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PROTECTION OF CHICKENS WITH MATERNALLY DERIVED ANTIBODIES AFTER CHALLENGE WITH VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS

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The protective value of maternally derived antibodies in broiler breeders and broilers was tested after challenge with very virulent infectious bursal disease virus. Chickens were infected on the 14th and 10th day of age respectively. According to the pathohistological lesions in the bursa of Fabricius protection was poor, namely 21.4% in both experiments. The primary antibody response after challenge measured by virus neutralisation test (VN) was correlated with the extensive pathohistological lesions in the bursa of infected chickens.

Key words: infectious bursal disease virus, maternal antibodies, pathohistology,

INTRODUCTION

Infectious bursal disease (IBD) is an important poultry disease because it is accompanied with mortality, high morbidity, immunosuppression and susceptibility of chickens to secondary bacterial and viral infections. The very virulent strains that emerged in the field about 13 years ago caused up to 30% mortality in broilers and up to 80% mortality in layer chickens (Chettle and Wyeth 1989, van den Berg *et al.* 1991).

The causal agent is infectious bursal disease virus (IBDV), a double stranded, bisegmented, non enveloped RNA virus that belongs to the Birnaviridae family (Dobos *et al.*, 1979). IBDV causes inflammation in the bursa of Fabricius followed by atrophy of the organ 7 days after infection (Winterfield *et al.*, 1972). The only way to cope with the disease is vaccination. From 1980 a strategy has been developed to treat parent flocks with oil emulsion vaccines in order to protect their progeny with maternal antibodies (Wyeth and Cullen, 1978). However, the protective ability of the maternal antibodies could be abolished due to the virulence of the challenge virus (van den Berg and Muelemans, 1991b).

Most published papers have dealt with the possibility to overcome a certain level of maternal antibodies by vaccine strains (Wyeth *et al.*, 1981, Lucio and Hitchner, 1979, Ide, 1979, Wyeth 1980, Wood *et al.*, 1981, Giambrone and Yu, 1982, Naqy *et al.*, 1982, Solano *et al.*, 1986, Gagić *et al.*, 1994, Gagić *et al.*, 1994, Lutticken 1997). However, it has been described that carefully selected vaccines given at the 14th day of age did not protect broiler chickens and layer pullets in terms of mortality and bursal lesions (van den Berg and Meulemans, 1991b) after

infection with very virulent IBDV. Therefore pathogenic strains can break through the antibody barrier and that creates a problem about how to vaccinate the progeny. In these experiments broiler breeder chickens and broilers were infected with very virulent IBDV, isolated in Yugoslavia. Our goal was to test the protective value of maternal antibodies, to measure the development of virus neutralisation antibodies (VN) 7 days after challenge with IBDV and to examine the relationship between antibody development with pathological lesions in the bursa of Fabricius.

MATERIAL AND METHODS

Chickens : Chickens from a broiler breeder flock (provenience Hybro) and one day old broiler chickens were brought to the Institute at day old. They were held in isolation. Water and feed were given ad libitum until the termination of the experiment.

Viruses: The virus CH/99 was used for the challenge test. CH/99 was isolated from IBDV outbreak in a flock of 6 week old laying pullets. Bursa homogenate was prepared in 1:10 ratio with PBS (pH 7.4) and inoculated in 5 week old broiler chickens that did not have detectable IBDV antibodies (tested by VN test). After 3 days, when 10% of the chickens had died, bursae from survivors were collected and inoculum for these experiments was prepared as described above.

Serology: The virus neutralisation test and AGP test were performed, according to Gagic *et al.*, 1995. The vaccine strain D-78 was used In the VN test. The titre of the virus was 10^5 TCID/50 in 1ml.

Histopathological examination: Bursae of Fabricius, removed from infected chickens that had been previously sacrificed, were collected, fixed in 10% buffered formalin and embedded in paraffin as described earlier (Aleksić-Kovačević *et al.*, 1999). Bursal sections were stained with hematoxylin and eosin. All examined bursal follicles with less than 20% lymphocyte depletion were considered healthy. Otherwise lesions in the bursa were scored as follows: 20% depletion was given the score 1, 20-50% depletion was assigned score 2, 50- 70% depletion was scored 3 and 70-100% depleted follicles were scored 4. The control group included 5 chickens. Their bursae were sampled and processed in the same way.

Experimental design: Two experiments were done. In the first experiment one day old broiler breeder chickens were marked and on the 14^{th} day of age infected with CH/99 IBDV virus by vent drop inoculation. Seven days after challenge, blood samples were taken from each chicken and, after they had been sacrificed bursae were taken for pathohistological examination. The second experiment was done in the same way, except that broiler chickens were used from a different hatchery and were challenged on the 10^{th} day of age.

