The Relative Levels of Neutralizing and Precipitating Antibodies Against Infectious Bursal Disease Virus and Their Correlation with Resistance to a Highly Virulent Challenge Strain.

S.M. Aghakhan, N. Abshar, C. Marunesi, Z. Sami and S.R. Fereidouni
Department of Avian Medicine, Razi Vaccine and Serum Research Institute, POBox 11365-1558, Tehran, Iran.

Summary
Maternal antibodies, even at relatively high levels protecting against mortality and gross bursal lesions, appeared to be inadequate in preventing infection with a highly virulent infectious bursal disease virus (IBDV) strain. A comparison between the sensitivity of a cell culture microtitre virus neutralization (VN) technique and a quantitative agar gel precipitation (QAGP) test, used to measure the relative antibody levels to IBDV, showed that the former technique was significantly more sensitive than the latter. False negative results, ranging from 2% to 100% (depending on VN antibody titres), were found in the QAGP tests, while false positive reactions were not observed . Evaluation of two IBD live vaccines, one relating to so-called " intermediate " and the other to " hot" strains, indicated that these lacked sufficient invasiveness in breaking through moderately high antibody titres. Study of the susceptibility of maternally immune birds to a challenge revealed that, within the scope of this investigation, the rate of bursal infection, at a given VN antibody titre, did not differ markedly in the two genetic lines of light and heavy breeds of chickens. The speculative significance of this finding , in relation to the differences existing between susceptibility of the layer and broiler type chicks to the disease, has been discussed.

Introduction

The emergence of a highly pathogenic strain of infectious bursal disease virus (IBDV) in the field inevitably calls for reevaluation of the parameters

involved in the process of infection. Virulence of the field challenge virus strain, genetic constitution of birds, antibody levels, and the age at which chickens become infected are important factors to be considered in this respect. In a previous article, the isolation and characterization of a new , highly virulent IBDV strain , capable of causing at least 85-90% mortality in SPF chickens, was reported (Aghakhan *et al.*, 1996). The virus was demonstrated as still belonging to the standard serotype 1 strains, based upon the results of serological studies and also the fact that some live vaccines derived from standard strains could protect SPF birds against challenge with the new isolate.

Progeny chicks almost invariably possess some levels of maternal antibodies to IBDV. These are the result of vaccination of doms with live as well as inactivated oil-based vaccines, and probably also field infections. Maternally derived antibodies (MDA) interfere with the replication of vaccinal strains and can also neutralize the field challenge virus (Lucio and Hitchner, 1981; Muskett *et al.*, 1979; Solano *et al.*, 1986; Winterfield *et al.*, 1980; Wood *et al.*, 1981; Wyeth, 1980). Therefore, it is highly valuable to quantitatively estimate MDA levels in order to assess the proper age for progeny vaccination. It is also important to investigate the relative effectiveness of MDA against the new, highly virulent virus.

This communication presents the results of an investigation into the following aspects of IBD infections : a) Determination of the role of MDA in conferring resistance against a highly pathogenic IBDV strain. b) Comparison of the relative sensitivity of a cell culture microtitre virus neutralization (VN) technique and a quantitative agar gel precipitation (QAGP) test in detecting and measuring MDA levels. c) Study of the rate of MDA decline, and d) Assessing the invasiveness of some live IBD vaccines in breaking through MDA levels.

Materials and methods

Viruses: The cell culture-adapted H13 strain of IBDV was supplied by a previously acknowledged source (Aghakhan *et al.*, 1996). The 2212/91 strain, a highly virulent virus, isolated in this laboratory and described elsewhere (Aghakhan *et al.*, 1996) was used for challenge purposes.

Cell culture: Primary chicken embryo fibroblast cell culture was prepared according to the standard techniques.

Eggs and chickens: The source of SPF eggs and chickens has been mentioned elsewhere (Aghakhan *et al.*, 1996). Conventional broiler or layer type chicks were obtained from apparently healthy breeder organizations.

