KINETICS OF ANTIBODIES IN SERUM, EGG YOLK AND DAY-OLD CHICKS AGAINST INFECTIOUS BURSAL DISEASE IN CHICKEN BROILER BREEDERS

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ABSTRACT

Ten broiler breeder flocks were selected for serum and egg yolk antibody titres against infectious bursal disease (IBD). Serum and yolk sac of the chicks hatching from the eggs of the same breeder flocks were also studied for antibody titres. Geometric mean titre of various flocks varied from 9 to 59 in serum and 20 to 127 in the eggs. The titres were maximum at 4 weeks post-vaccination which showed a drop at 8 weeks post-vaccination. Titre against IBD were comparatively lower in chicks than the corresponding titres in the parent flocks. GMT in yolk sac of the chicks revealed relatively higher values than the serum titres of the respective chicks.

INTRODUCTION

Poultry flocks do suffer from a variety of infectious diseases. Among these, infectious bursal disease (IBD) is the most common problem both in layers as well as in broilers and inflicts heavy economic losses. Since 1980, IBD has emerged as a havoc for the poultry industry in Pakistan (Siddique *et al.*, 1987). IBD is important besides direct pathological effects, IBD causes immunosupression. Once established in a poultry rearing area, it may recur in subsequent flocks if proper disinfection and biosecurity measures are not observed (Lee and Hin, 1992).

For the control of IBD, apart from the rigorous bio-security measures vaccination is the main tool. Currently, protection is achieved by hyper-immunizing the parent, flocks with live and inactivated IBD vaccines and vaccination of the commercial flocks (Sharma *et al.*, 1989). However, despite vaccination against IBD number of outbreaks have been reported in vaccinated chicken flocks. Variation in the antibody titres of day-old chicks may be one of the important factors (Abdu, 1988; Zaheer and Muneer, 1992). Different vaccination routes including sub-cutaneous, intramuscular and oral may have important influence on antibody response (Komine *et al.*, 1995).

The level of maternal antibodies to IBDV, decreases to 45 percent of the antibody titre in their respective dams. Maternal antibodies are found to disappear from the circulation of these crossbred chickens with a half-life of 6.7 days (Fahey *et al.*, 1987). A high correlation was observed between serum and yolk antibody titres against IBDV, IB and ND. In another study, Arpin *et al.* (1991) monitored antibody pofiles for four viruses (IBD,IB,ND and RV) in five vaccinated flocks from 27 to 57 weeks of age at six weeks intervals.

The present study was carried out to investigate the correlation of antibody titres between the egg yolk and serum in broiler breeding chickens as well as the transfer of antibody titres in serum and yolk of their offsprings.

MATERIALS AND METHODS

Ten broiler breeder flocks, five being at Mansehra, two at Abbotabad and three around Rawalpindi were selected. Detailed history including breed, freed, age of the birds, vaccination status and management was recorded Table 1. One hundred blood samples (ten from each flocks at random) were collected and stored in freezer till use. At the same time, one hundred eggs were opended aseptically, albumin was discarded and the yolk was poured in sampling bottles. After a period of 21 days, one hundred day-old chicks (ten from each flock) were obtained from the same batch of heart and serum was separated. The chicks were sacrificed and yolk samples were also collected aseptically in the sampling bottles. All the serum samples were heat inactivated (58°C, 30 minutes) and were subjected to indirect hemagglutination test using sonicated virus antigen and one percent sensitized sheep erythrocytes. Lyophilized live IBD vrius vaccine (BUR, 706, Rhone Merieux, France) was reconstituted with 20 mL of sterilized

phosphate buffered saline. The vaccinal virus was subjected to sonification in a jacketed vessel, using Rapidis 600 (Ultrasonic Ltd., Germany) at an intensity of 75 watts/cm^2 with tentanium probe (15 cm diameter) for five minutes to disintegrate the virus particles. The temperature was regulated at 20°C with cold water. The sonicated material was then centrifuged at 5000 rpm for 15 minutes and the supernatant was collected and used as sonicated antigen. Sensitization of sheep RBCs was done following the method of Bansal *et al.* (1986). The IHA was carried out in the titre tek microtitration plates (Flow Lab.).

RESULTS AND DISCUSSION

Serum and egg yolk antibody titres against IBD were studied in broiler breeders and serum and yolk sac of the day-old chicks from the same breeders.

