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Comparative efficacy study of Diftosec-CT® commercial fowl pox vaccine and DLS fowl pox vaccine in layer flock

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ABSTRACT

An investigation was conducted to determine the efficacy of Diftosec-CT®, a fowl pox virus vaccine imported by Advance Animal science co. Ltd., Bangladesh, in comparison to DLS (Department of Livestock Service) fowl pox vaccine in chicks. Six groups of chicks namely A, B, C, D, E and F where group A contained 20 chicks and used to determine the persistence of maternally derived antibody using PHA test. The MDA of chicks of group A showed a protective level of PHA titre at day 1 of age only and then gradually declined to and negligible titre was found from day 16. To compare the efficacy of these two vaccines group B, C, D and E each containing 10 chicks were used for vaccination and group F containing 20 chicks were used as unvaccinated control. DLS-FPV vaccine aged at day 22 and 18 (Group B and Group C) and Diftosec-CT® FPV vaccine aged at day 42 and 22 (Group D and Group E) were administered via IP route with an individual chick dose of 0.1 ml of the test vaccine. Blood samples were collected to obtain sera from each chick at 7, 14 and 21 days of post vaccination for the determination of antibody titre using PHA test. 'Take' reaction was recorded to assess the relationship with better immune response. The average percentage of 'take' reaction following vaccination was manifested 100% birds, 80% birds, 90% birds and 80% birds of groups B, C, D and E respectively. The mean PHA titres obtained from group B were 57.60 ± 8.38, 140.80 ± 31.35, 219.20 ± 18.79 and group C were 51.20 ± 10.61, 128.03 ± 35.05, 204.80 ± 20.90; group D were 51.20 ± 10.61, 134.40 ± 22.27, 217.60 ± 19.55 and group E were 46.40 ± 6.05, 121.60 ± 17.72, 198.40 ± 24.23 after 7, 14 and 21 days of vaccination respectively. Each chick from vaccinated group were challenged with field isolates of virulent fowl pox virus at the dose rate of 10-6 EID50/0.1ml after three weeks of vaccination along with 10 control birds. After challenge, the birds of group B and D showed 100% protection whereas, birds of group C and E showed 90% and 80% protection respectively against field isolates of virulent FPV except control group. It was also observed that the highest mean PHA titre was found in chicks of group B which was vaccinated at day 22 with DLS fowl pox virus vaccine than the chicks of group C, D and E vaccinated with DLS fowl pox virus vaccine at day 18 and Diftosec-CT® at day 42 and 22 respectively.

Introduction

Fowl pox (FP) is a common slow-spreading economically important viral disease of chicks of 4-6 weeks of age. It remains still a major threat to semi-urban and indigenous poultry of Bangladesh. Fowl pox is caused by a double stranded DNA virus belonging to the genus *Avipoxvirus* under the family *Poxviridae* which includes FPV, Turkey Pox Virus (TPV), Pigeon Pox Virus (PPV), Canary Pox Virus (CPV) and Mynah Pox Virus (MPV) (Calnek et al., 1997). Though each member of Avipox virus is host specific as well as antigenically distinguishable, yet a cross relationship has been demonstrated among the species (Calnek, 1997).

The disease is characterized by discrete nodular proliferative skin lesions on non-feathered parts or fibrino-necrotic lesions in mucous membranes of mouth, esophagus with intracytoplasmic inclusion bodies. It terminates with the formation of scabs and desquamation of degenerated epithelium (Calnek et al., 1997; Tripathy and Reed, 1997). Fowl pox is still a malady and an enzootic to the growing chicken of all ages, sexes and breeds either in organized or in backyard poultry farming system in Bangladesh (Siddiky *et al.,* 2004; Siddique *et al.,* 1997; FAO/OIE/WHO, 1995; Khandaker et al., 1994). It occurs most commonly during February to April with a high mortality rate of 30-50% in chicks. Economic losses incurred by this virus due to reduction in weight gain and transient drop of egg production in laying chicken.

Chicks may derive antibodies from parents due to recovery from naturally occurring infection or vaccination of parent chicks with live FPV vaccine under field conditions resulted in variable susceptibility to FP infection, is called maternal antibody. The levels of transferred maternal immunity decreased with the age of parents (Wyeth *et al.,* 1978).

The research work on Avipox virus so far been conducted in Bangladesh are the physical and cultural properties of FPV, experimental preparation of oral FPV vaccine; physicochemical properties of FPV and PPV; physicochemical, antigenic and immunogenic properties of PPV; persistence of maternally derived antibody (MDA); efficacy study of Poxine® vaccine (Novartis Ltd.) in comparison with DLS-FPV vaccine in experimentally reared backyard chicks and efficacy of the experimentally developed pigeon pox vaccine with the isolated

virus in chicken (Amin *et al.,* 1993; Siddiue, 1997; Rahman, 2000; Shil, 2005; Akhter, 2006; Islam, 2007; Das, 2007).

As a means of prevention and control of this economically important contagious viral disease in Avian species, strict bio-security measures along with vaccination in healthy flocks and antibiotic treatment in diseased flocks to prevent secondary bacterial infection are being practiced in any areas of the world in general.

