Arq. Bras. Med. Vet. Zootec., **v.58, n.3, p.327-332, 2006**

Antibodies specific to infectious bronchitis in broilers in Ceará state, Brazil

[*Anticorpos específicos para bronquite infecciosa em frangos de corte no Ceará*]

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ABSTRACT

The experiment was carried out to determine the antibody levels to infectious bronchitis virus (IBV) in 1120 broilers of two broiler flocks, both from the same parental flock and free from previous vaccination. Forty chicks of each line were alloted to the control group and the sera were tested by indirect ELISA. The vaccination program consisted on the administration of commercial vaccines against IBV at 10 and 25 days of age. Chicks with low levels of maternal antibodies (Mab) did not show significant titers to the first vaccinal stimulus. They presented a vaccinal response to the second vaccinal stimulus reaching the top around GMT 1100 at 45 days. Chicks with high Mab titers did not show significant titers to the primary and secondary vaccinal stimuli, reaching peak levels of GMT 500 at 45 days. No antibody response was detected after the primary vaccination at day 10. A delayed antibody response was detected after the secondary vaccination on day 25, indicating no previous priming. The maternal antibody titers can interfere on the response to the first and second vaccinal stimulus promoting the neutralization of the first vaccination and a different response to the second one, according to high or low maternal antibodies.

Keywords: broiler, infections bronchitis, antibody

RESUMO

Utilizaram-se 1120 pintos de um dia de idade, de duas linhagens, não vacinados, para determinar os níveis de anticorpos para o vírus da bronquite infecciosa (VBI) em frangos de corte no estado do Ceará. Quarenta aves de cada linhagem, colocadas em boxes isolados e não vacinadas, foram usadas como controle. As aves vacinadas contra VBI aos 15 e 25 dias foram submetidas a coletas de sangue periódicas para avaliação, pelo ELISA indireto, dos títulos de anticorpos para VBI. As aves com baixos títulos de anticorpos maternos (AcM) não apresentaram títulos significativos ao primeiro estímulo vacinal; para o segundo estímulo, o pico de resposta de GMT 1100 ocorreu aos 45 dias. As com elevado título de AcM não responderam significativamente à primeira vacinação e o pico de resposta ao segundo estímulo de GMT 500 ocorreu aos 45 dias. Não se verificou resposta de anticorpos para o primeiro estímulo vacinal, observando-se resposta tardia somente para o segundo. Os AcM podem ter interferido tanto no primeiro quanto no segundo estímulo, promovendo neutralização da primeira vacinação e resposta diferenciada para a segunda de acordo com o nível, elevado ou baixo, de AcM.

Palavras-chave: frango de corte, bronquite infecciosa, anticorpos

Recebido em 4 de julho de 2003
Aceito em 4 de maio de 2006
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INTRODUCTION

Infectious bronchitis (IB) was described for the first time by Schalk and Hawn in 1931, in the state of Dakota in the United States of America (Delaplane and Stuart, 1941) and since its discovery the IB is the major cause of disease in domestic chickens. It is endemic in the chicken populations throughout the world (Cunningham, 1970; King and Cavanagh, 1991).

Infectious bronchitis virus (IBV) is a positive sense, single-stranded RNA virus and the prototype member of the Coronaviridae family. The virus is a highly contagious pathogen of poultry that causes very significant morbidity and mortality. IBV primarily targets the respiratory tract, which can then predispose the bird to secondary bacterial infections. Other strains of IBV target the kidney or/and oviduct, resulting in nephritis and reduced egg production, respectively (King and Cavanagh, 1991; Cavanagh and Naqi, 1997).

IBV was isolated in Brazil for the first time by Hipólito (1957), identified a serotype Massachusetts isolate. The control of IB in the Brazilian poultry industry has been attempted with Massachusetts vaccine strains. However, Silva (1990) reported the existence of Massachusetts, Connecticut, JMK, Gray, Holte and Arkansas 99 isolates. IB can be controlled by vaccination of chicken flocks; nevertheless, outbreaks still occur in vaccinated flocks due to the lack of cross-protection against antigenically unrelated serotypes and variant strains of the virus (Gelb et al., 1991; King, 1988; Capua et al., 1994; Jia et al., 1995). Early diagnosis is essential to effective control of an outbreak. Vaccines with live or inactivated virus are used in the poultry (Cavanagh and Naqi, 1997). One dosis of 10^6 EID_{50} administered intraocularly induces antibody response in the tear and in the serum of vaccinated fowls (Toro et al., 1997).