Serum and egg yolk antibodies

Antibody titres in the serum and egg yolk of ten parent flocks have been summarized in Table 2. There was a considerable variation in the titres of birds from various flocks and even the birds from the same flocks. Different chicken lines are also likely to vary in their responsiveness to various vaccines of IBD. Geometric mean titre of various flocks varied from 9 to 59 in serum and 20 to 127 in the egg yolks. This wide variation in the antibody titres in the birds of the same flock might be due to inadequate vaccination procedural factors (age of the birds, management and feeding patterns) practiced by different farmers (Panisup *et al.*, 1984; Hahnewald *et al.*, 1989; Ismail and Saif, 1991; Akhtar *et al.*, 1992) and various immuno-suppressors present in the feed as well as in drinking water (Arshad *et al.*, 1993).

Determination of antibody titres in the egg yolk have been documented as a satisfactory method for screening of the breeders (Brown *et al.*, 1989, Zang *et al.*, 1987; Gassmann *et al.*, 1990). This procedure avoids unnecessary disturbance of breeders by pricking intravenously. The GMT of antibody tires in egg yolk against IBD almost correlated with the titres in the serum. The correlation of the titres in serum samples and egg yolk has also been indicated by Brown *et al.* (1989). Silim and Venne (1989) reported this correlation for IBD as r=0.9 and in the present study, this correlation was 0.916. Analyzing the results of antibody titres in the serum and egg yolk of parent flocks of various age groups, the values did not reveal any significant difference. However, post-vaccination time has a drastic effect Sharma *et al.* (1989). The maximum GMT (127) was recorded 4 weeks post-vaccination (Table 3).

The tires were maximum at 4 weeks post-vaccination and at 8 weeks post-vaccination, showed a drop in GMT. However, there was a slight increase in the flocks tested 12 and 42 weeks post-vaccination. This increase most probably may be due to some field exposure. Other factors like most favourable conditions for the persistence of antibodies may not be negated. A number of vitamins and minerals have been reported to assist the immune response of the birds (Fundenberg and Wybran, 1982). Generally, in the field, after the initial vaccination of chicks, during Ist week of 24 weeks, but to maintain a protective antibody titre in the chick, it is recommended that the breeders should be re-vaccinated again between 40 and 45 weeks of age.

Maternal antibodies in day-old chicks

Antibody titres in the serum and yolk sacs of day-old chicks which came from the eggs set during the same days when the sera were collected from the parent flock are presented in Table 4.

The comparison of tables 3 and 4 reveals that the titres of both serum and yolk were comparatively lower in chicks than the corresponding titres in the parent flocks (r=0.80). It has been reported by Fahey *et al.* (1987) that almost 45% of the antibody titre is transmitted to the next progeny, so the flocks having GMT more than 64 should be considered safe for the protection of the chicks, GMT in yolk sac of the chicks revealed relatively higher values than the serum titres of the respective chicks. There was quite significant difference ($P < 0.05$) in the GMT in yolk sac of chicks from different breeding flocks. Egg yolk antibody titres were quite high (GMT 37) 3 weeks post-vaccination which showed an increase in a flock 4 weeks post-vaccination. Titres of 1:32 is considered protective for the chicks upto 2-6 weeks of age (Menendez *et al.*, 1986; Knezevic *et al.*, 1987). Early vaccination in the presence of these high antibody titres may be one of the factors of the outbreak of IBD in many of the vaccinated broiler and layer flocks. Similar speculations have also been given by Zaheer and Muneer (1992).

The nature has blessed the day-old chicks with the yolk sac, not only for nourishment but also as an important reservoir for different immunoglobulins for the protection against various prevalent diseases (Kaspers *et al.*, 1990). In the present study, the antibody titres in the yolk sac were quite high (2-3 times) than the corresponding values in the serum of day-old chicks. Similar findings have also been reported by Brown *et al.* (1989). The immunoglobulins are constantly pouring

antibodies into the sera for the protection of susceptible chicks (Lucio and Hitchner, 1980). Infection of yolk sac leads to omphalitis which usually results in the hatcheries due to poor sanitary conditions and is quite prevalent under out conditions may be an important factor for the depletion of this important immunoglobulin reservoir and thus leaving the poor chicks susceptible, not only to IBD but also to other infectious diseases.

Age of the breeder flock did not affect the antibody titre in serum and the yolk sac but the post-vaccination interval had an important bearing. It can be inferred as per findings of this study that antibody titres in the sera and egg yolk of breeder correspond the titres in the next progeny. Considering this a baseline, the vaccination schedule of the commercial broiler and layer chicks may be formulated. It would be better if similar studies may be carried on Specific Pathogen Free (SPF) flocks.

