

Effect of *Cryptosporidium baileyi* infection on antibody response to sRBC in chickens

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Abstract: Hemagglutinin (HA) titers to sRBC were chronologically observed in chickens orally inoculated at 2 days of age with 5×10^5 oocysts of *Cryptosporidium baileyi*. All the infected chickens exhibited negligible HA titers by 44 days postinoculation (PI). The titers were elevated as time progressed, and peaked on day 52 PI, declined gradually thereafter, and eventually reached to normal titers on day 92 PI. On the contrary, the titers in uninfected chickens were higher in comparison with infected chickens during the experiment. Chickens infected with the protozoa showed normal oocyst shedding profiles during this period. These data suggest that *C. baileyi* infection suppress development of humoral immunity to sRBC in chickens. It is possible that impairment of the bursa of Fabricius by cryptosporidiosis rendered chickens vulnerable to other pathogens.

Key words: *Cryptosporidium baileyi*, hemagglutinin titer, sRBC, chicken, bursa of Fabricius

INTRODUCTION

It is well established that humoral antibody formation in the chicken depends on the bursa of Fabricius (BF), whereas delayed hypersensitivity and other manifestations of cellular immunity depend on the thymus for their development (Cooper *et al.*, 1966; Warner, 1967).

Cryptosporidium spp. appear to be present wherever avian hosts are raised commercially. Mild to heavy respiratory infection can be demonstrated and severe morbidity and sometimes mortality may result, but intestinal disease is usually mild. In a basic attempt to

elucidate the effects of *Cryptosporidium baileyi* infection on the development of immunity to other pathogens in chickens, light-microscopic lesions associated with experimentally occurring bursal cryptosporidiosis in chickens were examined in previous work (Rhee *et al.*, 1997). The typical morphologic lesions in *C. baileyi*-infected chickens were the marked diffuse chronic superficial purulent bursitis with mucosal epithelial hyperplasia (Rhee *et al.*, 1997). It suggests, therefore, that infection with *C. baileyi* may affect the development of humoral immunity to other antigens.

At present, hemagglutinin (HA) titers to sRBC following infection with *C. baileyi* in chickens were monitored to determine whether early exposure to the protozoa influences humoral immune response to sRBC because there have been few reports on the issue.

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MATERIALS AND METHODS

As humoral immune response to sRBC in chickens are dependent on aging of chicken, different routes of antigen administration, different doses of sRBC and stages of the response (McCorkle and Glick, 1980; van der Zijpp *et al.*, 1986; Kreukniet and van der Zijpp, 1990), a preliminary trial was performed to define criteria in regard to these factors.

Cryptosporidium baileyi oocysts used for the present study were the same origin of previous work (Rhee *et al.*, 1997). 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 oocysts. Meanwhile, a total of 150 uninfected age-matched chickens served as controls for the experiment. Following inoculation, feeding of chickens and examination of fecal samples were subjected to the methods previously described by Rhee *et al.* (1996).

HA titers were determined for each chick as suggested by Rhee *et al.* (1989). Thus, the sRBCs collected from a sheep were used as an antigen. The blood was preserved in Alsever's solution, washed three times in PBS, pH 7.0 and packed cells were diluted to concentrations of 1% in PBS (v/v). Chickens were intravenously injected once at an interval of 4 days with 0.5 ml of 1% sRBC ($1 \times$

10^7 /ml) from 3rd to 95th day postinoculation (PI). Five days following each immunization with 1% sRBC, blood were collected from 5 chickens in each stage of PI and HA titers measured by a microtiter technique. A U-bottom 96 well microtiter plate was used to make two fold dilutions of serum with a 25 μ l microdiluter. The volume transferred was 25 μ l, and the volume of 1% sRBC (2.5×10^5) was added to make the final volume of 50 μ l, respectively. After 2 hr in 37°C incubator, the titer was defined as the arithmetic average of the reciprocal of the highest positive HA dilution giving a visible agglutination.

The data obtained in the present study with three repetition showed similar results. HA titers to sRBC between uninfected and infected groups were analyzed by Student t-test.

RESULTS

As shown in Table 1, HA was detected at the 8th day PI in chickens infected with *C. baileyi*. The levels of HA, on the average, were gradually increased with day, peaked at the 52nd day PI and subsequently decreased. Meanwhile, HA titers in uninfected chickens showed a similar pattern with those in infected chickens, although the levels in uninfected chickens were significantly increased during the experimental period ($p < 0.05$).

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Table 1. Hemagglutinin titers to sRBC in chickens inoculated with *Cryptosporidium baileyi*

Days after inoculation	Uninfected	Infected	Days after inoculation	Uninfected	Infected
8	2.50 \pm 0.92	2.00 \pm 0.00	56 ^a	38.40 \pm 8.26	25.60 \pm 8.26
12	4.00 \pm 1.63	3.33 \pm 1.00	60 ^a	32.00 \pm 9.23	19.20 \pm 6.74
16	4.33 \pm 1.41	3.67 \pm 0.70	64 ^a	25.60 \pm 8.26	16.00 \pm 9.23
20	6.40 \pm 1.83	5.60 \pm 1.57	68 ^a	18.40 \pm 7.35	11.60 \pm 6.02
24	4.80 \pm 1.68	4.00 \pm 0.00	72 ^a	12.80 \pm 4.13	8.80 \pm 1.68
28	4.80 \pm 1.68	4.00 \pm 0.00	76 ^a	10.40 \pm 2.06	8.00 \pm 0.00
32 ^b	7.20 \pm 1.68	3.60 \pm 0.80	80	9.60 \pm 3.37	8.00 \pm 0.00
36	9.60 \pm 2.06	8.80 \pm 1.68	84	9.60 \pm 3.37	8.00 \pm 0.00
40	6.40 \pm 2.06	4.80 \pm 1.68	88	5.60 \pm 2.06	4.80 \pm 1.68
44 ^a	11.20 \pm 4.13	6.40 \pm 2.06	92	4.00 \pm 0.00	3.60 \pm 0.84
48 ^a	24.00 \pm 10.66	14.40 \pm 6.31	96	4.00 \pm 0.00	4.00 \pm 0.00
52	64.00 \pm 18.47	57.60 \pm 25.24	100	4.00 \pm 0.00	4.00 \pm 0.00

Each value represents the mean of three repetition of five determinations with the standard deviations.

