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Preparation and Evaluation of Oil Based Egg Drop Syndrome Virus Vaccine

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ABSTRACT

Oil based egg drop syndrome virus vaccine using local isolate (Pak-CVS-1 strain) was prepared and evaluated. The EDS virus was grown in nine days old duck embryos for 96 hours and allanto-amniotic fluid was harvested. A haemagglutination titre of the virus suspension in the allanto-amniotic fluid was $\log2^{10}$, while it's egg infective dose 50 was $10^{-8.4}$ per ml. The virus suspension was inactivated with 0.12% formalin. One part of the virus suspension was mixed with four parts of oil-base. The oil base contained four parts oil-emulsifier (Span-80:ICI), 1 part Tween-80 and 95 parts mineral oil. The vaccine thus prepared was found comparably antigenic with the imported one, in layers as wel as in broilers. The technique for production of an economical and quality egg drop syndrome virus vaccine as well as diagnostic antigen was established.

Key Words: Evaluation; Oil based; Egg Drop Syndrome Virus; Vaccine

INTRODUCTION

Egg Drop Syndrome 1976 (EDS) is a major cause of drop in egg production in ducks, geese and chickens (Van-Eck *et al.*, 1976). EDS, caused by haemagglutinating adenoviruses which are DNA containing particles 74 to 80 nm in diameter and replicate in the nucleus of host cells (Jordan, 1990). The EDS infection in chickens is characterised with loss of shell strength and pigmentation, thin and soft shell, shell-less and mis-shaped eggs (Yamaguchi *et al.*, 1980). The chicks from these infected eggs are latently infected and hence do not develop antibodies against the virus. At around peak egg production, the virus is reactivated, cycle of virus replication starts in the oviduct and induces the drop in egg production.

The seroprevalence study indicated the prevalence of the disease on many non-vaccinated commercial breeding and layer farms in Pakistan (Naeem, 1994; Muhammad & Rizvi, 1997; Saddique & Haque, 1997). Poultry farmers are spending substantial amount of foreign exchange to import costly vaccine to control the disease and diagnostic antigen for seromonitoring of the vaccinated or carrier birds. This paper describes the evaluation of locally prepared oil-based vaccine and formalinised diagnostic antigen from the indeginous isolate of egg drop syndrome virus.

MATERIALS AND METHODS

Propagation and characterization of egg drop syndrome virus. Local strain of EDS virus (Pak-CVS-1), obtained from Microbiology Section, College of Veterinary Sciences, Lahore, was grown in nine days old duck embryos (Senne, 1989). The allanto-amniotic fluid (AAF) and chorioallantoic membrane (CAM) of each embryo was harvested on 96 hours postinoculation. The EDS virus suspension was characterised as described by Muhammad and Rizvi (1997). The AAF and CAM was processed to determine its haemagglutination (HA) titre, egg infective dose-50 (EID 50), inactivation of the EDS virus, safety testing in duck embryos and sterility testing (Solyom *et al.*, 1982; Anonymous, 1993).

Preparation of oil-based EDS vaccine. Oil based EDS vaccine was prepared as described by Yaqub *et al.* (1998). One part of the virus suspension in AAF and CAM, having EID-50 $10^{-8.5}$and HA titre $\log2^{10}$ was mixed in four parts of oil-base. The oil-base contained four parts oil emulsifier (Span-80), 1 part Tween-80 and 95 parts liquid parafin. The virus suspension and oil base was mixed and homogenised in homogenizer for four minutes, packed in plastic bottle and stored at +4°C for stability test.

Efficacy of oil-based EDS vaccine. Sixty, day-old broiler birds were reared for 16 days in the experimental rooms. These birds were divided into three groups (20 birds in each group). The birds of group A were vaccinated with the local vaccine (0.5 ml/subcut in the neck), the birds of group B were vaccinated with imported oil-based (Rhone Poulenc) vaccine (0.3 ml/subcut in the neck, while the birds of group C were kept as non-vaccinated control.

The serum samples were collected from each of the birds of each group on 7, 14, 21 days post vaccination. The HI titer of the sera were determined

using 4 HA unit titre of the virus as described by Allan *et al.* (1978). The geomatric mean titer (GMT) was determined and compared (Villegas & Purchase, 1989).

Economics of the vaccine and diagnostic antigen production. Cost of production of each of the oil-based vaccine (1000 doses) and one ml diagnostic antigen was calculated.