Early study by Jungherr et al. (1956) showed that there are sufficient immunological differences among the strains; so that cross-protection would not occur. Other investigations showed that vaccines can elicit protection against some field challenges (Davellar et al., 1984; Parson et al., 1992; Afanador, 1994; Wang et al., 1997). Wang et al. (1996) showed that this occurs in function of the genetic variability of originating variant strains. Many authors propose that the inefficiency of the vaccination programs is due to the large diversity of antigenically different strains; because IBV presents the phenomenon of genetic recombination or the virus can suffer a mutation, generating new strains (Kouwenhoven and Davellar, 1989). Variant strains of IBV have been recovered from vaccinated flocks despite the use of combinations of several strains of live and attenuated IBV vaccines (Gelb et al., 1991).

The diagnosis of IB is based on the clinical signs, history, lesions, seroconversion or antibody levels, virus isolation and, more recently, by detection of viral RNA (Cavanagh and Naqi, 1997). The serology is efficient to the diagnosis of IBV in function of its specificity and it is based on the demonstration of ascending antibody titers in the serum, taking the first sample in the initial phase and the second, two or three weeks later (Villegas and Avellaneda, 1994). The existence of different IBV types can interfere on the results by serology; so it might be necessary to employ other serotypes. The ELISA is possibly the most frequently employed assay to measure IBV specific antibody levels nowadays.

The objective of the present study was to determine the curve of IBV specific antibodies through indirect ELISA test, in two lines of broilers reared in the Ceará State, Brazil.

MATERIALS AND METHODS

In order to determine the antibody levels in broilers vaccinated against IBV, 1120 one day-old chicks of two mixed lines from one flock of breeders and free from previous vaccination were used. Forty chicks from each of the two lines were used as control (these groups received no treatment and were placed in broiler house 1- H1), the remaining 1040 were placed in broiler house 2- H2. The boxes of H2 were all isolated. The H1 and H2 were completely isolated by an exterior fence covered with nylon curtain. These lodgings were located 60 meters apart and each lodging had its own personnel. The H2 was divided into 32 divisions of $2.25m^2$ each, to which 27 chicks/line were randomly allotted. This distribution resulted in a density of 14.22 fowls/m^2.

Chickens were organized into four groups as follows: A1 - line A, unvaccinated; B1 - line B, unvaccinated; A2 - line A, vaccinated; B2 - line B, vaccinated.

The weight and the mortality rates were observed weekly. The animals were fed on a balanced diet, according to the Nutrition Research Council. The vaccination program was performed as indicated in Table 1.

Table 1. Vaccination program against infectious bronchitis in Ceará

Age (days)	Vaccine	Strain and vaccinating route
1st	Infectious bursal disease	D_{78} / Intermediate intraocularly
8th	Newcastle disease	HB1 intraocularly
8th	Infectious bursal disease	D_{78} / Intermediate intraocularly
10th	Infectious bronchitis	H_{120} intraocularly
23rd	Infectious bursal disease	D_{78}/ Intermediate intraocularly
23rd	Newcastle disease	La Sota, intraocularly
25th	Infectious bronchitis	H_{52} intraocularly

The titer of the first IBV vaccine (H-120) was $EID_{50\%}$ $10^{3,2}$ and the second vaccine (H-52) was $EID_{50\%}$ $10^{3,5}$. All the vaccines were produced by the same laboratory and were administered by the same personnel.

The blood samples in all groups were colleted according to the following methodology: at hatching (n=25) and at every five days (n=25) until 50 days of age, resulting 275 samples of serum/group, and 1100 serum samples of the total. Total IBV antibodies were measured in individual sera and lavage fluids using a commercial kit of indirect ELISA[1], as based on manufacturer's conversion tables for converting optical density, and the titers were demonstrated in geometric mean of titers (GMT).