Serological tests- The cell culture microtitre virus neutralization technique and the quantitative and qualitative agar gel precipitation tests employed in this work have been described previously (Aghakhan *et al.*, 1996).

Study of the correlation of MDA levels detected by the VN and QAGP tests and their relationship to resistance against challenge- This study comprised two similarly designed experiments using broiler or layer type chicks :

Experiment 1: One hundred and fifty four layer type chicks, progeny of commercial breeders vaccinated against IBD, were reared in isolation up to 21 days of age. Blood samples were obtained at this time and the chicks were challenged with $10^{2.0}$ chicken infective dose 50 (CID_{50}) of the 2212/91 strain of IBDV, inoculated intraocularly in 0.05 ml. doses. The surviving birds were killed on the 4th. day following challenge and the bursae removed. These were then individually prepared as a 20% homogenate in PBS and examined for the presence of IBDV antigens using the AGP test. Bursae from SPF birds were treated similarly and used as the negative control antigen. All serum samples were tested for antibody levels to IBDV employing the cell culture microtitre VN and the QAGP techniques.

Experiment 2: One hundred and forty broiler chicks with MDA to IBDV were used in this study. Experimental design was similar to that described for the layer type birds (experiment 1).

Determination of the rate of MDA decline: Four hundred day-old broiler chicks with MDA to IBDV were maintained in isolation. Every other day, starting from day 1, blood samples were obtained from 22 chicks and after inactivation at 56°C for 30 min, the sera were stored at -20°C until tested. All serum samples were examined for antibody levels to IBDV using the cell culture microtitre VN and the QAGP techniques.

Comparison of the relative sensitivity of a cell culture microtitre VN technique and a QAGP test in detecting and measuring MDA levels - This study made use of the material obtained from the experiment concerning the rate of MDA decline, which was outlined above.

Interference of MDA with IBD vaccines: One hundred and fifty broiler chicks with MDA to IBDV were reared in isolation up to 14 days of age, at which time they were blood sampled and then vaccinated with vaccine B , an intermediate strain also employed in a previous work (Aghakhan *et al.*, 1996), administered in drinking water according to the manufacture's recommendation. After 21 days, blood samples were taken, the birds were killed and the bursae removed for histopathological examination. All sera were tested for antibody levels to IBDV using the cell culture microtitre VN technique. A control group, consisting of 40 chicks of the same age and from the same source but unvaccinated, were maintained separately and blood sampled in parallel with the vaccinated birds. A similar experiment

was also carried out using vaccine D, representative of the so-called " hot" strains.

Results

Study of the correlation of MDA levels detected by the VN and QAGP tests and their relationship to resistance against challenge . Tables 1 and 2 show the results of the experiments performed employing two different genetic lines of chickens. In experiment 1, involving white leghorn type chicks, no precipitin line was detected in serum samples possessing a VN titre of 2^6 or less. Above this level the rate of positive reactions increased, approaching 100% in serum samples with a VN titre of 2^{11}, while those with a titre of 2^{12} were all positive in the AGP test. Three days after challenge, 10 out of 150 birds died demonstrating the typical lesions of IBD. Seven of those had VN titres of 2^6 to 2^9 when challenged. The remaining chicks were killed 4 days after challenge and of these, 22 showed gross bursal lesions. They mainly (20 out of 22 chicks) represented a range of VN titres between 2^7 to 2^{10} at the time of challenge. Within the range of VN titres encountered at 21 days of age, there was no antibody level which could protect all respective birds against infection with the highly virulent isolate as evaluated by the presence of IBDV antigens in the bursae.

