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☐ **Brief Communication** ☐

Immunosuppressive effect of *Cryptosporidium baileyi* infection on vaccination against avian infectious bronchitis in chicks

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Abstract: Two-day-old commercial chicks were inoculated orally with 2×10^6 oocysts of *Cryptosporidium baileyi* and vaccinated with $10^{3.5}$ EID_{50}/head of a commercially available avian infectious bronchitis (IB) live virus vaccine at 4 and 14 days following inoculation. Chicks infected with *C. baileyi* were shown to have an immunosuppressive effect on IB virus. It is concluded that infection with the protozoon in early life may increase their susceptibility to IB.

Key words: *Cryptosporidium baileyi*, avian infectious bronchitis, immunosuppressive effect, hemagglutination-inhibition test, vaccination

Cryptosporidiosis caused by *Cryptosporidium baileyi* is a protozoan infection that produces marked diffuse chronic superficial purulent bursitis with a mild to moderate depletion of lymphocytes in the lymphoid follicles of young chicks (Rhee *et al.*, 1997). We had shown previously that infection with *C. baileyi* in early life depressed the ability of the chicks to respond to sRBC (Rhee *et al.*, 1998b). Recent investigations have demonstrated that 2-day-old chicks initially infected with *C. baileyi* tended to reduce the immune response following vaccination against Newcastle disease (Rhee *et al.*, 1998a).

Since the incidence of cryptosporidiosis appears to be widespread among domestic poultry, it is thought that its effect on the susceptibility to other important poultry diseases should be further examined. Avian infectious bronchitis (IB) is an acute, highly contagious viral disease of the respiratory tract of chicks, causing high mortality in young chicks. It is characterized by a lightning-fast spread and occurs worldwide.

It has been demonstrated that infectious bursal disease virus induced an immunosuppression against challenge infection with IB virus in chicks at 1 or 5 days of age (Pejkovski *et al.*, 1979). The objective of this study was to determine the effect of cryptosporidiosis on vaccination against IB in chicks in the field because the effect of the former is largely unknown, with all both diseases have been prevailing in Korea as well as other parts of the world.

Two-day-old commercial chicks (Dekalb-Warren, Sex-Sal-Link, male) with maternal antibody to IB virus were each orally inoculated with 2×10^6 oocysts of *C. baileyi* (infected group), as previously described by Rhee *et al.* (1997). Oocysts have been maintained in our laboratory since 1990. Age-

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matched chicks were served as uninfected control group. Each bird in both groups was orally given $10^{3.5}$ EID_{50}/head of a commercially available IB live virus vaccine at 4 and 14 days postinoculation (PI), as recommended by the manufacture. Following inoculation, the feeding of chicks and the examination of fecal samples were referred to the methods previously described by Rhee *et al.* (1995 & 1996). Furthermore, 10 commercial chicks immediately after incubation were allocated into intrinsic control group (uninfected and unvaccinated) to monitor maternal antibodies to IB virus.

The serum samples were collected weekly from the same 10 chicks per stage in the intrinsic, the infected and the uninfected groups, respectively. The immune response to IB vaccination was estimated by hemagglutination inhibition (HI) levels determined by the method described by King and Hopkins (1983) using Massachusetts 41 IB antigen prepared by 4 hemagglutinating units. The experiment in the present study was repeated at least three times with similar results. HI levels to IB virus between the uninfected and the infected groups were analyzed by Student's *t*-test.

In the intrinsic control chicks, the mean log_2 based HI levels of maternal antibody to IB virus at one day-old were 5.16 ± 1.39, subsequently decreased, and reached to negative seroconversion at 6 weeks of age. A negative relationship between the ages of chicks and the levels of maternal antibodies was linear, and in agreement with the linear equation $Y = -0.90X + 4.67$, showing that the age (X) was a parameter ($R = 0.9454$). As presented in Table 1, the mean levels of HI to IB virus were, in general, significantly lower in the infected group chicks compared with the uninfected group throughout the experimental period ($p < 0.05$). The mean levels were gradually decreased with day post-booster-vaccination (PBV), dropped at the lowest grade at 2 weeks PBV, and then increasingly raised with somewhat constant profiles thereafter in both groups. Such diminutions of the mean levels in initial stages of both groups were nearly in line with those of maternal antibody of the intrinsic control chicks.

In the infected group, oocyst output in the excrements revealed profiles similar to those of the previous study of Rhee *et al.* (1998b). However, the oocysts did not show up in the excremental samples of the intrinsic and the uninfected groups during this period.

Although IB viruses perform well as vaccines with SPF chickens, it is well known that maternal antibodies to IB block vaccine viruses and reduce the immune responses when vaccination is carried out at few days of age (Klieve and Cumming, 1988; Calnek,

Table 1. Fluctuations in log_2 based hemagglutination-inhibition titer against avian infectious bronchitis vaccination in chicks infected with *Cryptosporidium baileyi*

Weeks postvaccination	Uninfected	Infected	Significance of difference (p)
0	5.16 ± 1.39	5.16 ± 1.39	—
1	4.85 ± 1.25	4.15 ± 1.20	0.0166
2	4.74 ± 1.00	3.83 ± 2.11	0.0178
3	4.48 ± 1.52	3.58 ± 1.10	0.0045
4	3.85 ± 1.24	2.85 ± 1.95	0.0064
5	4.70 ± 2.20	3.55 ± 1.96	0.0234
6	6.10 ± 1.02	4.65 ± 0.93	0.0001
7	5.95 ± 1.43	3.70 ± 1.38	0.0001
8	5.70 ± 1.83	3.35 ± 2.00	0.0004
9	4.80 ± 1.36	3.25 ± 2.07	0.0081
10	4.90 ± 0.99	3.70 ± 0.67	0.0054
11	4.00 ± 0.66	3.30 ± 0.67	0.0314

Chicks were inoculated with *C. baileyi* at two days of age and vaccinated with a commercially available IB live virus vaccine 4 and 14 days following inoculation. Each value represents the mean of triple repetition of ten determinations with the standard deviations.