Table 1: Distribution of flocks on the basis of breed, age and vaccination schedule

Flock No.	Breed	No. of Birds	Age of Birds (weeks)	Age at last Vaccination Against IBD (weeks)	Sampling Period PV (weeks)	Egg Production (%)
1.	Indian River	3500	26	23	3	8
2.	Lohmann	8000	65	40	25	65
3.	Hubbard	3600	44	40	4	78
4.	Hubbard	3500	50	27	23	60
5.	Lohmann	3300	50	27	23	78
6.	Arbor Acres	3500	35	12	23	75
7.	Hubbard	3300	65	23	42	70
8	Indian River	4630	31	23	8	71
9	Arbor Acres	3080	31	23	8	60
10	Indian River	4180	35	23	12	65

Table 2: IBD antibody profiles in serum and egg yolks of broiler breeder parent flocks

Flock No.	Number of sera/egg yolk showing antibody titre								GMT Sera/Egg Yolk
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
1.	-/-	-/-	1/1	7/2	2/2	-/4	-/1	-/-	17/37
2.	-/-	-/-	2/1	5/1	3/4	-/3	-/1	-/-	17/37
3.	-/-	-/-	-/-	1/-	1/-	6/3	2/4	-/3	59/127
4.	-/-	-/1	5/2	-/3	5/2	-/1	-/1	-/-	16/20
5.	-/-	4/-	2/3	2/1	2/4	-/2	-/-	-/-	9/23
6.	1/-	-/-	2/1	2/2	1/4	1/2	1/1	-/-	22/52
7.	-/-	-/-	-/-	-/1	4/2	6/4	-/-	-/3	48/73
8.	-/-	-/1	2/1	4/1	3/3	1/4	-/1	-/-	18/39
9.	-/-	-/-	-/1	2/1	6/4	2/3	-/1	-/-	30/37
10.	-/-	-/-	-/-	4/3	4/2	2/5	-/-	-/-	28/37

Table 3: IBD antibody profiles in serum and egg yolks of parent flocks at various weeks post-vaccination (PV)

Sampling time postvaccination (weeks)	Flock. No	Number of sera/egg yolk showing antibody titre								GMT Sera/Egg Yolk
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
3.	1	-/-	-/-	1/1	7/2	2/2	-/4	-/1	-/-	17/37
4.	1	-/-	-/-	-/-	1/-	6/3	2/4	-/3	-/-	59/127
8.	2	-/-	1/-	2/2	6/2	9/7	3/7	-/2	-/-	23/38
12.	1	-/-	-/-	-/-	4/3	4/2	2/5	-/-	-/-	28/37
23.	3	1/-	4/1	7/5	4/6	10/10	1/6	3/2	-/-	15/26
25.	1	-/-	-/-	2/1	5/1	3/4	-/3	-/1	-/-	17/37
42.	1	-/-	-/-	-/-	4/1	6/2	-/4	-/-	-/3	24/73

Table 4: IBD antibody profiles in serum and yolk sac of day-old chicks from the respective parent flocks

Flock No.	Number of sera/egg sacs showing antibody titre								GMT Sera/Yolk Sac
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
1.	-/-	3/-	2/1	1/2	3/2	1/2	-/3	-/-	13/42
2.	-/-	2/-	1/1	2/1	3/5	1/2	-/1	-/-	16/34
3.	2/-	1/-	2/-	2/1	2/6	1/2	1/1	-/-	12/39
4.	2/-	-/-	2/1	3/2	2/4	1/3	-/-	-/-	12/30
5.	2/-	-/1	2/-	3/3	2/2	1/3	-/1	-/-	12/32
6.	-/-	2/-	3/2	4/4	1/2	-/2	-/-	-/-	10/28
7.	-/-	-/-	3/-	6/3	1/5	-/2	-/-	-/-	14/30
8.	2/-	3/-	2/1	1/1	2/5	-/2	-/1	-/-	7/34
9.	-/-	2/-	4/1	2/2	2/6	-/1	-/-	-/-	11/26
10.	-/-	1/-	4/1	4/2	1/4	-/3	-/-	-/-	11/30

REFERENCES

Abdu, P.A., 1986. Infectious bursal disease immunization failure in chickens in Nigeria. Trop Anim. Hlth. Prod., 18: 123-135.

Akhtar, S., S. Shahid and A. Hussain, 1992. Relative susceptibility of six broiler chicken strains to infectious bursal diseąse during an epidemic. Pakistan Vet. J., 12: 18-20.

Arpin, C., A. Silim and D. Venne, 1991. Kinetics of serum antibodies and yolk antibodies to four varuses in five broiler breeder flocks. Annales de Med. Vet., 135: 377-380.

