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THE IMMUNE RESPONSE AGAINST INFECTIOUS BURSAL VIRUS VACCINE IN CHICKEN

(With 4 Tables)

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الأستجابة المناعية ضد مرض الجامبورو فى الدواجن

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تم تحصين ثمانين من الدجاج بلقاح الجامبورو الحى المستضعف وذلك بجرعتين من اللقاح، أستخدمت الأولى منها كقطرة للعين عند سن ١٤ يوما وقدمت الثانية فى ماء الشرب عند سن ٢٤يوما (اى بعد ١٠ أيام من الجرعة الأولى). وقد أستخدمت ثلاث طرق سيرولوجية مختلفة لقياس المستوى المناعى فى هذه الطيور بعد تلك التحصينات. شملت الطرق السيرولوجية كل من أختبار الترسيب فى الآجار وأختبار المصل المتعادل وأختبار الأليزا. هذا وقد أوضحت التجارب العملية أن أختبار الترسيب فى الأجار يمكن استخدامه فى المسح السيرولوجى الشامل حيث أنه لا يعطى نتائج كافية فى حالة وجود مجال واسع من المستوى المناعى بينما كان أختبار المصل المتعادل أكثر دقة وحساسية منه ومساويا لاختبار الأليزا فى دقة تحديد معيار المستوى المناعى للطيور المحصنة والذى يمكن استخدامه لفحص عدد كبير من عينات أمصال الطيور.

SUMMARY

Eighty serum samples were screened from vaccinated chicken for infectious bursal disease virus (IBDV) antibody level after they had received a single dose and two doses of live attenuated IBDV vaccine (D_{7e}). The first dose was given at 14 days of age via eye drop route while the second dose was given via drinking water route. The antibody profile of primary and secondary were compared using agar gel precipitation test (AGPT), serum neutralization test (SNT) and enzyme linked immunosorbant assay (ELISA). The result revealed that the AGPT is useful for general screening purpose while the ELISA test is sensitive, specific, rapid and it consumes less time since large number of samples can be tested. The SNT appears to equal the

ELISA test in indicating flock immunity, but the SNT is more expensive and less in time consuming.

Key words: Immunity-Infectious Bursal Vaccine-Chickens.

INTRODUCTION

Infectious bursal disease (IBD) is an acute contagious viral disease of young chicken which was first described in 1962 *(COSGROVE, 1962)*. The virus affects primarily the bursa of Fabricius and other lymphoid organs and characterized by ruffled feathers, watery diarrhoea, trembling and severe prostration *(HOFSTAD et al., 1972)*. Chicken are most susceptible to clinical infection from 3 to 6 weeks of age *(LEY et al., 1983)*, although the disease has been reported to occur in 2 to 15 weeks old chicken *(LEY et al., 1979)*. Chicken that are susceptible and less than 2 weeks of age are subclinically infected, but the infection is usually led to immunosuppression *(HIRAI et al., 1980)*. Effective means for controlling IBD are needed because of the effect of early infection on the immune system. Early IBD infection decreases responses to vaccines and increases the susceptibility to other infections in young chicken *(ROSENBERGER and GELB, 1978)*.

The methods used commonly to detect flock exposure to IBD virus are the agar gel precipitin (AGP) and virus neutralization test (VN). The AGP test is economic and simple to perform although precipitins are sometimes not detectable even though birds are resistant to challenge with the virus while, VN test appears to be a better indicator of flock immunity *(WEISMAN and HITCHNER, 1978)*. The enzyme linked immunosorbant assay (ELISA) has been used in quantitating antibody to many agents *(ENGVALL and PERLMAN, 1971)*. The ELISA assay was more suitable than VN and AGP tests for detecting IBD virus antibody, however, its extreme sensitivity and variation in enzyme substrate reactivity time have imposed difficulties on data analysis *(BARLOUGH et al., 1983)*.

The present study was made to compare the ELISA sensitivity with that of the conventional SN and AGP tests in determining the immune response of vaccinated birds with IBD vaccine.

