Comparison between Micro and Tube Virus Neutralization Tests for Detection of Serological Responses to Infectious Bursal Disease Virus Following Vaccination

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Summary
Micro-Virus Neutralization (MVN) was used for the measurment of antibody titers in the sera from white-leghorn specific pathogen-free (SPF) and a flock of commercial broiler chickens that had been immunized with a tissue culture adapted IBD, intermediate Strain LZD-228TC, live vaccine.The level of antibody titers, obtained by MVN were compared with the antibody titers found by the tube-virus neutralization test.The results of both methods were approximately similar, but the MVN method was found be more practicable and reproducible.

Introduction

Infectious bursal disease (IBD) has been endemic in commercial chickens in Iran for many years. It is an immunosuppressive disease of young chickens characterized by sever damage of the bursa of fabricius (Allan *et al.*, 1972; Cheville, 1967; Moradian *et al.* 1990). The disease is caused by a bisegmented double-stranded RNA virus that is classified as a member of the Birnaviridae family (Dobos et al., 1979; Ismail *et al.*, 1978). Infectious bursal disease is a highly contagious disease of young chickens, which are most susceptible and show clinical disease between 3 and 6 weeks of age (Moradian *et al.* 1990). Several tests have been used for detection and measurment of antibody resposes to IBDV following immunization with IBD vaccines. Virus neutralization test (VNT) is widely used in estimation of antibody titers to IBDV (Reed *et al.*, 1989). The VNT is sensitive and can detect low levels of antibodies (titers of 1/16 to 1/64) (Giambrone, 197^{c}). However, micro-virus neutralization test is generally less expensive and less

time consuming than virus neutralisation test in tube and requires less amount of serum(Giambrone, 1979).

Materials and methods

Cell culture: A suspension was made from a 24-h primary culture of chick embryo fibroblasts (CEF). The growth medium was Eagles minimum essential medium (MEM) supplemented with 10% foetal calf serum, 0.75 g/l sodium bicarbonate and an antibiotic solution containing 200 IU/ml penicillin, 200 μg /ml streptomycin and 200 IU/ml kanamycin. The cell concentration was adjusted to 3-4 x 10^5 cells/ ml of the medium.

Virus: The virus used in the VNT must be adapted to cell culture. Therefore, vaccine strain LZD-228 which had shown a typical cytopathic effect (CPE) in CEF cells was used. 10^2 $TCID_{50}$ of the virus was used per microtiter plate well or each tube (Chettle *et al.*, 1985; Moradian *et al.* 1990).

Experimental design: At 2 separate experiments, the VNT was carried out for the measurement of IBD antibodies in the sera from chickens that had been vaccinated with the live tissue culture adapted IBD vaccine strain LZD-228 (intermediate).

Experiment 1: Thirty 2-week old SPF white Leghorn chickens were divided into 2 groups. *Group 1*: Twenty chickens were vaccinated with one dose of the IBD vaccine via drinking water. *Group 2*: Ten remaining chickens were kept in a separate place as unvacinated controls. Three weeks post vacciantion, both groups were bled. Serum samples were inactivated at 56°C for 30 min (Nancy Hebert *et al.*, 1982). Serum samples were stored at -20°C before testing (Nancy Hebert *et al.*, 1982; Reed *et al.*, 1989; Weisman and Hitchner, 1978).

Experiment 2: A flock of broilers in a commercial farm, approximately 10,000 chickens in one shed, were vaccinated twice with one dose of the live IBD vaccine strain LZD-228 via drinking water. Before vaccination 30 chickens, 7-day old and randomly selected, were bled in order to determine the level of maternal antibodies. The first dose of the vaccine was given at 8 days of age and were bled one week later. The second vaccination was administered 10 days after the first one. Thirty chickens randomly were bled three times at weekly intervals from 1 to 3 weeks after the second vaccination. All recovered sera were inactivated at 56°C for 30 min and stored at - 20°C before VN testing.

Virus neutralization test :VNT was carried out by microtiter and tube methods. 96-well flat bottomed microplates and tubes containing 24-36-h secondary confluent monolayer cell culture of CEF were used (Chettle *et al.*, 1985; Giambrone, 1979; Nancy Hebert *et al.*, 1982). For the neutralization,

serial towfold dilutions of each test serum were made in the MEM. The dilutions were mixed with equal volumes of the virus suspension, containing 100 $TCID_{50}$ per 25µl (Chettle *et al.*, 1985; Moradian *et al.* 1990). The serum-virus mixtures were incubated at the room temperature for 60 min to allow virus neutralizaion take place. For assay, 50 and 200 µl of the serum-virus mixtures were inoculated into each microplate well and tube, respectively(Skeells *et al.*, 1978). Five wells and five tubes were chosen for each dilution of the serum-virus mixture. Afterwards, 200 µl of maintenance medium was dispensed into each microplate well and 2 ml of the medium was added to each tube.The microplates were covered with polypropylene tape and were placed in a high humidity incubator at 37°C for 3-5 days (Chettle *et al.*, 1985). Tubes were covered with caps and incubated in the same manner. The monolayers were observed microscopically for CPE, the neutralization titers were determined on the base of the reciprocal highest serum dilution (Log_2) that did not show any CPE (Chettle *et al.*, 1985; Nancy Hebert *et al.*, 1982).