Table 1. *Correlation of VN titres, AGP tests and resistance to challenge in layer type chicks with MDA to IBDV*

Frequency of cell culture VN tests	Proportion of +ve sera in AGP tests	Macroscopic bursal lesions after challenge	Susceptibility to challenge (b)
$3x2^{12}$	$3/3^{(a)}$	$1/3^{(a)}$	$1/3^{(a)}$
$22x2^{11}$	21/22	3/22	10/22
$46x2^{10}$	40/46	5/46	31/46
$41x2^9$	24/21	7/41	30/41
$23x2^8$	6/23	7/23	19/23
$19x2^7$	1/19	8/19	18/19
$1x2^6$	0/1	1/1	1/1

a) Number positives / total tested
b) Based on the presence of IBDV antigens in the bursae

According to the results of the experiment, the level of 100% susceptibility to challenge corresponds to an MDA virus neutralizing titre of 2^5 or 2^6 while that of 50% susceptibility lies somewhere between the VN values of 2^{10} and

2^{11} . In experiment 2, an expected overall lower range of MDA neutralizing antibody titres was detected in the broilers at 21 days of age, compared to the layer type chicks in experiment 1. Accordingly , the overall incidence of gross bursal lesions following challenge was higher in this experiment than with the leghorn type chicks. However, considering a given MDA titre in both experiments , the rate of infection after challenge, evaluated by the presence of IBDV antigens in the bursae, did not seem to differ greatly in the two genetic lines of chicks. Despite a lower range of VN titres, only three out of 140 birds with antibody levels of 2^4 or 2^5 died of IBD three days post-challenge.

Table 2* - *Correlation of VN titres, AGP tests and resistance to challenge in broilers with MDA to IBDV*

Frequency of cell culture VN tests	Proportion of +ve sera in AGP tests	Macroscopic bursal lesions after challenge	Susceptibility to challenge (b)
$3x2^9$	1/3	1/3	2/3
$23x2^8$	5/23	10/23	18/23
$48x2^7$	2/48	20/48	43/48
$39x2^6$	0/39	27/39	37/39
$20x2^5$	0/20	16/20	20/20
$7x2^4$	0/7	6/7	7/7

* See footnotes to Table 1.

Determination of the rate of MDA decline in broiler chicks: Figure 1 illustrates the rate of MDA decline as measured by the cell culture microtitre VN technique and also the QAGP test. A value of 0 was given to a positive reaction with undiluted serum, and -1 to no reaction line in the QAGP test. The mean VN titre of day-old chicks was $2^{11.6}$ while the mean QAGP titre was $2^{2.5}$. A rise in MDA titre was detected by both VN and QAGP tests at 5 and 3 days of age, respectively . At 13 days of age, six out of 22 sera, and then only at the undiluted level, were positive in the QAGP test when the mean VN titre was $2^{9.0}$. There was no reaction line with the 22 sera from 15-day-old chicks except for two undiluted samples which produced faint lines. The VN antibody titre had fallen to $2^{2.4}$ by 39 days of age. The results of this experiment indicate that in broilers, MDA declines in a somewhat smooth linear fashion with a half-life of approximately three to three and a half days (99.99% confidence interval).

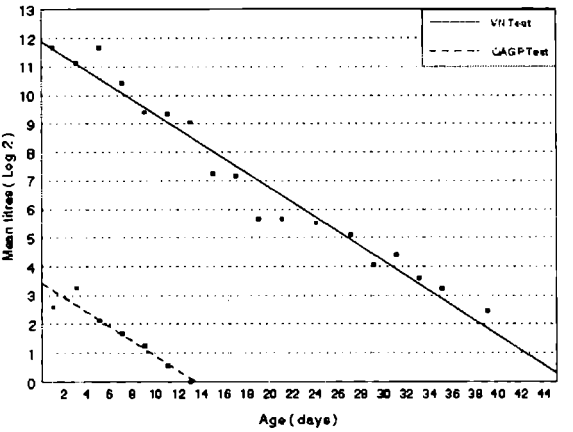

Fig 1 The decline of mean IBD maternal antibody titres measured by the cell culture VN and QAGP tests

Comparison of the relative sensitivity of a cell culture microtitre VN technique and a QAGP test in detecting and measuring MDA levels: Table 3 shows the frequency of VN and QAGP titres expressed as the Log $_2$ of the reciprocal end-point serum dilution. No precipitin was detected in serum samples which had VN titres of 2^6 or less. On the other hand, all sera with VN titres of 2^{12} and above showed reaction lines in the QAGP test. Between these two titre values, both positive and negative reactions existed at any antibody level, with sera having a titre of 2^{11} showing a frequency of precipitation lines close to 100%. The MDA could be detected for at least 26 days longer using the cell culture VN technique as compared to the AGP test.