Arshad, S., M.Z. Khan, M. Siddique and M.T. Javed, 1993. Studies on enzyme levels residual effects of aflatoxins in experimentally induced mycotoxicosis in broiler chicks. Indian Vet. J., 70: 898-902.

Bansal, R.P., R.C. Sharma, B. Bandy, S.K. Opadhyay and U. Chandra, 1986. Single radial haemolysis test for the detection of rinderpest antibodies. Anim. Hlth. Prod., 18: 133-136.

Brown, J., S. Resurrection, T.G. Kickson and A. Horne, 1989. The relationship of egg yolk and serum antibody: Infectious bursal disease virus. Avian Dis., 33: 654-656.

Fahey, K.J., J.K. Crooks and R.A. Fraser, 1987. Assessment by ELISA of passively acquired protection against infectious bursal disease virus in chickens. Australian Vet. J., 64(7): 203-207.

Fundenberg, H.H. and J. Wybran, 1982. Experimental immunotherapy. Basic and Clinical Immunology. 3rd ed., Los Angeles, California, Lang med. Pub.

Gassmann, M., T. Wwiser, P. Thomas and U. Hubscher, 1990. The egg yolk as a rich source of polyclonal antibodies. Schweizer Archiv. For Tierheikunde. 132: 289-294.

Hahnewald, R., R. Paesch and K. Ziedleir, 1989. Dynamics of antibody formation after vaccination of parent birds of laying lines against IBDV. Mon. Fur., Vet. 14: 233-235.

Ismail, N.M. and Y.M. Saif, 1991. Immunogenicity of infectious bursal disease virus in chickens. Avian Dis., 35: 460-469.

Kaspers, B., I. Ischranner and U.I. Osch, 1990. Immunoglobulin A in the yolk of chicken eggs. J.Anim.Phy.Anim. Nutr.,63: 30-37.

Knezevic, N., D. Rogan, V. Matovic and B. Kozlina, 1987. Persistence of maternal antibodies in the yolk and serum of chicks from parents vaccinated and infected with infectious bursal disease virus. Beterinarsk Glasnik, 41: 767-773.

Komine, K.I., H.Ohta, H. Fujii, Y. Watanabs, S. Kamata and M. Sugiyama, 1995. Efficacy of subcutaneous application of live infectious bursal disease vaccine in young chickens with maternally derived antibodies. J. Vet. Med. Sci., 57: 647-653.

Lee, I.H. and Y.P. Hin, 1992. A monoclonal antibody capture, enzyme linked immunosorbent assay for detecting antibodies to infectious bursal disease virus. J. Virol. Method, 36: 13-23.

Lucio, B. and S.B. Hitchner, 1980. Immunosuppression and active response by infectious bursal disease virus in chickens with passive antibodies. Avian Dis., 24: 189-196.

Menendez, N.A., M.A. Petrucceli, M.V. Piscopo, M.A. Risso, C.J. Perfumo, E. Brandettl and A.J. Garin, 1986. Studies of maternal antibodies derived from broiler breeders given a combined absorbed oil emulsion vaccine against infectious bursal disease and Newcastle disease. Revista de Medicina Veterinaria, Argentina, 67(1): 26-28.

Panisup, A.S., K.C. Verma and G.C. Mohanty, 1984. Age resistance of chickens against infectious bursal disease. Acta Veterinaria Scandianavia, 25(4): 561-566.

Sharma, J.M., J.E. Dohms and A.L. Metz, 1989. Comparative pathogenesis of serotype I and variant serotype I isolates of IBDV and their effect on humoral and cellular immuno-competence of SPF chickens. Avian Dis., 33: 112-124.

Siddique, M., T. Javed and M.A. Sabri, 1987. Incidence and pathology of various diseases prevalent in Faisalabad and surrounding districts. Pakistan Vet. J., 7(4): 148-154.

Silim, A. and D. Venne, 1989. Comparison of egg yolk and serum antibody titres of four avian viruses by enzyme linked immunosorbent assay using paired field samples. Avian Dis., 33: 643-648.

Zaheer, A. and M.A. Muneer, 1992. Role of maternal antibodies in protection against infectious bursal disease (IBD) in commercial broilers. M.Sc. Thesis, Deptt. of Vet. Micro. C.V.S., Lahore.

Zang, R.K., J. Wang and R. Xiao, 1987. Rapid detection of infectious bursal disease antibodies in chicken yolk using counter immunoelectrophoresis. Chines J. Vet. Sci. Tech., 5: 35-36.