MATERIAL and METHODS

1-Embryonated chicken Eggs(ECE):

The eggs were obtained as one -day-old from the United Company for Poultry Production (UCPP). They were incubated for 9-10 days before being inoculated. The eggs were used for virus propagation, titration and SNT test.

2- Chicken:

They were obtained as one -day- old baby chicks from UCPP. They were raised in isolated area untile they become four to eight weeks of age to be used in preparation of antiserum and antigens for AGP test.

3- Viruses:

a- Vaccinal IBD virus Strain (D78):

The virus was obtained from Intervet International, Boxmeer, Holland. Its titer was $10^{6.5}$ EID_{50} / ml. It was used for SN test and preparation of positive antiserum.

B. Virulent IBD virus strain:

The virus was a field isolate of IBDV maintained by passage in SPF chicken (Lohmann Co., Germany). Its titer was 10^3 EID_{50}/ dose. It was used in infection of the chicken to prepare the AGP antigen.

4- Preparation of viral antigens:

a- AGP antigens:

The antigen was prepared from a saline suspension of bursa from 4-6 weeks old susceptible chicken, 72 hours after ocular infection with IBDV containing 10^3 EID_{50}/ dose. Briefly 50% suspension was homogenized and then clarified by centrifugation at 2000 r.p.m. for 30 minutes. The supernatant was used as AGP antigen. It was checked for specificity and sensitivity against known positive and negative antiserum. It was stored at-70°C until used.

b- ELISA antigens:

Each of 30, 10-days-old embryos was inoculated by the chorioallantoic membrane (CAM) route with approximately 10^3EID_{50} of strain D78. Seventy two hours later, all the CAMs and the livers were harvested, ground in 5 ml of sterile broth containing 100 u penicillin and 5 ug streptomycin per ml. The emulsion was subjected to free-thaw for three times, and centrifuged at 3000 r.p.m. for 30 minutes. The supernatant was pelleted by ultracentrifugation at 30,000 r.p.m. for 3 hours. The pellete was resuspended in 2 ml saline and stored at- 70°C until used in the ELISA test.

5- Serum Neutralization Test:

It was carried out according to HWANG (1969) using constant serum-variable virus procedure.

6- Agar Gel Precipitation Test:

The test was done according to *CHUBB and CHURCHILL (1968).* The serum samples as well as positive and negative controls were placed in the peripheral 6 wells while the antigen was placed in the center well.

7- ELISA for IBD Antibody:

It was carried out according to *VOLLER et al. (1976).*

8- Preparation of antiserum against IBDV:

Four to five weeks old five susceptible chicken were inoculated intraocularly with 0.05 ml of a clarified 10% W/V bursal homogenate known to contain 10^3 EID_{50} /dose; 2 chicken were kept uninoculated. 28 days post inoculation all the inoculated and uninoculated chicken were bled and the sera were inactivated at 35°C for 30 minutes and stored at-20°C until used as positive and negative antisera.

9- Serum Samples:

Eighty blood samples were taken from apparently healthy vaccinated chicken from Private Farm in Qualiobia Province. The chicken were vaccinated with live attenuated IBD vaccine D_{78} strain. They received the first dose via the ocular route at 14 days old of age while the second dose was given at 24 day of age (10 days apart from the 1 st vaccination) via the drinking water route. The serum samples were taken after both vaccination as follows:

1- Twenty samples were taken one week after the 1 st dose of vaccination.
2- Twenty samples were taken10 days after the 1 st dose of vaccination.
3- Twenty samples were taken one week post 2 nd dose of vaccination.
4- Twenty samples were taken 2 weeks post 2 nd dose of vaccination.

All the serum samples were heated at 56°C for 30 minutes and stored at-20°C until used in the different serological tests.

