

Evaluation of Egg Drop Syndrome Virus Vaccines by Measuring Antibody Levels in Egg Yolk in Layers

M.A. ILYAS, I. HUSSAIN, M. SIDDIQUE, M.H. RASOOL, M.K. MANSOOR AND S. MANZOOR
Department of Veterinary Microbiology, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

Two inactivated vaccines were prepared from locally isolated and characterized egg drop syndrome (EDS) virus using mineral oil and aluminium hydroxide as adjuvants. The immunogenicity of oil-based, alum-adsorbed and a commercial oil-based vaccine were determined in commercial layers. A total of 40 commercial layers of 16 weeks of age were procured and divided into four groups i.e. A, B, C and D having 40 birds in each group. The birds of group A and B were vaccinated with 0.5 mL locally prepared oil-based and aluminium hydroxide gel adsorbed EDSV vaccines, respectively. A 0.5 mL of commercial oil-based vaccine (Nobilis, Intervet) was injected to the birds of group C. The group D was kept as unvaccinated control. The eggs were collected at 2, 4 and 6 weeks post-vaccination and haemagglutination inhibition (HI) test was performed from egg yolk. The maximum HI antibody titer (512) with GMT as 222.74 was obtained in group B at 4 week post-vaccination. In group A and C, maximum titer was 256 with GMT of 146.96 and 127.94 at 4 weeks post-vaccination, respectively. The GMT was lower at 2 weeks post-vaccination and further decreased at 6 weeks post-vaccination in all three groups. The antibody titer and GMT was zero in group D. A 50% of birds in each group were challenged at 22 weeks of age with virulent EDSV. The protection was 93, 96.5, 89.5 and 0% in group A, B, C and D, respectively. There was neither any drop in egg production, nor any change in egg quality in vaccinated groups. There was sudden drop in egg production with miss-shaped, soft-shelled and shell-less eggs in control group.

Key Word: Egg drop syndrome; Aluminium hydroxide; Oil-based; Vaccines; Haemagglutination inhibition; Egg yolk; Layer

INTRODUCTION

Egg drop syndrome (EDS) is the major cause of loss of egg production through out the world. It is posing a serious threat to layer industry world wide. The infected birds lay soft shelled, shell less, discolored and mis-shapen eggs without any change in the internal quality. In acute cases, there may be mild depression, however feeding, watering and general appearance of the affected bird's remains normal (Van Eck *et al.*, 1976; Yamaguchi *et al.*, 1980). The EDS is caused by haemagglutinating duck adenovirus-I which is a DNA virus, 74 to 80 nm in diameter and replicate in the nucleus of the host cells. Recently EDS virus has been placed in a new genus atadenovirus of family adenoviridae with bovine adenovirus type-7 and ovine adenovirus isolate 287 due to its close phylogenetic relationship with these viruses (Regenmortel *et al.*, 2000). EDS-76 virus may infect ducks, geese and fowls. Following experimental infection of chickens, the virus grows poorly in the nasal mucosae followed by viremia with viral growth in the lymphoid tissues. After 8th day of infection there is massive growth of virus in the pouch shell gland areas of oviduct with production of aberrant eggs. Diarrhea is mostly due to excessive oviduct secretion in the droppings. The hatchability of egg is also reduced (Brugh *et al.*, 1984). There is intermittent secretion of virus in droppings and disease may propagate in poultry houses up to 11 weeks after initial out break. The virus survives in the litter of an infected poultry house for many weeks. Duck family, both domestic and wild may act as a carrier and play a vital role

in the transmission of disease (Calnek *et al.*, 1991). The EDS virus haemagglutinate only avian but not mammalian erythrocytes. The most sensitive indicator system for EDSV growth is embryonated duck eggs. EDS-76 virus produces very high titer of haemagglutination when inoculated into the allantoic cavity of duck or geese eggs (Adair *et al.*, 1979). The haemagglutination inhibition (HI) is the test of choice for diagnosis of EDSV infection (Adair *et al.*, 1986).

The most susceptible age for EDS in layers is between 50% to peak egg production i.e. 17 weeks. For the prevention, an imported oil adjuvanted vaccine (EDS-76 Nobilis Intervet) is widely used and gives good protection against clinical disease. The birds are usually vaccinated between 14-16 weeks of age and antibodies can be detected by the 7th day with peak titers between 2nd and 5th week post-vaccination (Solyom *et al.*, 1982; Christensen, 1998).

The aluminium hydroxide gel adsorbed EDS-76 vaccine had advantages over the oil adjuvanted vaccine that it is easy to inject, induce less inflammatory response at the site of injection and economical to produce commercially (Garg & Garg, 1994). In the present study, oil-emulsion and aluminium hydroxide gel adsorbed vaccines were prepared from local isolates of EDSV to compare their efficacy in layers by measuring antibody levels in eggs.