RESULTS AND DISCUSSION

The EDS replicated in duck embryos upto 144 hours post injection. The embryos displayed normal blood vessels and embryonic motility. The reason of death of some of the embryos on different times of post-inoculation can not be exactly explained. Presumably, the virus agglutinates the host erythrocytes which might be incriminated to be the cause of embryonic death. The AAF harvested from the embryos showed agglutination of embryonic erythrocytes. Centrifugation of the fluid removed the clumps but might have resulted in loss of some of the virus. To avoid such loss of the virus, the inoculated embryos were chilled at +4 ^{0}C on post-incubation for 24 hours. This practice resulted recovery of clear AAF from the embryos. This fluid was having HA titer of 1:8192 to 1:23768. Propagation, as measured by HA production, was better in duck kidney, fibroblast and liver cells, than in fowl cell cultures, while growth in turkey cells was limited to kidney and liver cultures. There was no evidence of growth in a range of mammalian cells (Adair *et al.* 1979).

Formaldehyde, when admixed in the EDSV suspension at rate of 0.12%, inactivated the virus in 24 hours incubation at 37°C. The safety test in duck embryos and laying hens showed that the formalin is effective viricidal. However, formaldehyde (0.5%), glutaraldehyde (0.5%), b-propiolactone (4200 mg/ml) and Ethylenimine (23.2 mmol concentration) are effective to prolong the HA potential of the inactivated EDS virus (Solyom *et al.*,1982; Takai *et al.*, 1984).

Inactivated virus suspension, when injected to the birds, presumably gets absorbed from the inoculation site within few hours without suitable stimulation to immunocompetant cells. Moreover, such antigen might have not been processed by antigen processing cells (APC), hence development of suitable number of plasma cells or memory cells failed. Such birds also fail to show amnestic response to boosting with oil-based vaccine (Yaqub *et al.*, 1998). Therefore, to potentiate the immunity against such antigens, adjuvant (oil base) was added in the inactivated virus suspensions. The adjuvants are of various kinds such as mineral salts, oils, hydrophilic and hydrophobic block polymers, hydrocarbons, surface active agents liposomal membranes, lipopolysaccharides, each has different way of immunopotentiation (Dalsgaard, 1987). Oil-based or non-oil based adjuvants are common in many veterinary vaccines and induce a vaccine depot at the inoculation site (Stone *et al.*, 1983). One major constraint in developing oil-emulsion vaccines is the difficulty of preparing stable water-in-oil emulsions with low viscosity. The adjuvant effect of these vaccines depends on a stable emulsion of the water-in-oil type and low viscosity is essential to assure injectability and ease of handling, particularly in cold climates. There is a great influence of composition of the emulsion on its viscosity. Viscosity reduction through reduction of the aqueous-phase volume is achieved at the expense of an increased dilution factor for the antigen. Therefore, concentrations of the antigen in the aqueous phase must be higher to retain vaccine potency. In addition to emulsion composition, the mechanical method used to emulsify the aqueous and oil phases also influences the physical characteristics of emulsions (Becher, 1957). The emulsifiers such as Arlacel-A (mannide monooleate) and Span-80 (sorbitan monoleate) are used as 10% in mineral oils (Stone *et al.*, 1983). Addition of this mixture in aqueous phase of antigen resulted in milky white product (oil-based vaccine). Addition of surfactant (Tween-80) in the oil-base reduced the viscosity of the vaccine. The stability of the vaccine depended upon concentration of the oil emulsifier (Table I).

Table I. Effect of oil emulsifier concentration on the stability of the vaccine

Concentration of the emulsifier (%) in oil base	Physical nature of the vaccine
10	Separation of fluid at the top
4*	Uniformly homogenised
2.5	Separation at the bottom

*=This vaccine was used in all the future experiments. The vaccines were stored at 4°C and their stability was recorded.

In the present study, the vaccine had an aqueous-to-oil ratio of 1:4. The aqueous-to-oil ratios vary a great deal but the most commonly used values are 1:1, 1:2 and 1:4 (Stone *et al.*, 1983). This vaccine induced 223 and 137 HI antibody titres in broilers on 14 and 21 days postvaccination respectively (Table II). While titres more than 128 in the vaccinated birds on 21 days post

