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OLATION OF EGG YOLK IMMUNOGLOBULINS (IgY) BY LOROFORM POLYETHYLENE GLYCOL TECHNIQUE AND SAYING OF ANTIBODIES AGAINST AVIAN INFECTIOUS RONCHITIS

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e present research study was conducted to late the chicken egg yolk immunoglobulins Y) by Chloroform Polyethylene Glycol PEG) technique and measure antibody titres inst Avian Infectious Bronchitis (AIB). For purpose 300 eggs of broiler breeder were cured from 10 different flocks (30 eggs from h), at least 3 to 4 weeks postvacciantion inst Avian Infectious Bronchitis (AIB). The k of 3 eggs was pooled and subjected to the lation of IgY by CPEG technique. The centration of purified IgY was quantified by trophotometer and antibody titres against AIB re measured through haemagglutination ibition (H I) test.

an concentration of IgY in different flocks ged from 2.65 to 3.70 g/dl with overall mean .24 g/dl. The geomean antibody titres (GMT) inst AIB ranged from 147 to 388 with nulative GMT of 256. Correlation between centration of IgY and AIB antibody titres was puted to be 0.88.

RODUCTION

yolk of avian species contains a wide variety antibodies acquired either naturally or by immunization with suitable immunogen. The use of chickens as an alternative source for polyclonal antibodies has been described by Polson et al., (1980). The production of specific polyclonal antibodies in the chicken egg yolk provides double benefits. Firstly, these antibodies have protective effect on the newly hatched chicks against different diseases (Yokoyama et al., 1992). Secondly, these may be used for diagnosis and passive immunization of various diseases of poultry, livestock and human (Arshad et al., 1996).

Keeping in view the use of IgY as a diagnostic and therapeutic tool, the present study was conducted to isolate and quantitate IgY. A number of technhiques are used for isolation and purification of IgY (Losch et al., 1986) . Present study was conducted to standardize Chloroform Polyethylene Glycol (CPEG) for the isolation of IgY. Total concentration of IgY was measured and their presence was confirmed by measuring specific antibodies against AIB through haemagglutination inhibition (H I) test.

MATERIALS AND METHODS

Egg Collection:

A total of 300 eggs were collected from 10 commercial broiler breeder flocks (30 eggs from each flock), which were previously vaccinated

against Avian Infectious Bronchitis (AIB), Newcastle disease (ND) and Infectious bursal disease (IBD)). The eggs were collected 3 to 4 weeks post vaccination (PV) against AIB.

Preparation of Egg Yolk Homogenate:

Egg yolk was separated from the rest of egg contents as described by Aslam (1994). Yolks of three randomly selected eggs from the same flock were pooled together in a beaker, and homogenized with double volume of 100 mM phosphate buffer pH 7.6 on magnetic stirrer as described by Polson et al. (1985).

Isolation of Immunoglobulins:

A modified version of Chloroform Polyethylene Glycol (CPEG) extraction method as described by Polson (1990) was applied.

Preparation of 100 Mm Phosphate Buffer pH 7.6:

Phosphate buffer pH 7,6 was made by the method recommended by Rai (1985).

Antigens and Antisera:

The phspholipase C treated AIB virus antigen along with specific antisera were procured from Poultry diagnostic Lab., Rawalpindi. Antigen and anitisera were stored at -20°C till used.

Assaying Of Haemagglutination Inhibition Titres:

HI titre against AIB of each purified egg yolk immunoglobulin (IgY) sample was determined as described by Lashgari and Newman (1982).

Measurement of Total Protein (Immunoglobulins)

The concentration of purified IgY in phosphate buffer pH 7.6 was determined by the colorimetric method as described by Bradford (1976).

RESULTS

The mean concentration of IgY in the egg yolks of various flocks ranged from 2.65 to 3.70 g/dl with cumulative mean of 3.24 g/dl (Table I). When HI test was performed to assay the AIB antibodies in isolated IgY samples, cumulative mean titre of 356 was recorded, while the geomean antibody titres (GMT) were ranging from 147 to 388 in different flocks (Table I).

The results indicated that the birds in some flocks had relatively higher levels of AIB antibody titres than others. Most of the breeder flooks had GMT greater than 200 against Infectious bronchitis virus. This might be attributed to different factors associated with the field exposures or good vaccination. The highest GMT titre recorded was 388 in one flock. Only one of the ten flocks had GMT (147) lower than the protective level.

The eggs collected from the flock H were generally misshaped, having soft shells and very inferior quality of internal egg contents especially the watery albumens, but the presence of high antibody titres and laying of low quality eggs in flock H might be due to field exposure of AIB virus.

When the regression line was plotted on the data comprising HI titres of egg yolk of different

Table (1): IgY concentration and Geomean HI titers of Purified immunoglobulins against intectious Bronchitis in Broiler Breeders eggs.

Flock No.	Number of samples having HAI titer and mean conc. of IgY											
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2084	GMT	Total Protein (g/dl)
A	-	-	-	-	-	2	3	1	1	2	1	274	3.29
B	-	-	-	-	-	1	1	3	2	3	-	362	3.70
C	-	-	-	-	-	2	2	2	3	1	-	239	3.20
D	-	-	-	-	-	-	4	2	2	1	1	315	3.33
E	-	-	-	-	1	1	2	3	2	1	-	208	2.94
F	-	-	-	-	-	1	2	1	4	2	-	338	3.36
G	-	-	-	-	-	2	1	3	2	1	1	294	3.31
H	-	-	-	-	-	1	3	3	1	2	-	256	3.26
I	-	-	-	-	-	1	2	2	2	1	2	388	3.36
J	-	-	-	-	1	3	2	2	1	1	-	147	2.65
Cumulative		-	-	2	14	22	22	20	15	5	256		3.24

flocks with corresponding purified IgY concentration, the value of correlation coefficient (r) was computed out to be 0.88. It narrated a high positive correlation between the two.

