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Passive Immunization Against Infectious Bursal Disease in Chicks

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ABSTRACT

Infectious bursal disease (IBD) is a common problem in commercial unvaccinated birds, which is causing heavy economic losses to the poultry industry. Chicken layers (primed with oil based IBD vaccine at age of 13 weeks and boosted with the same vaccine at 15 weeks of age) showed high titre of yolk agar gel precipitating (AGPT) antibodies against IBD virus when tested on 21 and 28 weeks of age. Storage temperature (+4 or -20ºC) had undetectable effect on physical property (color and smell) and AGPT antibody titer of 0.5% formalin (V/V) containing hyperimmunised yolk. The AGPT antibody titer of the hyperimmunised yolk had good correlation with the enzyme linked immunosorbant assay (ELISA) titer of anti-IBD virus antibodies (r: 0.92). The IBD infected broilers (28 days old) when passively immunised with the yolk (one ml: 64 AGPT units of IBD antibody titre) induced 80% recovery while all the control (untreated) birds died. It is anticipated that the hyperimmunised yolk may be used as a therapeutic agent to cure the IBD infected birds.

Key Words: Passive immunization; Infectious bursal disease; Chicks

INTRODUCTION

Infectious Bursal Disease (IBD) is a formidable disease of poultry caused by IBD virus. The disease is common in layers, broilers and rural poultry during early periods of life (Anjum *et al*., 1994). Outbreaks in unvaccinated broilers and layers cause upto 100% morbidity and 50-80% mortality (Qureshi, 1999), heavy economic losses to the poultry industry (Chettle *et al*., 1989) and severe consternation to the poultry farmers.

Since there is no effective managemental remedy available for control and prevention of IBD, the disease is largely controlled by mass scale vaccination of chickens. However, the disease is not uncommon even in the vaccinated flocks (Muhammad *et al*., 1996). The failure in the induction of protective immune response may be due to a number of reasons including immuno-suppression caused by the vaccinal strains of IBDV themselves, faulty immunization techniques and exposure to more virulent field IBDV strains.

Early chick mortality could be reduced by rearing day-old chicks with high level of maternal antibodies which is possible by vaccinating breeders with oil based IBD vaccines at least 15 days before the point of lay (Hahrewald *et al*., 1989). Passive use of hyperimmune serum in ailments such as in Foot and Mouth Disease (FMD), tetanus and canine viral diseases is well documented (Rimmelzwaan & Osterhaus, 1997). Although, information using immunoglobulins in ailment of chicken diseases is scanty, but Yushen *et al*. (1997) have attempted hyperimmunised yolk for curing IBD infected birds with promising results.

This paper describes production of hyperimmunised yolk and its effect on the IBDV infected broilers under local managemental and climatic conditions.

MATERIALS AND METHODS

Production of hyperimmunized yolk. Ten Golden Breeder Chicken Layers (10 weeks old) were purchased and reared in cages in an experimental room, Microbiology Section, College of Veterinary Sciences, Lahore. The layers were primed with oil-based IBD vaccine (0.5 ml dose/bird with a subcutaneous injection at neck) at 13 weeks of age. On 14 days postpriming, the birds were boosted with the same vaccine as previously. At 21 and 28 weeks of age, the eggs were collected and the yolk of each egg was separated and processed for titration of IBD antibodies through agar gel precipitating test (AGPT) and Enzyme linked Immunosorbant assay (ELISA) as described by Piela *et al*. (1985). The relationship of the antibody titre determined through AGPT and ELISA was worked out as described by Nicholas *et al*. (1985).

Effect of storage on hyperimmunised yolk. The yolk (75 ml) containing four AGPT units antibody titer was mixed with formalin (Formaldehyde: 37% :Merck at rate of 0.5%: v/v). The sample was divided into three parts and each part was further subdivided into five replicates. Each was stored at room temperature (24ºC), 4ºC and -20ºC. On 0, 7, 14, 21 and 28 days post storage, the yolk samples (each time three samples, one from each storage temperature) were removed, brought to room temperature and processed for AGPT (qualitative assay). Each of the remaining samples was checked for any smell or change in color and discarded.