The titers were submitted to normality tests and the variance analysis through the SAS (User`s… 1995). As the number of samples in each treatment was not balanced, the analysis by linear geometric mean was applied. In the analysis of variance, a possible interaction between the poultry company and the treatment was considered. GMT were calculated using the standard procedures (Villegas and Purchase, 1989), whereas the mean titers for the treatment groups were compared using the Student's t-test (Bishop, 1966).

RESULTS

Fig. 1 shows the maternal antibody (Mab) titers of the unvaccinated groups (A1 and B1) and Fig. 2 shows the maternal and vaccinal antibody titers to vaccinated groups (A2 and B2).

The A1 group presented low Mab titers in the first day, around GMT 200. Titers decreased to zero at approximately the 5th day of age and remained around zero until the end of the experiment.

The B1 group presented high passive antibody titers in the first day, around GMT 5500. Titers decreased to zero around the 15th day of age, and remained zero until the end of the experiment.

The fowls of the A2 group presented Mab of GMT 400, which decreased to zero at approximately the 5th day of age and remained with basal titer levels up to 40 days of age, and increased after that, reaching the peak around GMT 1100 at 45 days. A decrease in the titers (GMT 500) was detected by 50 days of age, when the experiment finished.

The fowls of the B2 group presented Mab of GMT 5500. The titers decreased and reached zero by day 15, remaining at this level up to day 35, when there was an increase of titers, reaching the peak at 45 days around GMT 500 and decreased to around GMT 300 by 50 days, at the end of the experiment.

[1] Kirkegaard and Perry Laboratories, USA

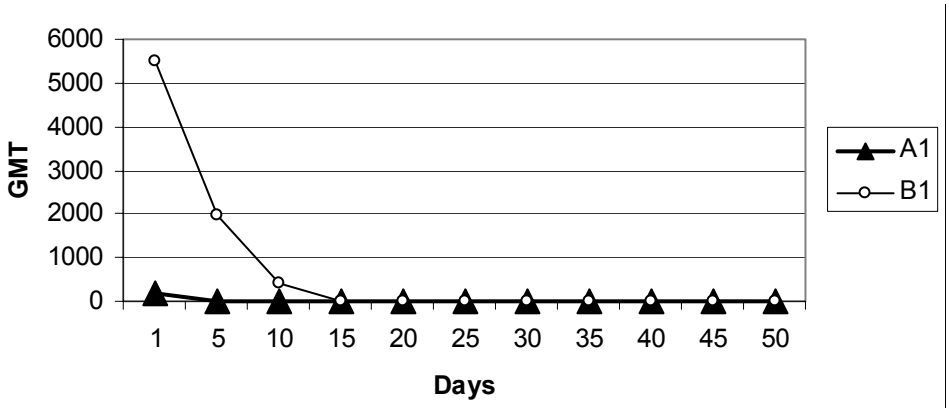

Figure 1. Maternal antibody titers (geometric means of titers - GMT) of unvaccinated groups of broilers in Ceará

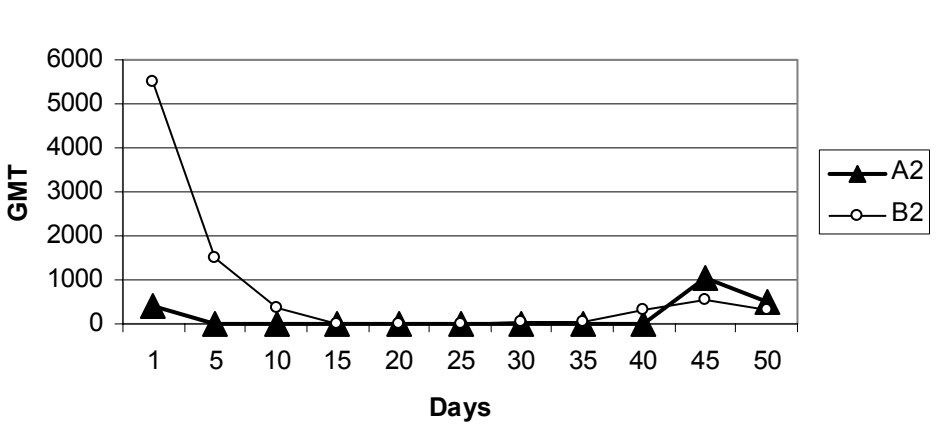

Figure 2. Maternal and active antibody titers (geometric mean of titers - GMT) of vaccinated groups of broilers in Ceará