MATERIALS AND METHODS

Virus preparation. The locally isolated and characterized egg drop syndrome virus (EDSV) was procured from Department of Veterinary Microbiology, University of

Agriculture, Faisalabad (Rasool *et al.*, 2002). A 0.2 mL virus was inoculated in each of 50 embryonated, 11 days old duck embryos through allantoic cavity route (Senne, 1989). After 6 days of incubation, the allanto-amniotic fluid (AAF) was harvested and pooled together. The haemagglutination activity of AAF was determined (Allam *et al.*, 1978). The infectivity of virus was determined by calculating egg infective dose 50 (EID₅₀) (Reed & Munch, 1938).

Inactivation of virus. The AAF was transferred to a 500 mL sterilized glass bottle and formalin was added at a rate of 0.12%. The contents were properly mixed and incubated at 37°C for 48 h. The fluid was transferred to refrigerator and processed for safety and sterility tests.

Sterility test. 10 mL of AAF was centrifuged at 600xg for 15 minutes. The sediment was streaked on Nutrient, Blood and MacConkey's agar and inoculated into mycoplasma broth (PPLO). The agar plates were incubated at 37°C for 48 h while PPLO broth was incubated at 37°C for 10 days and observed daily for microbial growth.

Safety test. A 0.2 mL of AAF was inoculated into 11 days old embryonated duck eggs via allantoic cavity route. After 6 days of incubation, the embryos were chilled in refrigerator. The AAF was harvested and haemagglutination activity was checked (Solyom *et al.*, 1982).

Preparation of oil-based EDSV vaccine. The AAF with EID₅₀ 10^{-9.3} was diluted 10 times in sterilized saline. The diluted fluid was having EID₅₀ 10^{-8.3} and HA titer as 1024. The one part of diluted AAF was mixed with 4 parts of oil base which contained 4 part span-80, 1 part tween-80 and 95 parts liquid paraffin. The mixture was homogenized in homogenizer for 3 minutes. The vaccine was packed in plastic bottles and stored at 4°C for 6 months to check the stability of vaccine i.e. homogeneity of diluted AAF and oil-based (Baxendale *et al.*, 1980; Christensen, 1998).

Preparation of alum-adsorbed EDSV vaccine. The inactivated AAF was used to prepare an aluminium hydroxide gel adsorbed vaccine (Hassan *et al.*, 1992). An aluminium hydroxide gel was prepared by dissolving 1000 mL of 10% potassium aluminium sulphate solution in a flask containing 1000 mL of IN solution of sodium hydroxide. After thorough mixing, a white gelatinous precipitate of aluminium hydroxide was formed which was allowed to settle down at 4°C. The supernatant was discarded and precipitate was reconstituted in distilled water after every 24 h and washed for 3-4 times until complete removal of sulphate ions. The virus was adsorbed on the washed gel and adsorption was checked after 48 h. The pH of the vaccine was checked after every week. The viscosity and stability of vaccine was also observed by storing it at 4°C for 2 months.

Evaluation of vaccines. A total of 40 layers of 16 weeks of age were kept in experimental animal house of the Department of Veterinary Microbiology, University of Agriculture, Faisalabad. They were divided into four groups i.e. A, B, C and D having 10 birds in each group. The birds of group A were vaccinated with 0.5 mL of local oil-

adjuvanted EDSV vaccine through subcutaneous route. The birds of group B were vaccinated with 0.5 mL of local alum-adsorbed EDSV vaccine through intramuscular route. The birds of group C was injected with imported oil-based EDSV vaccine (Nobilis, Intervet) @ 0.5 mL subcutaneously. The group D was kept as unvaccinated control. The 5 eggs were collected from each group at 2nd, 4th and 6th week post-vaccination. The yolk of each egg was diluted in distilled water as 1:4 and haemagglutination inhibition (HI) titer was determined in each sample and Geometric mean titer (GMT) was calculated (Allan *et al.*, 1978). A 50% of birds from each group were challenged at 22nd week of age with virulent EDSV through oral route (Holmes *et al.*, 1989). The protection percentage was recorded in each group.