RESULTS

Table (1) summarized the results of the development of ELISA titer of vaccinated chicks with IBDV vaccines. It was found that the GMT was 2.35 one week post 1 st vaccination as the ELISA titers ranged from 1:4 to 1:16 (six samples out of 20 had 1:4 ELISA titer and four sample out of 20 had 1: 16).Ten days post vaccination, the GMT was 3.9 where the titer raised till 1: 32 with three samples out of 20. One week post 2 nd dose of vaccination, the

GMT was 5.2 as the ELISA titer reached 1: 128 with six samples. Two weeks post vaccination, the GMT was 3.0 (7 samples out of 20 has a titer of 1: 4 and 10 samples out of 20 had 1: 16).

Table (2) showed the results of serum neutralization test as the GMT of neutralizing indices in serum samples taken one week post 1 st dose of vaccination was 1.9, as it ranged from 1.4 till 3 as one samples out of 20 samples has NI (3). Ten days after the 1 st vaccination the GMT of NI was 2.76 where NI ranged from 1.3 to 4.8 as two samples had 2.5 and 2 samples has NI 3.7 and two samples showed NI 3.9 and one sample showed NI 4.8. While, the NI in serum samples taken one week post 2nd dose of vaccination was 4.1 as it ranged from 3.1 till 6 as 6 samples out of 20 have NI (3.9). Two samples showed NI 4 and one sample had NI (6). Two weeks post 2 nd dose of vaccination, the NI declined till 1.7 as it ranged from 1.3 till 3.5.

Table (3) summarized the results of AGPT as the percentage of serum samples that gave positive precipitin antibodies one week post 1st dose of vaccination was 50% while the 80% positive serum samples was gained 10 day post 1st dose and one week post 2 nd dose of vaccination while 2 weeks post 2nd vaccination showed no precipitating antibody except in 5% of the serum samples.

Table (4) summarized the percentage of positive and negative results of SNT indices, AGPT as well as ELISA titer of sera of vaccinated chicken. The results showed that one week post 1st dose of vaccination the percentage serum samples was 55%, 80%, 50% as indicated by SNT, ELISA and AGPT test respectively. Ten days post 1 st dose of vaccination, the percentage of positive serum samples was 75%, 100% and 80% as showed by SNT, ELISA and AGPT respectively. One week post 2 nd dose of vaccination, the positive percentage of serum samples was 100% in both SNT and ALISA tests while the percentage of positive serum in case of AGPT was 20%. Two weeks post 2 nd vaccination the AGPT showed 5% positive serum samples while the SNT and ELISA tests showed 95% and 100% positive serum respectively.

DISCUSSION

Effective means to control IBD are needed to overcome its early suppressive effect on the immune system of chickens. The most practical one of these means is the vaccination of chicken flocks. There are two methods of vaccination either by vaccinating the parent stock; there by providing

passive immunity for their offspring, or by vaccinating the young birds using a live IBD vaccine *(MAZARIEGOS et al., 1990).*

The immune response of vaccinated birds could be evaluated by different serological methods such as AGPT *(WEISMAN and HITCHNER, 1978)* and ELISA *(ENGVALL and PELMAN, 1971, VAN WEEMAN and SCHURRS, (1971) and ENGVALL (1977).*

The present work compared between three serological tests; AGPT, SNT and ELISA; used to detect the immune response of chickens vaccinated with 2 doses of a live attenuated IBDV vaccine (strain D78). The first vaccination was applied at the age of 14 days as eye drops while the second one was applied at the age of 24 days via the drinking water.

The experimental results showed that AGPT was not accurate to measure a wide range of antibody level. The test revealed positive results in 80% of the flocks vaccinated at 10 and 7 days post first and second vaccination respectively, while, positive results were 5% only in vaccinated flocks at 38 days post the second vaccination. High levels of SNT and ELISA antibodies were detected in the last group of birds.