DISCUSSION

Polyclonal antisera are usually produced by blood collection and serum separation from immunized mammals. An alternative to this conventional antiserum production is the production of antibodies in chicken eggs. The egg yolk of immunized chicken is a rich and inexpensive source of specific polyclonal antibodies (Gassmann et al., 1990). Like other mammalian only IgG is able to cross the vitelline membrane in case of hen. Failure of young chickens to develop expected levels of immunity after vaccination is often attributed to immune interference from passively acquired maternal antibody, which is transferred from hens to progeny via the egg yolk. Maternal antibody interferes with active immunization, presumably by sequestering vaccine antigen or restricting replication of vaccine virus. To improve immune response in the presence of maternal antibody, it would be desirable to have a readily available source of chickens with uniform and predictable levels of passive immunity. This may be accomplished by intramuscular or subcutanceous injection of immune serum or egg yolk immunoglobulins (Stone et al., 1992).

IgG transfer to the ovarian follicle is receptor dependent which allows the selective transport of all IgG subpopulations presented by the maternal

blood (Locken and Roth 1983). Keeping in view the importance of IgY as a modern diagnostic tool for disease control a convenient and cost effective purification protocol was designed to achieve a flexible means of obtaining purified antibodies against AIB virus. Egg yolk immunoglobulins were purified through CPEG technique. This is a two step procedure, the first step involves the delipidation process of the yolk using the principles of lipid separation and in second step, precipitation of antibodies results in substantial recovery of antibodies from egg yolks with a high degree of purity as assessed by haemagglutination inhibition test and total protein estimation.

The major deterrent for working with egg yolk is the necessity for removing the lipid, which constitutes one-third of the egg yolk before protein extraction. A number of methods are available for extraction the protein from the lipid, most of them do not provide an adequate yield of antibodies for detection by serological tests (Meslar and White, 1978).

The electrophoretic immunochemical (immunodiffusion, immunoelectrophoresis and radial diffusion) methods have been employed to assay for purity only, not for purification of immunoglobulins. Chromatography requires elaborated equipments and is somewhat lengthy and cumbersome technique. For successful results in alcohol precipitation of gamma globulins, strict adherence to specifications of temperature, pH and ionic strength is necessary . Most of the other methods require organic solvents, sophisticated equipment and are time consuming . Meslar and White (1978) concluded that ammonium sulphate precipitation procedure for proteins was not excellent, because some lipoprotein fraction flo and may carry many immunoglobulins with Furthermore, Wallmann et al. (1990) claimed th during the precipitation of IgY by ammoniu sulphate method, a loss of antibody may occur.

Ether and acetone solvent extraction is mor difficult to work with and required overnigh incubation at 37°C . Chloroform extraction yield a clear water soluble fraction in a relatively shor period of time. Nevertheless, the relatively higher titres obtained with the chloroform extracted samples might have ebeen due to the absence of lipid in such samples. Polson et al. (1985) and Afzal et al. (1996) successefully used polyethylene glycol as protein precipitants in the purification of immunoglobulin fractions from the egg yolks.

In present research studies IgY from the egg yolk was purified by CPEG method. High yields of antibodies in the present studies were obtained and it was concluded that the usual extraction of specific antibodies by crude methods may be replaced by CPEG technique. The purification of immunoglobulins by CPEG was found to be quite economical and easier than other methods. Polyethylene glycol has multiple hydroxyl groups which becomes highly reactive in aqueous phase. In aqueous phase it acts as nucleophile i.e. anions and can attract positively charged substances, so it produces electrostatic changes in the water. Therefore, in a PEG medium the hydrophobic interaction of antibodies molecules is considerably enhanced which makes them separate from other proteins of the suspension.

n view of the critical review from the relevant iterature, inference of the study made by Losch et l. (1986) seemed of much significance indicating xclusively the presence of IgG (IgY) in the yolk nder normal instances. The concentration of IgG the fresh egg yolk of an average size was easured to be 3.4 ± 0.89 g/dl. This should have eant that estimation of total protein oncentration in egg yolk gave a rationale for the gG present in the yolk. After immunization of aying hens the IgG antibodies produced are ransferred into the egg follicle. In this way large mounts of specific antibodies can be produced vith little effort .

A number of immunologist had already described he application of yolk antibodies in the detection nd characterization of a large number of tructures, viruses, receptors, etc. Chicken can be sed to determine fecal viruses, as they would roduce no false positive result due to the resence of protein A in the feces (Brandt et al., 1981). The fact that yolk antibodies are exclusively IgG-isotype antibody and the fact that mammalian structures are highly immunogenic in the phylogenetically distinct avian are both distinct advantages.

Purification of egg yolk antibody offers the advantages of greater ease of injection and greater stability on storage. Furthermore, there would seem to be no good reason why the laying hen could not be used as a general source of specific antibody. The antigens used to elicit such antibody which would be transferred to the egg, need not necessarily be confined to avian pathogens so long as the immune response which occurs does not interfere with laying. The oral administration of antigen specific IgG has been reported as a promising method for prevention of gastrointestinal or dental infections. The purification of IgY described here is quite practical and IgY production from immunized hen eggs on a large scale may well promote wider utilization of chicken antibodies from passive immunization therapies and minimize the dependency on antibotics as the drug of choice against many infectious animal and human diseases.

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