Sterility test. The hyperimmunised yolk was diluted aseptically in saline solution (0.85% sodium chloride aqueous solution) to achieve dilutions containing undetectable, 32, 64, 128, and 256 units of anti-IBDV antibody titre. Each of the dilution was admixed with formalin at rate of 0.5% (v/v). After 24 hours incubation at 30ºC, a loopful material of each

dilution was inoculated in sterilised nutrient broth (Difco, 5 ml/vial). The vials were examined for bacterial growth after 48 hours of incubation at 37ºC. No growth indicated that the yolk was sterile.

Effect of hyperimmunised yolk on IBDV infected birds. A commercial broiler farm with problem of IBD was visited. Antemortem and postmortem examination of the birds was performed. The bursa of fabricious from five dead birds was collected and processed for confirmation of the disease through AGPT. The homogenized bursal tissue (1:4) was used as AGPT antigen. The sick birds (28 days old) on the farm were divided into A, B, C, D and E groups. Birds of each of these groups were treated with diluted yolk containing undetectable, 16, 32, 64, 128 units of anti-IBDV antibody titer, respectively. These birds were examined for any mortality for 10 days post-immunization and results were recorded.

RESULTS AND DISCUSSION

Active immunization of birds with an oil based IBD vaccine induced detectable level of anti-IBD antibodies. The same method is used to induce hyperimmunised serum in animals and birds (Anonymous, 1992). The oil based vaccine make a depot at the inoculation site and released slowly through antigen processing cells (APC) such as macrophages (Unanue, 1984). The APC phagocytose, process, and present the antigens to T and B cells which respond in form of lymphoproliferation and development of effector cells for cytokine production and plasma cells for antibody secretion (Vanio *et al*., 1988). The memory cells when interact with the antigens presented by APC again enter the cycle of antibody production. Depot of the antigen is a source of autoboosting in the birds. The golden layers vaccinated at age of 13 and 15 weeks of age had high level of AGPT and ELISA antibodies in developing yolk when tested at age of 21 and 28 weeks.

The AGPT is used as a diagnostic and qualitative test i.e. to detect the presence of either antibodies or antigen of IBDV (Rosenberger, 1989). This is a crude, economical, reliable, specific test but is less sensitive and time consuming (Shakya & Joshi, 1997). The test is also used for quantitative study of anti-IBDV antibodies in the yolk and titration of the bursal IBDV antigen (Saeed, 1988). The yolk collected at the age of 21 or 28 weeks of the vaccinated chickens had the same level of AGPT antibodies i.e. upto 128 (Table I); while yolk from eggs collected from the layers which had an experience of IBD outbreak had AGPT antibody titer upto 1024. It could be due to persistence of the virus in the tissues (in intestine) of the recovered birds. This virus continuously activates the immunocompetent cells over a long period of time. These results are in line with those of Saeed (1988) who raised antibodies in the parent flock and then determined the egg yolk antibodies which were quite high.

ELISA is a routine test for diagnosis of IBDV in the field conditions and for titration of serum antibodies (Marquardt *et al*., 1980). The AGPT titer of IBDV antibodies is directly correlated with that of ELISA titer in the same yolk sample (r=0.92) and is sensitive to detect ELISA anti-IBDV antibodies in yolk sample containing 0.0625 to four AGPT antibody units (Table II & III). The ELISA is not sensitive to quantitate the ELISA antibodies in the yolk having more than four or less than 0.0625 AGPT antibody units. These results are supported by Denzin and Henrion (1996) who raised antibodies in the egg yolk against a bacterium, *A. salmonicida* and found linear relation between AGPT antibody titer and ELISA antibody titer.