RESULTS

One chicken died in the first experiment three days after infection with CH/99. The antibody titre at the time of challenge ranged from 128 to 4096. However, in most of the chickens atrophy of the bursa was noticed and a high score of pathohistological lesions was recorded. Chickens with antibody titres lower than 2048, experienced a rise in antibody titre 7 days post infection. One chicken (number 13) had lesions in the bursa (score 4) in spite of a relatively high antibody

titre at the time of challenge (Table 1). The antibody titre continued to fall 7 days after infection in chickens number 10, 12, and 14. All these chickens had low bursal scores (1-2).

Table 1: Antibody titer for IBDV in broiler breeder chickens and bursa scores seven days after infection with very virulent virus

Chick . No.	Ab titer upon infection	Bursa score	Ab titter 7 DPI	Chick . No.	Ab titer upon infection	Bursa score	Ab titer 7 DPI
1	128	4	4096 ↑	8	1024	4	1024
2	128	4	512 ↑	9	1024	4	4096 ↑
3	512	4	4096 ↑	10	2048	2	1024 ↓
4	512	died*		11	2048	4	> 4096 ↑
5	512	4	4096 ↑	12	4096	2	256 ↓
6	512	4	4096 ↑	13	4096	4	2048 ↓
7	1024	4	1024	14	4096	1	512 ↓

*chick died three days after infection with vv IBDV

Similar results were obtained in the second experiment where broiler chickens were infected on the 10th day. The antibody titre ranged from 128 to 8092.. Atrophy of the bursa was detectable in most of the chickens and pathohistological examination showed destruction of almost all follicles (score 3-4) in 10 chickens. A higher antibody titre 7 days post challenge could not be detected in chickens number 8, 10, 11, 12, 13 and 14, although the bursal score ranged from 2-4 (Table 2).

Table 2. Antibody titer for IBDV in broiler chickens and bursa score seven days after infection with very virulent virus

Chick No.	Ab titer upon infection	Bursa score	Ab titer 7 DPI	Chick No.	Ab titer upon infection	Bursa score	Ab titer 7 DPI
1	128	4	4096 ↑	8	512	3	256 ↓
2	128	4	4096 ↑	9	512	4	1024 ↑
3	128	3	4096 ↑	10	1024	4	1024
4	128	4	1024 ↑	11	1024	4	128 ↓
5	128	4	512 ↑	12	2048	4	128 ↓
6	128	4	256 ↑	13	4096	4	1024 ↓
7	128	4	8092 ↑	14	8092	2	4096 ↓

Microscopic lesions

On the seventh day of infection in parallel with depletion and atrophy of follicles in the medulla, cystic cavities were found (Figure 1).

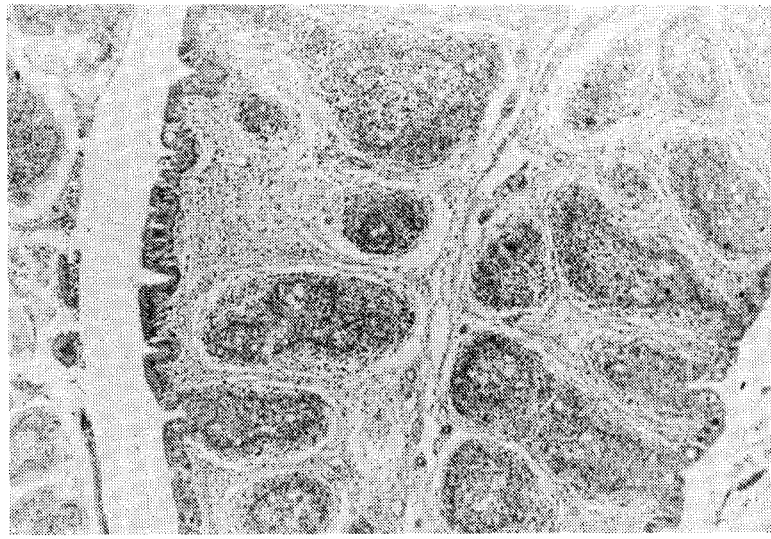

Figure 1. Bursa of Fabricius, sampled 7 days post infection. Cystic cavities, depletion and atrophy of follicles.

Necrosis in the medulla was detectable as well as phagocytosis. The most prominent change in the follicles was depletion, and some of the lymphocytes showed apoptosis. At the same time, proliferation of connective tissue was observed (Figure 2).