Results and Discussion

Experiment.1: Table 1 and Fig. 1 show the comparison of the rate of VN antibody titers (Log_2) which measured by both the microplate and tube methods. The antibody titers by MVN method varied between 6 to 8 with an average titer of 7.15. The titers of VN antibody determined by tube method also varied between 6 to 8 and the average of 7.35. Antibody responses in unvaccinated control chickens were negative.

Experiment 2: VN antibody titers of the sera that had been taken from commercial broiler chickens were determined by microplate and tube methods. The results and comparison of VN antibody titers are summerised in Table 2. Pre-vaccination maternal antibody titers were measured in one-week chickens. The average titer (Log_2) of pre-vaccinated chickens were 6.45 by the MVN test and 6.66 by the VN test in tubes. The average of VN antibody titers (Log_2) of 15-days old chickens were 3.36 by the MVN methods and 3.66 by the tube method. The average titers of antibody (Log_2) after 1, 2 and 3 weeks after the second vaccination were shown to be 4.34 , 6.23 and 8.30 by the MVN method and 4.53 , 6.43 and 8.53 by the VN test in tube. The level of VN antibody titers are compared in Fig.s 2 to 6, respectively. The level of VN antibody titers were measured by the microplate and the tube systems indicated that the titers of antibody were approximately identical. However, the VN test by microculture method is more applicable, reproducible and generally less time consuming. It also requires less amounts of serum or the virus. The results from experiment 2 demonstrated that the commercial broiler chickens that already carried

maternally derived antibody exhibited less humoral antibody than susceptible SPF chickens after the first vaccination.

Table 1 VN antibody titres of in SPF chickens 3 weeks post vaccination with live IBD vaccine intrmdiate LZD-228TC

Group	Age (Days)	No.	VN Antibody Titres* Micro Tube								Average titres*
			3	4	5	6	7	8	9	10	
Vaccinated	(14)	20				4 2	9 9	7 9			7.15 7.35
Control	(14)	10	Neg . Neg .								

* Reciprocal of highest Log_2 dilution of serum to inhibit CPE

Table 2 VN Titres of sera in broiler chickens prior and after vaccination with live IBD vaccine imermediate Strain LZD-228TC

Vaccination (age:days)	Serum collection (age: days)	No. tested	VN Antibody Titres* Micro Tube								Average titres*
			3	4	5	6	7	8	9	10	
	Prevacciation (7)	30				16 14	11 12	3 4			6.56 6.66
1st (8)											
	(15)	30	20 12	9 16	1 2						3.36 3.66
2nd (18)											
	(25)	30	2 2	17 14	9 10	2 4					4.34 4.53
	(32)	30			4 3	16 13	9 12	1 2			6.23 6.43
	(39)	30					4 1	14 14	11 13	1 2	8.30 8.53

* Reciprocal of highest Log_2 dilution of serum to inhibit CPE

This indicated that maternally derived antibody interferes with the development of active antibody (Solano *et al.*, 1985; Winterfield *et al*, 1979; Wyueth and Chettle, 1990). The results also indicated that the higher level of active antibody appears 3 weeks after the second vaccination. In our study, we also concluded that the commercial broiler chickens revealed adequate humoral active antibody titers (Log_2 >8) after second vaccination. The highest titer of the antibodies were obtained 3 weeks after the second vaccination.