Table 3. *Frequency of MDA titres detected and measured by cell culture VN and QAGP techniques.*

VN titres	Frequency	QAGP titres (Log $_2$)							
		-ve	0	1	2	3	4	5	6
14	2	-	-	-	1*	1	-	-	-
13	18	-	-	1	5	7	3	1	1
12	20	-	-	3	4	7	2	3	1
11	34	1	2	8	15	4	4	-	-
10	31	4	5	9	5	6	2	-	-
9	28	12	4	7	3	2	-	-	-
8	26	18	2	4	2	-	-	-	-
7	22	18	2	1	1	-	-	-	-
6	11	11	-	-	-	-	-	-	-
5	9	9	-	-	-	-	-	-	-

* *Number of chicks.*

Interference of MDA with live IBD vaccines: The initial (pre-vaccination) VN titre ranged from 2^7 to 2^{11} in the vaccine B trial, and from 2^7 to 2^{12} in the experiment on vaccine D. Seroconversion could not be detected in any serum sample obtained 21 days post-vaccination. Instead, all sera showed a normal decline in MDA titres. This implies that, at least under the conditions of these experiments, the vaccines B and D virus strains lack sufficient invasiveness in breaking through moderately high antibody titres.

Discussion

The results of the work reported herein clearly indicate that although MDA may protect against mortality or even gross bursal lesions after challenge with a highly virulent strain of IBDV, it cannot prevent infection as evidenced by the presence of viral antigens and microscopic lesions in the bursa. In fact, even the highest levels of maternally derived VN antibodies detected in the experiment, i.e. titres of 2^{11} and 2^{12} , could not protect 100% of the chicks against challenge. This explains the significantly higher mortality rates caused by the new , highly virulent strains of IBDV in the field compared to the standard viruses, and is at variance with the findings reported by chettle *et al.* (1985), which indicated that VN titres of 2^9 and higher protected 100% of the birds against a classic, virulent strain of IBDV. An interesting point in the experiments on susceptibility to a challenge was that, at least within the scope of this work, the rate of bursal infection at a given VN antibody titre did not differ greatly in the two genetic lines of white leghorn and heavy breeds of chickens. The bursa has been considered as the target organ in the IBDV infections. In all probability the largest quantity of virus / viral antigens is produced at this site. Nevertheless, taking into account the greater susceptibility of the layer type of chickens to the disease as compared to the heavy breeds (Van Den Berg and Meulemans, 1991), it seems unlikely that all or the most crucial episodes involved in the pathogenesis of the virus, culminating in clinical signs and death, take place in the bursa of Fabricius. Even the possibility of a slightly higher B : BW value (bursa: body weight ratio) in the light breeds can hardly offer an explanation for their greater susceptibility to the disease. It may be that , in different breeds, the chain of events leading to clinical disease and death, progress in a more or less similar manner up to the level of bursal infection. It is probable that other steps involved in the course of viral pathogenesis, occurring in other organs/ tissues, under physiological conditions specific to particular breeds, and controlled by the genetic constitution of the bird, determine the rate of susceptibility to clinical signs and death. It seems that further elucidation of the processes involved in viral pathogenesis may be able to shed some light on this matter.