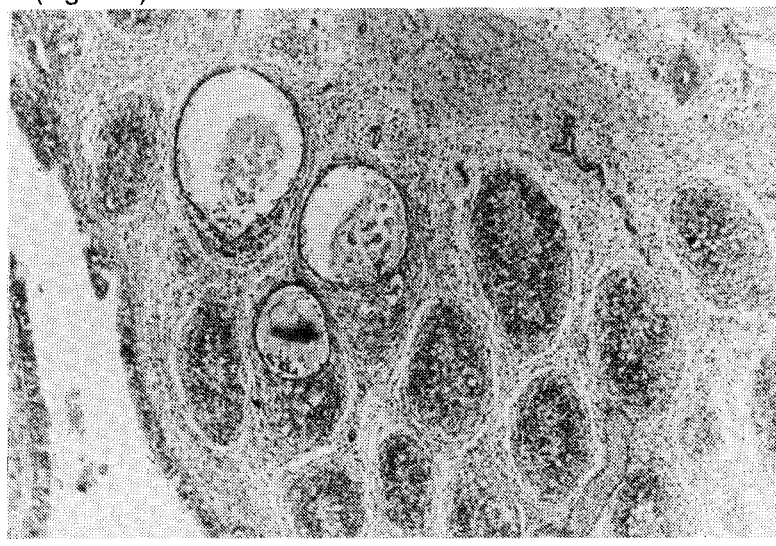

Figure 2. Bursa of Fabricius, sampled 7 days post infection. Necrosis in medulla and proliferation of connective tissue.

DISCUSSION

The most important result from these experiments is that broiler breeder chickens at 14 days old as well as broiler chickens at 10 days old were poorly protected from the challenge with a very virulent IBDV strain CH-99. In the first experiment one chicken died but in both groups the bursa was significantly damaged in most of the chickens (score 4). According to pathohistological data, protection in the first and second experiment was 21.4%. These results differ from the findings of Lucio and Hitchner (1980) who observed that maternal antibodies could protect bursa from atrophy after infection with pathogenic IBDV strain 73688, 7 and 14 days post challenge. Our experiment differs from the above mentioned in four points: 1) Lucio and Hitchner administered passive antibodies to 6 day - old chickens, 2) the challenge was done with a classical IBDV strain that does not belong to the group of very virulent IBDV strains, 3) pathogenic virus was administered occularly and 4) the authors used the bursa/body index to measure bursa atrophy.

It is known that maternal antibodies can suppress the active immune response of the chickens (reviewed by Lasher and Shane, 1994). In these experiments a primary antibody response after challenge could be detected within 7 days in most of the chickens that had an original antibody titre lower than 1024. The bursa from these chickens was depleted of lymphocytes (score 4).

Bursae from chickens with high levels of maternal antibodies were protected from the lymphocidal effect of the pathogenic virus, showing that pathohistological data were in good correlation with protection. Consequently, the delay in antibody development in those chickens might be connected with the influence of maternal antibodies on secondary viremia that develops right after IBDV replicates in the bursa (Weiss and Kaufer-Weiss, 1994).

Our data confirm the findings of van den Berg and Mulemans (1991b) and indicate the need for multiple vaccination in the field preferably as described by Woodger (1990). Also, the need for early vaccination in such circumstances, as underlined by Winterfield *et al.* (1980) with selected IBDV vaccines of intermediate virulence is recommended.

Upon pathohistological examination the most prominent change in the bursal follicles was depletion. Parallel with the described changes in the bursae, some of the follicles from the chickens used in these experiments showed signs of repopulation. This is important because Winterfield *et al.*, (1972) noted that bursae, damaged by pathogenic viruses, never reached normal size. Furthermore, Kim *et al.* (1999) found that repopulating with lymphocytes in the bursae occurred faster after infection of SPF chickens inoculated with vaccine virus compared to infection with the pathogenic strain IM. According to Edwards *et al* (1982) after severe damage of the bursa architecture, 50% of full repopulation is necessary to obtain a normal follicular structure. Whether maternally immune chickens are under immunosuppression after infection with the very virulent strains has to be elucidated in the future.

In conclusion, the results of the experiments provide evidence that 10 day old broiler chickens and 14 day old broiler breeders were poorly protected against infection with vvIBDV, strain CH/99. A primary antibody response occurred in chickens with extensive pathohistology lesions 7 days after infection. However, when compared with the results published by authors who used other IBDV

strains, this experimental model with maternally immune chickens does not give a clear difference between classical and vvIBDV.

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ZAŠTITNA VREDNOST MATERNALNIH ANTITELA POSLE INFEKCIJE PILIĆA VRLO VIRULENTNIM VIRUSOM INFEKTIVNOG OBOLJENJA BURZE

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja zaštitne vrednosti maternalnih antitela kod brojlerskih roditelja i brojlerskih pilića posle infekcije vrlo virulentnim virusom infektivnog oboljenja burze. Pilići su inficirani 14. i 10. dana života a na osnovu patohistoloških lezija u burzi Fabricii zaključeno je da je zaštita pilića bila slaba (21,4% u oba eksperimenta). Primarni imunološki odgovor posle infekcije, ustanovljen virus neutralizacionim testom, je bio u visokoj korelaciji sa patohistološkim lezijama ustanovljenim u burzama inficiranih pilića.