RESULTS AND DISCUSSION

The locally isolated and characterized EDSV grew well on 11 days duck embryos. The inoculated duck embryos were found to be live 144 h post-inoculation. The haemagglutination titer of harvested allanto-amniotic fluid was 1:1024. The EDS virus mediated haemagglutination did not show elution at 37°C even after 24 h this property might be due to the lack of neuraminidase molecule on the surface of EDS virus (Spalatin *et al.*, 1970). Previous studies also revealed that EDS virus did not grow in chicken embryos rather it grow on duck or geese embryos. This may be due to the fact that the cells in developing chicken embryos are devoid the receptors for adsorption with the ligand of virus which is the primary stage of virus replication (Zsak *et al.*, 1982). The selective growth of virus on duck embryos and its stable haemagglutination property with high virus titer on duck embryos reconfirmed the already characterized local EDS virus. The EID₅₀ of the virus was 10^{-9.3}. The formaldehyde @ 0.12% completely inactivated the virus with 48 h incubation at 37°C. The lack of HA activity of AAF harvested from duck embryos after the inoculation of inactivated virus showed that formalin is an effective virucidal. The higher concentration of formalin presumably mitigates the antigenicity of virus, while lower concentration may necessitate the prolong incubation of formalin-virus mixture. However, formalin is carcinogenic above 12 mg/dose (Solyom *et al.*, 1982).

There was no growth on Nutrient, Blood or MacConkey's agar plates after 24 or 48 h of incubation. Similarly there was no pellicle like growth in PPLO broth even after 10 days of incubation. These showed that the virus suspension was completely inactivated and had no secondary bacterial contamination.

The immunogenicity of vaccines was determined by comparing the HI antibody titer in egg yolk at 2, 4 and 6 week post-vaccination. In group A, the maximum titer was 256 with GMT of 127.94, 146.96 and 111.38 at 2, 4 and 6 week post-vaccination, respectively. In group B, the highest antibody titer was 512 with GMT as 146.96, 222.74 and 127.94 at 2, 4 and 6 week post-vaccination, respectively. In

group C, the maximum titer was also 256 with GMT as 111.38, 127.94 and 96.96 at 2, 4 and 6 week post-vaccination, respectively. The antibody titer and GMT was zero in control group (Table I).

These results showed that in all three vaccines, the maximum GMT was achieved at 4 week post-vaccination. The GMT was less at 2 week and further decreased at 6 week post-vaccination in all three groups. The overall results revealed that locally prepared alum-adsorbed vaccine was most immunogenic followed by local oil-based and imported oil-based vaccines, respectively in terms of HI antibody titers in egg yolk in layers. This was a first attempt to prepare an alum-adsorbed vaccine against EDS virus in Pakistan using the local virus. Hassan *et al.* (1992) prepared an inactivated aluminium hydroxide gel adsorbed Newcastle disease (ND) virus vaccine and an oil-based NDV vaccine. They compared these vaccines with imported oil-based NDV vaccine. They found that both the local vaccines were safe and immunogenic. The oil-based vaccines are unsuitable for field use due to high viscosity of oils.

On 22nd weeks, 50% layers in all four groups were challenged orally with virulent EDS virus. The protection was 93, 96.5 and 89.5% in group A, B and C, respectively. There was neither any change in egg quality nor any decrease in egg production in any of the three vaccinated groups. In unvaccinated control group, there was a sudden drop in egg production. The quality of eggs was also deteriorated as miss-shaped, soft-shelled and shell-less egg was given by the layers in control group. Hassan *et al.* (1992) reported that aluminium hydroxide gel adsorbed vaccine gave 93% protection for 6 months whereas oil emulsified vaccines gave only 80% protection.

On the basis of overall results, it is concluded that local aluminium hydroxide gel adsorbed EDSV vaccine is more immunogenic, safe and economical than local oil-based and imported oil-based EDSV vaccines. There is not a significant difference in antibody titers in all three vaccines but the imported vaccines are very costly compared to local vaccines. Therefore, the indigenous EDS virus isolates should be used in future to produce inactivated EDS virus vaccines locally. These local vaccines will not

only save the foreign exchange but will also be more immunogenic and will definitely help to implement the better disease control programme in the country.

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Table I. Antibody titers against egg drop syndrome virus vaccines in layers

Groups	Time post-vaccination (weeks)	Distribution of birds on the basis of antibody titers								GMT	
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256		1:512
A	2	0	0	0	0	0	2	1	2	2	127.94
	4	0	0	0	0	0	1	2	2	2	146.96
	6	0	0	0	0	1	1	1	2	2	111.38
B	2	0	0	0	0	0	2	1	1	1	146.96
	4	0	0	0	0	0	1	1	1	1	222.74
	6	0	0	0	0	1	1	1	1	1	127.94
C	2	0	0	0	0	1	1	1	2	2	111.38
	4	0	0	0	0	0	2	1	2	2	127.94
	6	0	0	0	0	1	1	2	1	1	96.96
D	2	0	0	0	0	0	0	0	0	0	00.00
	4	0	0	0	0	0	0	0	0	0	00.00
	6	0	0	0	0	0	0	0	0	0	00.00