^a $p < 0.05$; ^b $p < 0.01$.

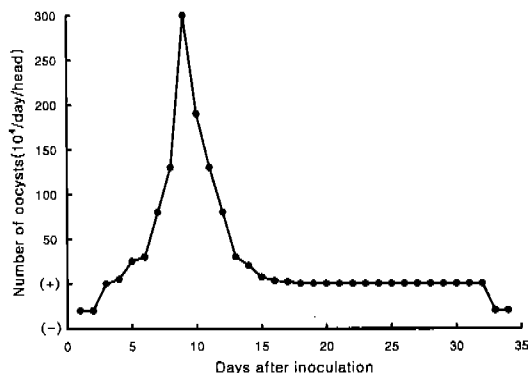


Fig. 1. Pattern of oocyst shedding in chickens inoculated with 5×10^5 oocysts of *Cryptosporidium baileyi*. (+) and (-) indicated that less than 10^3 oocysts were detected and oocysts were not detected, respectively.

protozoa was 2 days and peak oocyst production (10^6 /day/head) occurred between days 8 and 11 PI with a patent period of 30 days (Fig. 1). While the oocysts did not show up in the fecal sample of uninfected chickens during this period.

DISCUSSION

The objective of the present study was to investigate the antibody response in immunity to cryptosporidiosis by using *C. baileyi* and a chicken model of infection. The progenitor cells of B-cell enter the bursal epithelium by transiting through the surrounding basement membrane, and settle down in the bursal medulla. Most of the progenitor cells arrive in BF sometime between the eleventh and fourteenth day of embryonic life. BF continues to function as the B-cell source throughout life, although its activity gradually declines with age (Klein, 1990).

This study revealed that the pathological changes such as hyperplasia and thickening of the epithelium of BF after the ceasing of oocyst production observed by Rhee *et al.* (1997) were correlated with high levels of HA titers. The relationship may explain that hyperplasia of the epithelium is a disturbance of growth characterized by an absolute increase in number of cells. In addition, proliferative replacement of body cells is a natural defence

mechanism that can be used by a host to facilitate elimination of a noxious stimulus, as described by Rhee *et al.* (1997). Meanwhile, there was no relationship between the number of oocysts in daily fecal samples and HA titers in infected chickens. It is a natural outcome due to a short patent period (30 days) of *C. baileyi* infection in chickens.

In the present study, the levels of HA in infected chickens were significantly decreased in comparison with uninfected chickens throughout the experimental period ($p < 0.05$). In the light of diffuse chronic superficial purulent bursitis with mucosal epithelial hyperplasia in chickens infected with *C. baileyi*, as described previously (Rhee *et al.*, 1997), it is supposed that outcome of the present study attributed to the effect of the protozoa infection on bursal epithelium and a mild to moderate depletion of lymphocytes in bursal lymphoid follicles.

The antibody titers to sRBC, a T-cell dependent antigen, were significantly influenced by age but not sex. The total sRBC agglutinin titers increased significantly from 1 to 6 months of age and then declined by 12 and 24 months to levels that were not significantly different from 1-month-old New Hampshire chickens (McCorkle and Glick, 1980). In the present study, HA titers of uninfected chickens were inconsonant to those of McCorkle and Glick (1980). It is thought that such phenomenon may attribute to differences in breeds of chickens, the levels of sRBC dose and immunoresponse periods. Differences in responses are the subject of future work.

The present study showed a depression in antibody response to sRBC in chickens when infected with *C. baileyi*. Therefore, cryptosporidiosis in chickens has resulted in a tendency to immunosuppression to sRBC, which might render the host vulnerable/or more susceptible to other pathogens. Further studies are in progress to clarify the impact of *C. baileyi* infection on humoral immunity to other pathogens.

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=초록=

닭에 있어서 닭와포자충 감염이 sRBC에 대한 항체반응에 미치는 영향

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닭에 있어서 닭와포자충 감염이 면역억제에 미치는 영향을 규명하기 위한 일환으로 150마리의 2 일령 SPF 병아리에 5×10^5 의 닭와포자충 오오시스트를 경구 투여하였다. 경구투여 후 3일부터 95일까지 4일 간격으로 5마리씩의 병아리 정맥 내에 1×10^7 의 sRBC를 주입한 다음 각각 5일 후에 혈액을 채혈하여 sRBC의 응집소가를 측정하였다. 감염군에 있어서 분변 속의 오오시스트 배설은 정상적인 양상이었으며, 응집소가는 시일이 경과함에 따라 감염, 비감염 양군 모두 점점 높아져 52일에 최고에 이른 다음 점점 낮아졌다. 한편, 실험기간을 통하여 응집소가는 오오시스트를 투여하지 않은 비감염군에 비하여 오오시스트를 투여한 감염군이 의외있게 낮았다 ($p < 0.05$). 이상의 결과로 미루어 보아 닭와포자충 감염닭은 비감염닭에 비하여 항체반응이 저하되는 경향이 있다.

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