

Immunosuppressive effect of *Cryptosporidium baileyi* infection on vaccination against Newcastle disease in chicks

Jae Ku RHEE*, Hyeon Cheol KIM, Sang Bork LEE and Sim Yong YOOK

Bio-Safety Research Institute, Chonbuk National University, Chonju 561-756, Korea

Abstract: Hemagglutination-inhibition titers (\log_2) to Newcastle disease (ND) virus were chronologically observed in chicks, which were orally inoculated with 5×10^5 oocysts of *Cryptosporidium baileyi* at 2 days of age and subsequently vaccinated with inactivated ND virus at 4 and 21 days postinoculation. In general, the titers were considerably lower in the infected chicks than those in the uninfected control throughout the experimental period ($p < 0.01$), and rapid negative seroconversions were observed in the infected chicks. The titers reached a peak on weeks 2 and 4 post-booster-vaccination in the control and infected chicks, respectively. Thus, *C. baileyi* infection was shown to have an immunosuppressive effect on ND vaccination when the agent was given to 2-day-old chicks. It is suggested that *C. baileyi* infection in chicks may increase the host susceptibility to ND virus.

Key words: *Cryptosporidium baileyi*, Newcastle disease, immunosuppressive effect, hemagglutination-inhibition test, vaccination, chicks

INTRODUCTION

It has been shown that a considerable number of microorganisms provoke an immunosuppressive effect in humans as well as animals. Regardless of well-known human immunodeficiency virus in humans, under circumstances not completely understood, infection with Marek's disease virus may increase the susceptibility of chicks to coccidial infection (Biggs *et al.*, 1968). Early exposure to infectious bursal disease virus (IBDV) in chicks has resulted in an increased susceptibility to Newcastle disease (ND)

(Faragher *et al.*, 1974), Marek's disease (Cho, 1970), and several bacterial infections with *Escherichia coli* and *Salmonella typhimurium* (Wyeth, 1975). In addition, Anderson *et al.* (1977) demonstrated that *Eimeria tenella* infection is more severe when chickens are infected with IBDV at one-day-old.

Guy *et al.* (1988) and Snyder and Current (1987) intimated that *Cryptosporidium baileyi* infection appeared to enhance reovirus replication in dually infected chickens. They suggested that cryptosporidiosis may potentiate the pathogenicity of reovirus infection and the possibility of immunologic modification in chickens. Therefore, it is supposed that impairment of the bursa of Fabricius (BF) by *C. baileyi* will result in defects on the ontogeny of humoral immune response and will render chicks vulnerable to other infections.

As a series of studies to elucidate the immunosuppressive effect of *C. baileyi*

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* Corresponding author (e-mail: jkr@moak.chonbuk.ac.kr)

infection on humoral immunity to other infections in two-day-old chicks, we have shown previously that *C. baileyi* infection had an impact on the histopathologic findings in BF and that antibody response to sRBC in chicks was depressed by *C. baileyi* infection (Rhee *et al.*, 1997 & 1998). The objective of this study was to determine whether the immunosuppressive effect of *C. baileyi* infection directed against ND vaccination in chicks.

MATERIALS AND METHODS

Cryptosporidium baileyi oocysts used in the present study were previously described (Rhee *et al.*, 1997). Two-day-old SPF chicks (Dekalb-Warren, Sex-Sal-Link, male) were each orally inoculated with 5×10^5 oocysts of *C. baileyi* (experimental group). Meanwhile, age-matched chicks served as the uninoculated control group. Each bird in both groups was given an intramuscular injection of the field dose of a commercial inactivated ND vaccine (gel adsorbed) at 4 and 21 days of postinoculation (PI). Following inoculation, the feeding of chicks and the examination of fecal samples were subjected to the methods previously described by Rhee *et al.* (1995 & 1996). And, each of ten additional one-day-old SPF chicks free of maternal antibody to ND virus served as an intrinsic control (uninfected and unvaccinated).

The serum samples were collected at a week intervals from ten chicks per stage in both groups throughout the experimental period, and the antibody response to ND vaccination was estimated by hemagglutination-inhibition (HI) levels. The HI titer was determined by the method of Beard *et al.* (1975). In brief, 25 μ l of phosphate buffered saline was added into a U-bottom 96 well microtiter plate. Twenty-five μ l of inactivated test serum was added to each well in A1 to G1 lines and the same volume of positive serum to each well in H1 line, then the volumes transferred were diluted in wells of the plate in two-fold steps from 1:2 to 1:1,024. Twenty-five μ l of antigen, inactivated ND virus (LaSota strain) prepared by 4 hemagglutinating units, was added into each well, and then the plate was agitated by a

micromixer. Following sensitization for 20 min at room temperature, 25 μ l of 1% chick RBC suspension was added to each well. The plate was covered by a tray and left at room temperature for 40 min. HI titers were defined as follows giving a visible agglutination-inhibition: agglutination-inhibition refers to falling of chick RBC as a tight button to the bottom and agglutination occurs as a spread of aggregates around the bottom of the well.

In the present study, each trial was repeated at least three times with similar results. HI titers to ND virus between the infected and the control group were analyzed by Student's *t*-test.

RESULTS

In general, the HI levels in Table 1 show a significantly higher response in the control chicks. In the experimental chicks infected with *C. baileyi*, the HI response is lower than that of the control ($p < 0.01$), which indicates a greater degree of immunological impairment in the infected chicks.

The HI levels gradually increased with days after booster-vaccination, peaked at 2- and 4-week post-booster-vaccination in the control and the experimental group, respectively, and decreased thereafter. The period for negative seroconversion in the experimental chicks was slightly shorter than in the control. The sera of intrinsic control chicks obtained at one day of age had no antibodies to ND virus (Table 1).

