig. 1).

The bursa indices throughout the experimental time period were less than 1.0 in most cases by 0.5204320, and few cases were greater than 1.0 by 1.2455042 (Table 1).

DISCUSSION

Histopathological changes of *C. baileyi*-infected bursa most often appeared for a long term in superficial layer. The lesions were scattered randomly with inflammatory cells and hyperplastic response throughout the bursa. Particularly, the presence of large numbers of heterophils in the epithelium was also a striking feature in the parasitized bursa. As shown in Table 2, the typical morphologic lesion in *C. baileyi* infected bursa was marked diffuse chronic superficial purulent bursitis with mucosal epithelial hyperplasia, as fragmentarily described by Guy *et al.* (1988) and Goodwin and Brown (1989). Hyperplasia is a disturbance of growth characterized by an absolute increase in number of cells. This lesion typically indicates a response to relatively prolonged injury. Proliferative replacement of body cells is a natural defense mechanism that can be used by a host to facilitate elimination of noxious stimulus (parasitism).

Goodwin and Brown (1989) intimated that the often-made assumption that bursal cryptosporidiosis is dependent on lymphocyte damage (and therefore, immunosuppression) is not correct. In the present study, as the findings and suggestion of Guy *et al.* (1988), functional impairment may occur as a result of interference with particle uptake possibly on account of the physical presence of the parasite and/or the consequent thickening of the epithelium and adjacent lamina propria, since alternations in bursal follicles were minimal.

Precursors of B-cell enter the epithelium by passing through basement membrane, and are induced to become B-cell, which proliferate in the medulla of developing follicles in embryonic life of chicken. BF continues to function as the B-cell source throughout life, although its activity gradually declines with age (Klein, 1990). From the present results, therefore, it is supposed that such alternations of superficial layer in *C. baileyi*-infected bursa may give rise to impairment of immunity against the other pathogens.

As of now, it is a well-known phenomenon that mucosal mast cells proliferate rapidly in helminths infection (Chai *et al.*, 1993; Rhee *et al.*, 1994), nevertheless available information on the kinetics of mucosal mast cells in protozoan infection has not been published. There are numerous suggestions relating to the molecular mechanisms of mucosal mast cell function in helminths infection (Lee *et al.*, 1986), and their precise role in the expulsion process of worms is, however, still not clearly elucidated. Moreover, inasmuch as host-parasite relationships in helminths may be much different from those in protozoa, there is a question whether a similar mechanism of mastocytosis would occur in protozoan infection.

In the current study, regardless of the actual mechanism of the expulsion of the protozoa, appearance of numerous mucosal mast cells was followed by a dramatic loss of cryptosporidia in BF, although mast cell deficient W/W^v infant mice were similar to normal mice in their susceptibility to and recovery from infection with *C. parvum* (Harp and Moon, 1991). It is reasonable that the distribution density of developmental stages of the protozoa in BF yields comparable pattern in oocyst shedding in droppings. But fluctuations in numbers of the mast cells in BFs from initial stages of infection did not correspond with those of the parasites, so further investigations are needed to clarify the factors affecting the numbers of the mast cells. This study described the first attempt to use a protozoa, *C. baileyi* concerning mastocytosis as a pathogen in chicken. It seems self-evident that worm expulsion must in some way be causally correlated with mucosal mast cells, but it is still not clear exactly what the correlation is. Therefore, intensive studies on the functional aspects of mucosal mast cell during the expulsion process of the protozoa are needed in future.

The bursa of chickens infected with

infectious bursal disease virus markedly increased the size and weight owing to edema and hyperplasia at initial stage (Cheville, 1967). However, the bursa indices in the present communication indicated that bursal cryptosporidiosis may result in histologic lesions but does not result in gross lesions.

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=초록=

닭에 있어서 닭와포자충 감염이 파브리시우스낭에 미치는 영향

이재구, 김현철, 박배근

전북대학교부속 생체안전성연구소

닭에 있어서 닭와포자충 감염이 면역반응에 미치는 영향을 규명하기 위한 기초적 연구의 일환으로 파브리시우스낭의 병리조직학적 소견을 경시적으로 조사하고자 150마리의 2일령 SPF 병아리(Dekalb-Warren, Sex-Sal-Link)에 닭와포자충의 오오시스트 5 × 10^5를 한 번에 경구투여하였다. 분변 속의 오오시스트 배설양상은 정상적이었으며, 파브리시우스낭 지수는 전 실험기간에 걸쳐 거의 변동이 없었다. 많은 수의 원충체가 접종 후 4-16일에 이 낭상피의 미세융모 가장자리에서 관찰되었으며, 많은 수의 비만세포가 출현한 다음 원충체가 급격하게 소실하였다. 원충체의 분포 상황과 상피 및 인접점막 고유층의 위호산구 침윤은 일치하였다. 이 병소는 상피, 인접 점막고유층의 위호산구 침윤과 점막상피 증식을 동반한 미만성 만성 표재성 화농성 파브리시우스낭염의 병리조직학적 소견이었다. 이러한 파브리시우스낭염은 면역억제를 유발할 것으로 생각된다.

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