The 80% positive AGPT, is nearly similer with the findings of *MOHAMED EL-SHORBAGY (1992)* who obtained 90% positive results in the detection of immune vaccinated birds. The 10% difference between the two results could be attributed to the period (postvaccination) when the test was carried out. There is also an agreement as regards the positive ratio (5%) on 35 days post second vaccination (i.e. more 5 weeks) where he obtained a ratio of 23% on 4 weeks period post vaccination. In the same respect *WALTER and PHILLIPS (1981)* obtained positive AGPT with a similar ratio of 81.5% of tested chicken sera

Regarding the serum neutralization test, the present results agree with that of *WINTERFIELD (1969) and WEISMAN and HITCHNER (1978)* who stated that SNT was more sensitive than AGPT to detect the higher levels of serum antibodies in vaccinated chickens. Also *MOHAMED EL- SHORBAGY (1992)* detected higher levels of neutralizing IBDV antibodies using SNT on 7, 14, 21 and 28 days post vaccination.

In relation to ELISA, the present work cleared that the immune response of vaccinated birds could be detected on a period of 1 week post the first dose of vaccination with a positive ratio of 80%, so, it is more sensitive test if compared with positive results of 55% in case of SNT and 50 % in case of AGPT. Moreover, the ELISA test could be applied to examine a large number of serum samples saving the time consumed with other methods. The present results are in agreement with that of *MARQUARDT et al. (1979) and*

SALANO et al. (1985) who concluded that the ELISA test was an efficient sensitive and rapid test suitable for testing a large number of samples.

Therefore, it could be concluded that the SN and the ELISA tests are more sensitive than the AGPT in measuring the immune level of vaccinated chickens.

REFERENCES

Barlough, J.E.; Jacobson, R.H.; Downing, D.R.; Marcella, K.L.; Lynch, T.J. and Scott, F.W. (1983): Evaluation of computer- assisted, kinetics- based enzyme- linked immunosorbent assay for detection of corona virus antibodies in cats. J. Clin. Microbiol., 17: 202-217.

Chubb, R.C. and Churchill, A.E. (1968): Precipitating antibodies associated with Marek's disease. Vet. Rec., 83: 4-7.

Cosgrove, A.S. (1962): An apparently new disease of chicken- avian nephrosis. Avian Disease, 6: 385- 389.

Engvall, E. (1977): Ouantitative enzyme- immuno- assay (ELISA) in microbiology. Medical Bio., 55: 193-200.

Engvall, E. and Perlman, P. (1971): Enzyme- linked immunosorbant assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry, 8: 871-874.

Hawang, J. (1969): Duck hepatitis virus neutralization test in chicken embryo. Am. J. Vet. Res., 30: 861-869.

Hirai, K.; Kunihiro, K. and Shimakura (1980): Characterization of immunosuppression in chicken to infectious bursal disease virus. J. Virol., 32: 323-329.

Hofstad, S.; Caleek, B.W.; HimboldW.M. and Yoder, H.W. (1972): Eds. Iowa State Univ. Press, Ames Iowa.

Ley, D.H.; Storm, N.; Bickford, A.A. and Yamamoto, R. (1979): An infectious bursal disease virus outbreak in 14 and 15 week old chicken. Avian Dis., 23:; 235- 240.

LEY, D.H.; Yamamoto, R. and Bickford, A.A. (1983): The pathogenesis of infectious bursal disease: Serologic, histopathologic and clinical observation. Avian Dis., 27: 1060-1085.

Marquardt, W.W.; Johnson, R.B.; Odenwald, W.F. and Schlottober (1979): An indirect enzyme linked immunosorbent assay (ELISA) for measuring antibodies in chicken infected with infectious bursal disease virus. Avian Dis., 24 (2): 375-385.

Mazariegos, L.A.; Lukert, P.D. and Jhon, B. (1990): Pathogenicity and immunosuppressive properties of infectious bursal disease "Intermediate" strains. Avian Dis., 34: 203-208.

Mohamed El- Shorbagy (1992): Studies on infectious bursal disease in chicken in Qualubia Province. M.V.Sc. Thesis (Poultry Diseases), Fac. Vet. Med.,Zagazig Univ.