DISCUSSION

The A1 group presented low Mab titers, in the first day, around GMT 200. Some authors observed Mab levels in broilers with a variation between GMT 3000 and 5000 (Villegas and Avellaneda, 1992) being similar to B1 Mab levels, around GMT 5500 (Fig. 1). The difference of Mab levels can occur by factors like the vaccinal strains, vaccination programs, production systems and bird line. Mondal and Naqi (2001) showed the importance of high Mab titers in chicks challenged with IBV, observing that chicks which hatched with high Mab levels had excellent protection (>95%) against IBV, challenged at one day of age, but not at seven days (<30%).

The results showed that the initial Mab levels interfere on the persistence of antibody titers. The B1 group reached basal levels by 15 days (Fig. 1). These results are in accordance with the ones by Mondal and Naqi (2001), that studied the role of IBV maternal antibodies on the development of active immunity to vaccine, finding that the Mab declined gradually, remaining on perceptible levels up to 17 days. Darbyshire and Peters (1985) observed Mab that decayed linearly with a mean half-life of five to six days. However A1 reached basal levels by five days. The explanation to this fast titer

decrease can be the low levels of initial Mab titers in this group.

The A2 and B2 group birds presented no appreciable antibody response to the first vaccinal stimulus (10^{th} day) by the vaccine $H_{120,}$ in spite of low serum Mab by 10 days. However Ladman et al. (2002) observed IBV vaccinal serum response (GMT) ranging from 184 to 1354, 2-week-old chicks vaccinated with attenuated IBV strains, including H_{120}, but it is important to observe that they used IBV Mab-free chicks. The Mab levels may interfere directly with the response to vaccination, aspects of which have been the subject of a number of investigations (Winterfield et al*.,* 1976; Davelaar and Kouwenhovem, 1977; 1980; Davelaar et al*.,* 1982; Marius et al*.,* 1983).

It is known the existence of IBV systemic and local Mab, the first one is responsible for protection against spread of IBV from the respiratory tract to internal organs via blood stream and the local Mab (respiratory tract) are responsible for protection against IBV challenge (Modal and Naqi, 2001). The vaccinal virus neutralization was probably performed by local Mab, which were not assessed in this experiment.

Maternal antibodies are chiefly of the IgG class and become systemic after the intestinal absorption of the yolk. IgG from the mother can be found in the respiratory tract of the progeny, but its role in the protection, as compared to the IgA, is less efficient.

Both vaccinated groups responded to the secondary vaccinal stimulus (25^{th} day) by the vaccine $H_{52.}$ It is interesting to observe that A2 presented a higher antibody response, around GMT 1100, in comparison to B2, around GMT 500, as observed by day 45. The superior response of A2 is considered to be associated to the initially antibody titer (passive). Similar results have been found in the literature, such as a better secondary vaccination response in antibody free flock, subjected to vaccination at days 1 and 21 of age (Mondal and Naqi, 2001)

The Mab levels are important parameters related to immunity against IBV infections. In this experiment, it was observed that the Mab influenced on IBV vaccinations, which are essential to the control of IBV infections. According to De Herdt et al. (2001), economic losses associated with IBV infections in broilers occurred predominantly in flocks hatched with low and erratic maternal antibody titers, concluding that IBV vaccination strategies should aim at high and uniform antibody titers in the broiler breeders. On the other hand, for the adequate vaccination, Mab antibodies titers should be taken into account, in order to allow a proper vaccinal infection.

CONCLUSIONS

No antibody response was detected after the primary vaccination at day 10. A delayed antibody response was detected only 25 days after the secondary vaccination on day 40-45, indicating poor or no previous priming. The maternal antibody titers can interfere on the response to the first and second vaccinal stimulus promoting the neutralization of the first vaccination and a different response to the second one, according to high or low maternal antibodies.

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