vacination qualify the efficacy of the vaccine (Anonymous, 1993). Adjuvant containing vaccines cause irritation, recruit immunocompetent cells (lymphocytes and antigen presenting cells at the injection site leading to a noticeable grnuloma (Unanue, 1984). These cells phagocytose, process and present the antigen on their surface in association with self immune associated (Ia) antigen (Vanio *et al.* 1988). The thymus dependent lymphocytes (T-cells, an important component of the cell mediated immunity) can only recognize the antigen when presented on the surface of antigen presenting cells (APC) with Ia antigen (Vanio *et al.* 1988). Such antigen-stimulated T-cells transform into lymphoblasts and populate in different primary and secondary lymphoid organs such as spleen, mucous membrane associated lymphoid tissue, etc. (Vanio *et al.* 1988; Muhammad *et al.* 1994). These cells secrete lymphokines which potentiate the activity of Bursal dependant lymphocytes (B-cells). Layer chickens (15 weeks old) showed antigenic response to locally prepared oil based EDSV vaccine (Table III). Aforementioned immunomechanism might be a logical reason of high level of HI antibodies for long time in the sera of the layers vaccinated with adjuvant containing local vaccine. The inactivated EDS vaccines containing oil-base as an adjuvant have been proved to be effective in layers (Baxendale *et al.*, 1980: Rampin *et al.*, 1980: Zanella *et al.*, 1980). These results are in agreement to Calnek *et al.* (1991) who has reported that oil-based EDSV vaccine induces HI titres upto 256 in uneffected birds while HI titres upto 16384 in previously exposed birds.

The price of the vaccine depends on the cost of duck embryos, oil base components, depreciation cost of instruments and building and indirect expenditures. The price of duck embryos varies depending on the prices of the feed. The price of the emulsifier (oil-base) is Rs 120/Kg at the source of production but in Pakistan, it costs Rs 2000.00/Kg. Cost of production of 1000 doses of EDS vaccine was calculated as Rs 463.00 (this does not include the depriciation cost of instruments and building) in contrast to Rs. 1650.00 for the similar number of doses of imported oil based EDS vaccine. The split prices of different items used for preparation of vaccine are given below:

1. Cost of 30 fertile embryonated duck eggs=120 ml AAF/30 eggs

($10^{-8.4}$ EID_{50})

Total AAF =120 ml @ Rs. 10/embryo Rs. 300.00

Table II. Comparative antigenic response of broilers to local and imported oil-based egg drop syndrome virus vaccines

Source of Vaccine	Time post-vaccination (days)	Distribution of birds on the basis of EDS-antibody titres								GMT
		2^{-0}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	2^{-9}	2^{-10}	
Local	07	16	0	0	0	0	0	0	0	0
	14	0	0	1	0	5	6	4	0	222.9
	21	0	0	3	0	7	4	2	0	137.2
Imported	07	16	0	0	0	0	0	0	0	0
	14	0	0	0	1	3	3	5	4	415.9
	21	0	2	2	0	4	5	1	2	147.0

Table III. Immune response of layers to oil-based local egg drop syndrome virus vaccine

	Weeks Post-vaccination	Distribution of birds on the basis of HI titres							GMT
		2^{0}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	
Vaccinated birds	04	0	0	0	2	1	5	0	84.4
	08	0	0	1	2	4	1	0	48.5
	12	0	1	1	2	2	2	0	36.8
Un-vaccinated birds	04	0	0	0	0	0	0	0	00.0
	08	0	0	0	0	0	0	0	00.0
	12	0	0	0	0	0	0	0	00.0

2.	Liquid paraffin 370 ml	Rs. 040.00
3.	Tween-80 10 ml	Rs. 020.00
5.	Span-80 40 ml	Rs. 018.00
6.	Cost of bottle+cover,	Rs. 004.00
7.	Label	Rs. 001.00
8.	Indirect cost	Rs. 080.00
	Total	**Rs. 463.00**

Similarly, highly economical diagnostic antigen was prepared. An average of 5 ml of the AAF was harvested per embryo but each of the chorioallantoic membranes and embryos was admixed with 10 ml normal saline, ground and filtered. This 20 ml tissue material along with 5 ml AAF made total volume of 25 ml EDS virus suspension. The mixture of these components contained more than 1448 HA activity (log $2^{10.5}$). The cost of one ml antigen was Rs 2.00/ml. This antigen was sufficient to monitor 1000 serum samples. The price of the imported antigen varies from Rs 600 to 2200/one ml vial which is also sufficient to monitor 1000 serum samples. The split prices of different items used for antigen preparation are given below:

1.	Cost of one duck embryo	Rs. 010.00
2.	Indirect Cost	Rs. 030.00
3.	Label+vial	Rs. 010.00
	Total cost for 25 ml antigen	**Rs. 050.00**
	Total cost of one ml antigen	**Rs. 002.00**

CONCLUSION

The locally prepared EDS vaccine as wel as diagnostic antigen is comparably effective and more economical to the imported one.

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