Rosenbeerger, J.K. and Gelb, J.Jr. (1978): Response to several avian respiratory viruses as affected by infectious bursal disease virus. Avian Dis., 22: 95-105.

Salano, W.; Giambrone, J.J. and Panangala, V.S. (1985): Comparison of a kinetic based enzyme linked immunosorbent assay (ELISA) and virus neutralization test for infectious bursal disease virus. I. Quantitation of antibody in Whit Leghorn hens. Avian Dis., 29 (3): 662- 671.

Van Weeman, B.K. and Schurrs, H.W. (1971): Immunoassay using antigen enzyme conjugates. FEBS LETTERS, 15: 232-236.

Voller, A.; Bidwell, D. and Bartlett (1976): Microplate enzyme immuno assay for immunodiagnosis of virus infections. Manual of Clin. Immunol. ASM Washington, D.C.P. 506- 512.

Walter, E. and Phillips, Jr. (1981): Comparison of precipitin antibodies and virus neutralization antibodies to infectious bursal disease virus. Avian Dis., 25 (4): 1093- 1097.

Weisman, J. and Hitchner, S.B. (1978): Virus precipitation versus agar gel precipitin tests for detecting serological response to infectious bursal disease. Avian Dis., 22: 598-603.

Winterfield, R.W. (1969): Immunity response to infectious bursal agent. Avian Dis., 13: 548-557.

Table (1) : ELISA antibody titer of vaccinated chickens with one and two doses of IBVD vaccine.

Period after vaccination	No. of birds with ELISA antibody titer								GMT
	2	4*	8	16	32	64	128	512	
One week post 1 st dose	-	** 6	5	5	-	-	-	-	2.35
Ten days post 1 st dose	-	4	5	3	5	3	-	-	3.90
One week post 2 nd dose	-	-	-	2	6	4	2	6	5.20
Two weeks post 2 nd dose	-	-	7	1	10	1	-	-	3.00

* Two fold dilution of serum samples.
** No. of tested sera showing the above dilution.

Table (2) : Serum neutralization indices of vaccinated chickens after 2 successive vaccination.

Period after vaccination	Neutralization indices (NI)	GMT
One week post 1 st vaccination	(3) (2) (2) (3) (3) 0, 1.4, 1.5, 1.9, 2, 2.3, 2.5, 2.7, 2.8, 3	1.9
10 days post 1 st vaccination	(3) (2) (2) (2) 1.3, 1.5, 2, 2.3, 2.5, 2.6, 3, 3.5, 3.7, 3.9, 4.1, 4.8	2.76
One week post 2 nd vaccination	(6) (2) (3) 3.1, 3.3, 3.4, 3.5, 3.9, 4, 4.1, 4.3, 4.5, 4.8, 5.1, 6	4.1
Two weeks post 2 nd vaccination	(5) (2) (2) (2) (2) (2) Not tested, 1.3, 1.5, 2.1, 2.2, 2.4, 2.7, 2.8, 3, 3.2, 3.5	1.7

Table (3) : The results of AGPT of vaccinated chicken with 2 successive doses of IBVD.

Period after vaccination	No. of positive / Total No.	% of positive AGPT
One week post 1 st vaccination	10/20	50 %
10 days post 1 st vaccination	16/20	80 %
One week post 2 nd vaccination	16/20	80 %
Two weeks post 2 nd vaccination	1/20	5 %

Table (4) : Comparison between the percentage of positive results of sera of vaccinated chicken examined by the three tests.

Period after vaccination	Age of birds at time of vacc.	NI more than 2	ELISA	AGPT
One week post 1 st vaccination	21 days	11/20 * (55 %)	80 %	50 %
Ten days post 1 st vaccination	24 days	15/20 (75 %)	100 %	80 %
One week post 2 nd vaccination	31 days	100 %	100 %	80 %
Two weeks post 2 nd vaccination	38 days	19/20 (95 %)	100 %	5 %

* No. of tested sera with positive results over the total No. of samples.