The value of the quantitative estimation of antibodies to IBDV is three fold : 1) It is important to accurately measure the level of maternal antibodies in order to assess the proper time of progeny vaccination. 2) It is highly desirable to evaluate the effectiveness of vaccination in both breeders and progeny , and 3) It is sometimes employed as a means of diagnosing the disease. Therefore, an attempt was made to compare the relative sensitivities of two methods, i.e. a cell culture microtitre VN technique and a QAGP test, in measuring antibodies to IBDV. Analysis of the results showed that while false negative reactions, ranging from 2% to 100% , depending on virus neutralizing antibody titres, could be observed in the QAGP test, false positive reactions were not detected. In this respect, our results are in accordance with those reported by Chettle *et al.* (1985), which indicated that the cell culture virus neutralization technique was considerably more sensitive than the AGP test. Indeed , Van Den Berg *et al.* (1991) found that the former technique was even more sensitive than the Enzyme-linked immunosorbent assay (ELISA) in detecting antibodies to IBDV. At present, the cell culture VN technique is probably the most sensitive serological method available and, in fact, the only method which can be employed for differentiation of the two serotypes and also serotypes of the virus. However, there are some drawbacks in using this technique for routine purposes. It is time consuming, laborious and costly. On the other hand, the agar gel precipitation test, while being relatively simple , inexpensive and less time consuming, is considerably less sensitive. Nevertheless, we believe that, provieded the factors including all physical test parameters , antigen preparation and concentration, and also reading are standardized, the QAGP technique can be effectively employed for estimation of antibodies to IBDV. At least, the test can give some indication of antibody levels. A point which can add greatly to the homogeneity and reproducibility of the results is the inclusion of a reference preparation serum in each test.

Study of the invasiveness of so-called "intermediate" and "hot" IBD vaccine strains (vaccines B and D, respectively) in both layer and broiler type chicks showed that, under our experimental conditions, they were incapable of breaking through moderately high levels of virus neutralizing MDA i.e. 2^7-2^8 for the intermediate and 2^8-2^9 for the hot strain. In a previous communication (Aghakhan *et al.*, 1996) we reported that vaccine B was superior to vaccine C, also an intermediate strain, in conferring immunity against challenge with the highly virulent IBD virus. The possibility that seroconversion might have been detected with an antibody level follow-up extending beyond 21 days post-vaccination i.e. the time interval considered in the experiment, cannot be ruled out. Lukert *et al.* (1982) reported that in maternally immune birds, attenuated vaccine strains of IBDV may persist in the bursa and possibly other organs until MDA levels decline , whereupon

an active antibody response may be observed as late as several weeks after vaccination. However, the objective of the experiment was to examine the practical aspects of the interference of MDA levels with live vaccines, applicable to field conditions. In this respect, we were concerned with the time interval for antibody response and the route of vaccine administration. Virus neutralizing MDA titres significantly higher than those mentioned above cannot prevent infection with the highly virulent strains of IBDV.

At present, the situation of IBD imposes one of the most severe worldwide challenges upon the poultry industry. Although there is considerable variation in the clinical and pathological features of the disease in different geographical regions, the economic impacts of the problem, resulting from immunosuppression , morbidity and mortality are markedly similar. Due to the genomic constitution of the virus and also vaccination pressure, the possibility of further changes leading to increased virulence may be anticipated. Since the emergence of the highly pathogenic IBDV strains , it has become increasingly difficult to formulate an effective vaccination program. All efforts should be aimed at 1) hyper-immunization of breeders using both live and high potency inactivated vaccines 2) monitoring MDA levels to assess the proper age for vaccination , and 3) immunization of progeny employing appropriately selected live vaccines which best fit the requirements of particular areas or premises . Biosecurity, however, must be regarded as the cornerstone in any control programme, without which most if not all vaccines and vaccination regimen would fail.

Under the present conditions and until the era of new generations of vaccines (Kilenge et *al.*, 1988), engendering higher levels of immunity and offering a much wider spectrum of safety, a sound approach to minimizing losses due to IBD can be based solely on the measures already referred to.

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