Table I. Agar gel precipitating titer of of infectious bursal disease virus antibodies in hyperimmunised yolk

Group of eggs	No of samples	Distribution of samples on the basis of AGPT titres				
		64	128	256	512	1024
A	10	–	10	–	–	–
B	8	–	8	–	–	–
C	8	–	8	–	–	–
D	10	–	–	–	–	10

A= The eggs were collected from the vaccinated commercial layers on 21 weeks of age (The Golden layer birds were primed at 13 weeks of age and boosted at 15 weeks of age with oil based IBD vaccine (0.5 ml/bird at neck subcutaneously: RM), B= The eggs were collected from the same flock but on 28 weeks of age, C= The eggs were collected from a vaccinated broiler breeder flock on 35 weeks of age, D= The eggs were collected from a commercial flock at 21 weeks of age. This flock had an outbreak of IBD at 35 days of age.

Table II. Enzyme linked immunosorbant assay titer of infectious bursal disease virus antibodies in the hyperimmunised yolk

Serial No.	Dilution	ELISA titer (n=3)	Mean
1	1:32	11739, 14204, 21089	15666.6
2	1:128	13599, 12827, 13685	13333.3
3	1:512	7790, 7237, 7080	07302.3
4	1:2048	3321, 3534, 2948	03267.3

The hyperimmunised yolk with AGPT titer of 4 units was diluted and subjected for ELISA titer.

Table III. Correlation of ELISA titer with AGPT titer of IBD* antibodies in hyperimmunised yolk**

Serial No.	Dilution	ELISA titer (O.D. values)	AGPT** titre (units)
1	1:32	15666.6	4
2	1:128	13333.3	1
3	1:512	07302.3	0.25
4	1:2048	03267.3	0.0625

*ELISA= Enzyme linked immunosorbant assay, **AGPT= Agar gel precipitation test, ***IBD= Infectious bursal disease

The antibodies in the hyperimmunised yolk were resistant to wide range of storage temperature and formalin (Table IV). The hyperimmunised yolk can be stored in refrigerator (+4ºC) or freezer (-20ºC) for more than 28 days. At room temperature (25ºC), fermentation reaction is so strong that the yolk gave bad smell on 7th day post storage and color of the yolk was also changed to white. The reaction could be due to exoenzyme

of the contaminating bacteria or enzymes of the yolk. Thawing of the yolk in boiling water resulted in coagulation of yolk; while it can be thawed at +4ºC or at room temperature/water bath (25ºC).

Table IV. Effect of storage temperature on the physical property and AGPT titer of yolk infectious bursal disease virus antibodies

Storage temperature (ºC)	AGPT titres of IBD antibodies on storage (days)				
	0	**7**	**14**	**21**	**28**
24	+	–	–	–	–
+4	+	+	+	+	+
–20	+	+	+	+	+

The yolk having one unit of AGPT antibodies, was admixed with 0.5% formalin (v/v). The diluted yolk was stored upto 28 days. Each time each sample was processed for AGPT assay (qualitative assay) and physical changes (smell and colour change)

Hyperimmunised yolk is effective to cure the IBDV infected birds (Table V). These results are in line with those of Anonymous (1992), Rimmelzwaan and Osterhaus (1997) and Yushen *et al*. (1988) who used immunoglobulins as hyperimmunised serum or yolk in a number of diseases such as rabies, avian arthritis virus, FMD, IBDV and canine parvo virus infection. The maternally derived yolk antibodies in first few weeks of chicken's life induce protection from infectious diseases such as Newcastle disease (ND), IBDV, and infectious bronchitis virus (Rimmelzwaan & Osterhaus, 1997). Muhammad *et al*. (2001) showed that maternally derived yolk antibodies can control ND in broilers upto three weeks of age. These antibodies neutralize the virus particles and inhibit their replication in the tissues.

Table V. Effect of hyperimmunised yolk on the recovery of infectious bursal disease infected birds

S. No. (n=10)	Antibody titer (AGPT units)	Recovered	(%)
1	Control	0	0
2	16	4	40
3	32	6	60
4	64	9	90
5	128	8	80

The hyperimmunised yolk was diluted to achieve the above mentioned units. Each of the diluted yolk was given to a group of 10 IBD infected birds and the response was recorded for 8 days. The recovery was noted in form of percentage.

It is concluded that hyperimmunised yolk can be produced in layers, diluted, stored and be used to cure IBD infected layers/broilers.

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