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☐ **Brief Communication** ☐

Verification of immunosuppression in chicks caused by *Cryptosporidium baileyi* infection using *Brucella abortus* strain 1119-3

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Abstract: Humoral immune response of young chicks to *Brucella abortus* strain 1119-3 inoculation was monitored to verify the degree of immunosuppression caused by infection with *Cryptosporidium baileyi*. Young chicks (2-day-old) were orally inoculated each with 2 $\times 10^6$ oocysts of *C. baileyi*, and then injected intramuscularly with 0.3 ml *B. abortus* strain 1119-3 containing 1×10^9 living organisms on day 14 postinoculation (PI). Serum samples were tested by plate agglutination test on day 17 PI onwards at an interval of 3-6 days over a period of 36 days. Infected chicks with the coccidium showed significantly lower antibody titers than those of uninfected controls ($P<0.05$). These findings document that *C. baileyi* infection in early life stage may predispose chicks easily to other potential poultry diseases.

Key words: *Cryptosporidium baileyi*, *Brucella abortus*, immunosuppressive effect, chick

Cryptosporidium baileyi appears to be present wherever avian hosts are raised commercially. It is a primary pathogen that can produce respiratory and/or intestinal disease resulting in morbidity and mortality and generally invades the epithelium of the cloaca and the bursa of Fabricius, the essential organ for the development of humoral immunocompetence in young chicks (Claflin *et al.*, 1966; Cooper *et al.*, 1966).

In a series of studies to document the immunosuppressive effect caused by the protozoan infection on humoral immunity to other poultry infections, we have shown previously that infection with *C. baileyi* in early life stage depressed the ability of the chicks to respond to sRBC (Rhee *et al.*, 1998b). Moreover, recent investigations have also demonstrated that 2-day-old chicks initially infected with the protozoon showed a decreasing tendency of the immune responses following vaccination against Newcastle disease (ND) virus and avian infectious bronchitis (IB) virus, respectively (Rhee *et al.*, 1998a & 1998c). Such immunosuppression to sRBC, ND virus and IB virus in chicks appears to be attributed to a marked diffuse chronic superficial purulent bursitis caused by *C. baileyi* (Rhee *et al.*, 1997). Taken together, it is suggested that cryptosporidiosis could predispose chicks to secondary invasion by other important pathogens. Therefore, the present study examined this possibility using *B. abortus* strain 1119-3 as a corroborative indicator in *C. baileyi* infected chicks to verify the status of immunosuppression.

Oocysts used in the present study have been maintained in our laboratory since 1990, as previously described by Rhee *et al.* (1997).

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Bacteria were kindly provided by the Department of Veterinary Public Health, Chonbuk National University and cultured in *Brucella* agar and *Brucella* broth (Difco Laboratories, Detroit, USA) at 37°C using 10% CO_2 in descending order. Two-day-old SPF chicks (Dekalb-Warren, Sex-Sal-Link, male) were divided into three experimental groups; infected (infected with both *C. baileyi* and *B. abortus*), uninfected (infected with *B. abortus*, but not with *C. baileyi*) and intrinsic control groups (negative control: uninfected with both *C. baileyi* and *B. abortus*). Chicks were orally inoculated each with a single dose of 2×10^6 oocysts of *C. baileyi*, and 15 chicks in each group were injected intramuscularly each with 0.3 ml of *B. abortus* strain 1119-3 containing 1×10^9 living organisms on 14 days after *C. baileyi* infection. Procedures used for the feeding of chicks and the examination of fecal samples were referred to those described previously (Rhee *et al.*, 1997).

The serum samples were collected from each bird at an interval of 3-6 days over a period of 36 days postinoculation (PI) with the bacteria and the serum agglutination titers to *B. abortus* were estimated by buffered plate agglutination test (PAT) using a commercially available antigen prepared by *B. abortus* strain 1119-3 for PAT, as recommended by the manufacturer (Dae Sung Microbial Laboratories, Seoul, Korea). To prepare the antigen, *B. abortus* strain 1119-3 concentrate was diluted to a final concentration of 0.5% phenol (0.5 g phenol in 100 ml of normal saline solution) along with crystal violet and brilliant green. Thus doubling dilutions of serum from 1.25 to 80 μl were transferred to 10 well glass plates marked in 1.5×1.5 cm, respectively. Aliquots (30 μl) of the antigen were added to appropriate wells of the previously prepared plates. The plates were rotated three times in a tilting motion, and then incubated for 4 min in a humid chamber at an ambient room temperature (20-25°C). They were then incubated again after three times rotation as described above. At this point, the plates were removed, rotated and observed for visible agglutination.

The test in each isolator was repeated at least three times with similar results. PAT levels to *B. abortus* between infected and uninfected groups were analyzed by Student's *t*-test.

The results of the serum agglutination titers to *B. abortus* strain 1119-3 between *C. baileyi*-infected and uninfected chicks were summarized in Table 1. The titers were not detected at any time in the samples from 15 intrinsic control chicks, indicating that the titers in both infected and uninfected chicks had developed specific antibody in response to the bacterial infection and that the bacteria did not spread to the intrinsic control chicks.