In this case, oocyst shedding in droppings was likely to exhibit profiles similar to those of previous study in chicks, as previously described and figured by Rhee *et al.* (1998). The oocysts did not show up in fecal samples of the control chicks during this period.

DISCUSSION

Newcastle disease, an acute, highly contagious viral disease of chicks and other birds, is characterized by respiratory, nervous and digestive disorders and often shows high mortality. The disease has been considered to cause worldwide economic losses in the poultry industry, and has been prevailing in Korea since 1929. The virus sometimes causes

Table 1. Fluctuations of hemagglutination-inhibition titers (\log_2) after vaccination against Newcastle disease in *Cryptosporidium baileyi* infected chicks^{a)}

Weeks postinoculation	Uninfected	Infected	Significance of difference (p)
0	0	0	
2	1.90 ± 0.3162	1.40 ± 0.5026	0.0078
3 ^{b)}	2.40 ± 0.5163	1.90 ± 0.3077	0.0024
4	3.00 ± 0.0000	2.40 ± 0.5026	0.0008
5	5.00 ± 0.0000	3.66 ± 0.4923	0.0001
6	4.47 ± 0.5163	3.65 ± 0.4925	0.0001
7	4.60 ± 0.5070	3.88 ± 0.6658	0.0009
8	4.40 ± 0.5163	3.30 ± 0.4701	0.0001
9	4.20 ± 0.6761	3.36 ± 0.4898	0.0001
10	3.40 ± 0.5163	2.75 ± 0.4442	0.0012
11	2.90 ± 0.3162	2.72 ± 0.4608	0.2891
12	1.80 ± 0.4216	1.82 ± 0.3929	0.8848
13	0.30 ± 0.4830	0	0.0155

^{a)}Chicks were inoculated with *C. baileyi* oocysts at two days of age and vaccinated primarily with ND vaccine four days following inoculation. ^{b)}Each bird was given a booster injection of ND vaccine. Each value represents the mean of triple repetition of ten determinations with the standard deviations.

conjunctivitis in humans (Timoney *et al.*, 1992). While IBDV infection exerts an immunosuppressive effect in young chicks on vaccination against ND virus infection (Faragher *et al.*, 1974), the effect of cryptosporidiosis on vaccination of chicks against ND virus infection has not been investigated. Meanwhile, Rhee *et al.* (1991) reported that *C. baileyi* oocysts were detected in 2-54% (15% on the average), depending on chickyards, of four-week-old chicks in Korea.

In the present study, *C. baileyi* infected chicks had a significantly ($p < 0.01$) lower antibody response to vaccination against ND virus throughout the experimental period. This may be due to functional defects of BF, which might result from *C. baileyi* infection. In a previous study, we reported that *C. baileyi* infection caused a disturbance of the ontogeny of B cells by hyperplasia and thickening of bursal epithelium and a mild to moderate depletion of lymphocytes in bursal follicles (Rhee *et al.*, 1997). IBDV causes severe immunosuppression in young chicks by destroying immature B lymphocytes within the BF (Kibenge *et al.*, 1988). We have also demonstrated that *C. baileyi* infected chicks exhibited low hemagglutinin titers to sRBC (Rhee *et al.*, 1998). Taken together, *C. baileyi*

has an immunosuppressive effect and thus renders the host vulnerable to vaccine agents by mediating low antibody response.

It is possible that an increased incidence of ND among chicks in Korea is due to cryptosporidiosis, despite a routine vaccination program both at field and farm levels against ND (Seong *et al.*, 1993). On a block basis, the effect, for example, may be of significance in maintaining foci of infection with ND virus.

Since inapparent cryptosporidiosis is widespread among domestic poultry in Korea (Rhee *et al.*, 1991) and the impairment of BF in chicks by *C. baileyi* with concomitant decrease in humoral immunity may correlate with the increase of susceptibility to other pathogens, their effect on the incidence and severity of other important poultry diseases should be further examined.

With the widespread incidence of inapparent cryptosporidiosis and ND in chick flocks, this results suggest that potential field problems can only be counteracted by good management and disease prevention practices.

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=초록=

닭와포자충 감염이 닭의 뉴캐슬병 예방접종에 대한 면역억제 효과

이재구, 김현철, 이상복, 육심용

전북대학교부속 생체안전성연구소

닭와포자충 감염이 다른 병원체에 대한 닭의 체액성 면역에 미치는 영향을 규명하기 위한 연구의 일환으로 2일령 SPF 병아리에 5×10^5 의 닭와포자충 오오시스트를 한 번에 경구투여한 다음 4일과 21일 두 번에 걸쳐 뉴캐슬병 불활화 (사독) 백신을 접종하였다. 오오시스트 접종 후 2주부터 1주 간격으로 13주까지 채혈하여 혈구응집억제반응으로 ND HI \log_2 역가를 경시적으로 측정하여 대조군과 비교, 검토하였다. 일반적으로 뉴캐슬병 바이러스에 대한 HI가는 전 실험기간을 통하여 대조군에 비하여 실험군이 상당히 낮았으며 ($p < 0.01$), 보다 빨리 음전하는 경향이였다. 백신의 보강주사 후 역가는 점점 높아져서 2주 (5.00 ± 0 ; 비감염 대조군)와 4주 (3.88 ± 0.6658 ; 감염 실험군)에 최고치에 이른 다음 점점 낮아졌다. 한편, 분변 내의 오오시스트 배설 양상은 통상적인 감염례와 같았다. 이로 미루어 보아 병아리가 닭와포자충에 감염되면 건강한 병아리에 비하여 뉴캐슬병 바이러스에 대한 면역억제 현상이 일어나 감수성이 증가할 수 있을 것으로 생각된다.

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