1991). After booster-vaccination such a temporary depression of HI titer is supposed to be due to the interference phenomenon of maternal antibody. This suggests that the vaccines' efficacy is diminished when birds with maternal antibody are vaccinated with the virus of the same serological subtype. Overall, the data presented confirm reports that maternal antibodies reduce the activity of vaccine reaction.

Based on previous findings (Rhee *et al.*, 1997, 1998a & b) and the present result, it is thought that cryptosporidiosis has an immunosuppressive effect on important poultry disease such as Newcastle disease and IB owing to disturbance of the ontogeny of B-cells by hyperplasia and thickening of bursal epithelium and a mild to moderate depletion of lymphocytes in bursal lymphoid follicles. Therfore, the results give rise to suggestion that the infection with *C. baileyi* tends to increase the susceptibility of chicks to other important poultry diseases.

The mean HI titers of IB virus were kept up high during 20 to 60-week-old chicks ascribed to several vaccination against IB virus in Korea (Seong *et al.*, 1993). Moreover, the disease is characterized by epizootiological character, as previously described and has been prevailing in Korea (Rhee *et al.*, 1986). Hence, chicks with maternal antibody to IB virus were used as an experimental animal model for the present study to document immunosuppressive effect on other important poultry diseases of chicks at field and general poultry farms. Therefore, the outcome obtained from the present investigation can effectively execute disease prevention practices. It indicate that the managers of poultry flocks have to counteract the incidence of cryptosporidiosis initially by good managements prior to prevent important poultry diseases.

REFERENCES

Calnek BW (1991) Diseases of Poultry. 9th ed. p471-484, Iowa State University Press. Ames, Iowa, USA.

King DJ, Hopkins SR (1983) Evaluation of hemagglutination-inhibition test for measuring the response of chickens of avian infectious bronchitis vaccination. *Avian Dis* **27**(1): 100-112.

Klieve AV, Cumming RB (1988) Infectious bronchitis: safety and protection in chickens with maternal antibody. *Aust Vet J* **65**(12): 396-397.

Pejkovski C, Davelaar FG, Kouwenhoven B (1979) Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. *Avian Pathol* **8**: 95-106.

Rhee JK, Jang BG, Park BK (1995) Oocyst production and immunogenicity of *Cryptosporidium baileyi* in chickens and mallards. *Korean J Parasitol* **33**(1): 45-54.

Rhee JK, Kim HC, Lee SB, Yook SY (1998a) Immunosuppressive effect of *Cryptosporidium baileyi* infection on vaccination against Newcastle disease in chicks. *Korean J Parasitol* **36**(2): 121-125.

Rhee JK, Kim HC, Park BK (1996) Chronologic change of serum IgG antibody response in chickens reinfected with *Cryptosporidium baileyi*. *Korean J Parasitol* **34**(4): 255-258.

Rhee JK, Kim HC, Park BK (1997) Effects of *Cryptosporidium baileyi* infection on the bursa of Fabricius in chickens. *Korean J Parasitol* **35**(3): 181-187.

Rhee JK, Kim HC, Park BK (1998b) Effect of *Cryptosporidium baileyi* infection on antibody response to sRBC in chickens. *Korean J Parasitol* **36**(1): 33-36.

Rhee YO, Kim JH, Kim JH, *et al.* (1986) Outbreaks of infectious bronchitis in Korea. *Korean J Vet Res* **26**(2): 277-282.

Seong HW, Kim JH, Song CS, Mo IP, Lee JW, Kim SH (1993) Serological survey against several avian diseases in breeder farms in Korea. *RDA J Agri Sci* **35**(2): 604-611.

=초록=

닭에 있어서 닭와포자충 감염이 닭전염성기관지염 예방접종에 대한 면역억제 효과

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닭에 있어서 닭와포자충 감염이 다른 중요 가금 질병에 대한 체액성 면역억제 현상을 규명하기 위해 2일령의 보통 병아리에 2×10^6의 닭와포자충 난포낭을 한번에 경구 투여한 다음 4일과 14일 두 번에 걸쳐 닭전염성기관지염 생독백신을 접종하여 1주 간격으로 혈구응집억제반응으로 항체가를 측정하였다. 감염 및 비감염군 모두 평균 항체가는 점점 낮아져 2차 예방접종 후 2주에 최저에 이른 다음 점점 높아져 일정한 수준을 유지하였다. 일반적으로 평균 항체가는 비감염군에 비하여 감염군이 의의있게 낮았다 ($p<0.05$). 한편, 이 두 병원체를 접종하지 않은 진정 대조군의 1일령의 모체이행항체가 log_2 5.16 ± 1.39는 시간이 경과함에 따라 점점 낮아져 6주령에 이르러 완전히 음전하였다. 이는 직선적인 변화를 하였으며, 주를 함수 (X)로 한 $Y = -0.90X + 4.67$의 일차방정식에 잘 부합하였으며, 실측치와 추정치와의 상관계수는 $R = 0.9454$이었다. 닭와포자충 감염 병아리는 건강한 것에 비하여 닭전염성기관지염 바이러스에 대한 면역억제현상이 일어나 아마도 감수성이 증가할 것으로 생각된다.

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