Table 1. Fluctuations of $\log_2$ based serum agglutination titers to *Brucella abortus* in *Cryptosporidium baileyi* inoculated chicks

Days postinoculation with *B. abortus*	Uninfected with *C. baileyi*	Infected with *C. baileyi*	Significance of difference (P)
0	0	0	0
3	0	0	0
6	1.07 ± 0.44	0.87 ± 0.50	0.2711
9	2.07 ± 0.57	1.13 ± 0.50	0.0001
12	2.93 ± 0.68	1.73 ± 0.85	0.0003
15	3.27 ± 0.68	2.07 ± 0.77	0.0001
18	2.07 ± 0.77	1.40 ± 0.49	0.0108
21	1.73 ± 0.57	1.27 ± 0.44	0.0227
24	1.87 ± 0.62	1.07 ± 0.68	0.0029
30	1.13 ± 0.53	0.93 ± 0.39	0.0041
36	1.13 ± 0.34	0.53 ± 0.50	0.0008

Each value represents the mean of triple repetition of fifteen determinations with the standard deviations.

The titers were not observed at day 3 PI in samples from both infected and uninfected chicks.

The serological immune response to the bacterial infection of chicks which were previously infected with the protozoon was significantly lower than that of uninfected chicks throughout the experiment ($P<0.05$), never reaching a mean greater than $\log_2$ based titer of 2.07 without an evident peak. The difference in peak titers between infected and uninfected chicks at 15 days PI was exceedingly significant ($P=0.0001$). It is supposed that *C. baileyi* infection caused a disturbance of the ontogeny of B-cell by hyperplasia and thickening of bursal epithelium and a mild to moderate depletion of lymphocytes in bursal follicles (Rhee *et al.*, 1997).

Since the humoral immune response to *B. abortus* was observed to assess the effect of surgical and chemical bursectomy (Claflin *et al.*, 1966; Cooper *et al.*, 1966), a number of researchers have used this bacterium to monitor the antibody response in chicks (Hopkins *et al.*, 1979; van der Zijpp *et el.*, 1986; Kreukniet *et al.*, 1992). Although the serological response to the bacteria is not high in chicks, aside from ND and IB, the current work showed that measurement of the immune response to *B. abortus* is a suitable, satisfactory method for assessing immunosuppression caused by cryptosporidiosis. In addition, the result suggests that the serum agglutination test may be applicable for the measurement of immunosuppression in field situations where it would not be possible to use other avian pathogens because of interferences of maternal antibody or natural infection to the test stimulant. Moreover, the serum agglutination test may have simple, practical advantages over the hemagglutination inhibition test in detection of ND and IB antibodies.

In the infected chicks, oocyst output in the excrements was presumed to show similar profiles to those of our previous study (Rhee *et al.*, 1998b). However, oocysts were not detected in the excremental samples of the intrinsic control and the uninfected chicks during this period.

Although infectious bursal disease had a significant effect on response to ND virus vaccination (Faragher *et al.*, 1974), it is corroborate that cryptosporidiosis leads to a significant reduction in the antibody response after vaccination with ND virus and IB virus based on the results from the present and previous studies (Rhee *et al.*, 1998a & 1998c). Taken together, the present study highlights immunosuppression attributed by cryptosporidiosis in early life stage of chicks, which is presumably the first consideration worldwidely. Therefore, we propose that cryptosporidiosis should be eradicated preferentially by proper sanitation and immunization to prevent important infectious diseases in poultry industry, even though there are no proven recommended programs. Finally, further work is required to determine whether the degree of immunosuppression could have a practical effect upon the severity of other avian disease.

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=초록=

닭와포자충 감염닭에서 *Brucella abortus*의 이차감염에 대한 면역저하 현상의 입증

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닭와포자충에 감염된 닭에서 다른 병원체의 이차 침입에 대한 면역억제능을 입증하기 위하여 2일령 SPF 병아리에 2×10^6의 닭와포자충 난포낭을 한 번에 경구투여한 다음 14일 후에 1×10^9의 *Brucella abortus* strain 1119-3 부유액 0.3 ml를 근육주사하였다. 그 다음 3-6일 간격으로 36일간에 걸쳐 채혈한 후 평판응집반응으로 이 세균에 대한 면역반응을 관찰하였다. 전반적으로 감염군과 비감염군 모두 3일 후까지는 이 세균에 대한 혈청응집반응이 전혀 일어나지 않았으나 그 후 혈청응집역가는 비감염군에 비하여 감염군이 의의있게 낮았다 ($P<0.05$). 세균 접종 15일 후 감염군과 비감염군에 있어서 최고 역가는 매우 의의있는 차이를 보였다 ($P=0.0001$). 한편, 진정대조군은 전 실험기간을 통하여 이 세균에 대한 혈청응집반응을 보이지 않았다. 이 연구결과 닭와포자충에 감염된 닭에서 미생물의 침입에 대한 면역반응이 억제됨을 확인하였다.

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