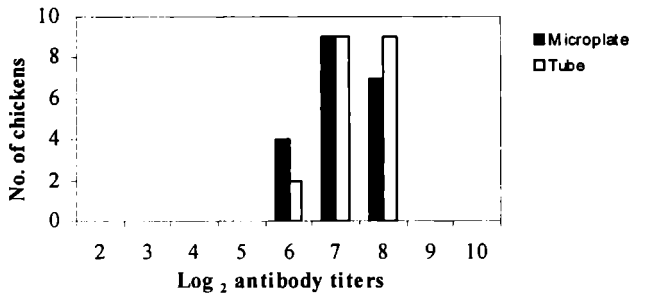

Fig.1 VN antibodies to IBDV in SPF chickens 3 weeks post vaccination

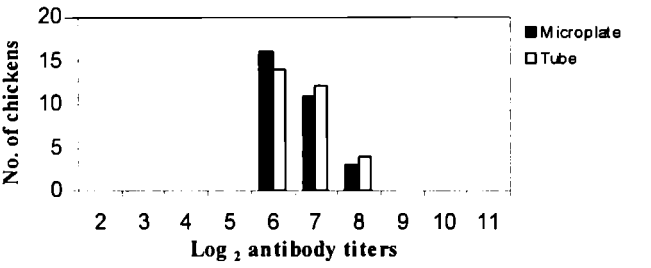

Fig.2VN antibodies to IBDV in the sera of 7-day old broiler chickens

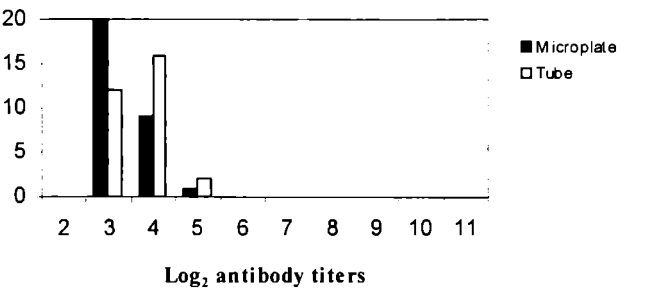

Fig.3 VN antibodies in 15-day old broiler chickens 7 days after first vaccination

References

Allan, W. H., J. T. Faragher, and G. A. Gullen. (1972). Immunosuppression by the infectious bursal agent in chickens immunised against Newcastle disease. Veterinary Record. **9**: 511-512.

Chettle, N. J., R. K. Eddy and P. J. Wyeth. (1985). Comparison of virus neutralizing and precipitating antibodies to infectious bursal disease virus and their on susceptibility to challeng. British Veterinary Journal. **141**: 146-150.

Cheville, N. F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken. American Journal of pathology. **51** : 527-551.

Dobos, P., B. J. Hill, R. Hallett, D. T. C. Kells, H. Becht, and D. Teninges. (1979). Biophysical and biochemical characterization of five animal viruses with bisegmented double-straded RNA genomes. Journal of Virology. **32** : 593-605.

Giambrone, J. J. (1979). Microculture Neutralization Test for serodiagnosis of three Avian Viral Infetcions. Avian Disease. **24** : 284- 287.

Ismail, N. M., Y. M. Saif, and P. D. Moorhead. (1978). Lack of pathogenicity of five serotype 20 infectious bursal disease viruses in chickens. Avian Disease. **32** : 757-759.

Ismail, N. M., Y. M. Saif, W. L. Wigle, G. B. Havenstein and C. Jackson. (1978). Infectious bursal disease virus varient from commercial leghorn pullets. Avian Disease. **31** : 766-770.

Moradian, A., J. Thorsen, and R. J. Julian. (1990). Single and combined infection of Specific-Pathogen-Free chickens with Infectious bursal disease virus and an intestinal isolate of reovirus. Avian Disease. **34** : 63-72.

Nancy Hebert, C., N. E. Reed, J. C. Muskett, and Denise, H. Thornton. (1982). Factors affecting reproducibility of the serum neutralization test for infectious bursal disease. Journal of Biological standardization. **10** : 221-229.

Reed, N. E., Denise, H. Thornton, C. Nancy Hebert, J. C. Muskett, and G. W. Wood. (1989). An International collaborative study of anti-infectious bursal disease virus reference serum. Journal of Biological standardization. **17** : 361-369.

Skeels, J. K., P. D. Lukert, O. J. Fletcher, and J. D. Leonard. (1978). Immunization studies with a cell-culture-adapted infectious bursal disease virus. Avian Disease. **23** : 456-465.

Solano, W., J. J. Giambrone, J. C. Williams, L. H. Lauerman, V. S. Panangala, and C. Garces. (1985). Effect of maternal antibody on timing of initial vaccination of young white leghorn chickens against infectious bursal disease. Avian Disease. **30** : 648-652..

Weisman, J., and S. B. Hitchner. (1978). Virus neutralization Versus Agar-Gel precipitin test for detecting serological Response to infectious bursal disease virus. Avian Disease. **22** : 598-603.

Winterfield, R. W., A. S. Dhillon, H. S. Thacker, and L. J. Alby. (1979). Immuno response of white leghorn chicks from vaccination with different strains of infectious bursal disease virus and in the presence of maternal antibodies. Avian Disease. **24** : 179-188.

Wyeth, P. J., N. J. Chettle. (1990). Use of infectious bursal disease vaccine in chichs with maternally derived antibodies. The Veterinary . Record. **126** : 577-578.