In Bangladesh, chick raisers are acquainted with using DLS-FPV vaccine at the age of 3-4 weeks of age, whereas most of the commercial company including Advance Animal Science Co. Ltd. Bangladesh (Diftosec-CT®), Novartis Bangladesh Limited (Poxine®), Intervet (Nobilis Ovodiphtherin® and Nobilis® A.E+Pox) and ACI Limited (CEVAC® MASS L), suggested vaccinating the chicks at 5-6 weeks of age. But FP is frequently reported in previously vaccinated flock from every corner of the country despite FPV vaccination (personnel communication) causing serious problem to poultry raisers. The increase in incidence of FP might be due to emergence of 'variant' strains or the appearance of previously unrecognized strains of virus or antigenic differences of FPV from attenuated vaccine strains, persistence of MDA which stand on the way to produce immunity, inability of current vaccines to induce adequate immunity in poultry and improper vaccination schedule are also important (Olufemi and Reed, 1996; Singh and Tripathy*,* 2000; Shivaprasad *et al.,* 2002; Singh *et al.,* 2005; Nakamura *et al.,* 2006 and Odoya *et al.,* 2006). Effective and proper vaccination of chicken with a dependable FPV vaccine and the proper schedule of vaccination are the actual means to control the frequent outbreaks of this disease among the chicken population in Bangladesh.

Before introduction of any biological or vaccines in a country it must be monitored by a respective controlling agency. Unfortunately, in Bangladesh though there is the existence of such an agency under the Ministry of Health and Family Welfare but practically this agency is inactive. In this regard, the present research work was designed to study the efficacy of imported FPV vaccine in comparison with DLS-FPV to recommend alternative FPV vaccine to user's level. Keeping the above ideas in mind the present study was conducted to study the persistence of maternally derived antibody (MDA) in experimentally reared chicks and to compare the level of antibody production in chicks vaccinated with DLS fowl pox vaccine and Diftosec-CT® fowl pox vaccine with their protective efficacies.

Materials and Methods

The research work was carried out in the experimental poultry shed of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. Laboratory tests were performed in the Virology laboratory of the same Department of BAU, Mymensingh.

Samples

Fowl pox virus samples

The infected tissues containing scabs or nodules appearing on different parts of the body of chicks (clinical infection) were collected aseptically as per as practicable with the help of scissors, scalpel and forceps and kept in screw capped test tube or vials containing 50% buffered glycerin. The materials were carried out to the laboratory and preserved at -20^{0}C until processed for isolation.

Fowl pox virus vaccine

Lyophilized live attenuated FPV vaccine produced from LRI, Mohakhali, Dhaka, was collected from LRI, Mohakhali and Freeze dried live Diftosec-CT® vaccine was collected from regional office of Advance Animal Science Co. Limited, Mymensingh maintaining cool chain system. The vaccine was stored at 4-8^{0}C until use.

Chicken Eggs

Fertile eggs of white leg horn (WLH) breeds of chicken were procured from Bangladesh Agricultural University (BAU) poultry farm, Mymensingh. These eggs were incubated for 10-12 days at 37^{0}C for the development of embryos. Embryos which were found well developed and active by candling were selected for the propagation of pigeon pox and fowl pox viruses and titration of these viruses.

Experimental birds and rabbit

A total of 80 day-old chicks of Fayoumi, Sonali Breed (SB) and WLH breed were purchased from the BAU Poultry Farm, Mymensingh. These baby chicks were reared in the poultry house of the Department of Microbiology and Hygiene, BAU, Mymensingh. The birds were provided with recommended feed and other management requirements and were reared under strict bio-security measures.

All media and buffers were prepared freshly Antibiotics (Streptopen® Renata Bangladesh Ltd.) was purchased from local market. Apparently healthy adult rabbit obtained from the Department of Animal Nutrition, Faculty of Animal Husbandry, BAU, Mymensingh and selected for the collection of rabbit serum which was used for PHA test.

Experimental design

Eighty day old Fayoumi, SB and WLH chicks were divided into six groups namely A, B, C, D, E and F where group A and F contained 20 chicks but B, C, D and E each group contained 10 chicks. Of these, group A was used to determine the persistence of maternally derived antibody (MDA). Group B, C, D and E were used for vaccination with DLS-FPV and Diftosce-CT® vaccine through usual and suggested schedule of vaccination. Group F kept as unvaccinated control. In order to determine the

persistence of MDA in chicks of vaccinated origin, 20 chicks were reared in Group A. Blood samples were collected for the preparation of sera from randomly selected 15 chicks at day 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34 and 40 of age and subjected of PHA test.

To study the comparative efficacy of Diftosec-CT® and DLS-FPV vaccine following usual and suggested schedule of vaccination in chicks, remaining 40 chicks were divided into four groups namely B, C, D and E and each group contained 10 chicks, Chicks of group B and C were vaccinated with DLS-FPV vaccine applying usual and suggested schedule of vaccination at 22 and 18 days of age respectively, on the other hand, the chicks of group D and E were vaccinated with Diftosec-CT® FPV vaccine applying usual and suggested schedule of vaccination at 42 and 22 days of age respectively.

To determine the antibody response against FPV vaccine, blood samples were collected to obtain sera at 7, 14 and 21 days of post vaccination from the birds of each vaccinated group and at the same time blood samples were also collected 5 birds of unvaccinated control group. Each bird of vaccinated group and 10 from unvaccinated control were challenged with 10^{-6} EID_{50}/0.1 ml virulent Fowl pox virus (FPV) after 3 weeks of age.

Antibody titres of the collected sera were determined by passive haemagglutination (PHA) test to assess the efficacy of experimentally developed FPV vaccine (Siddiky et al., 2004).

Preparation and inoculation of the virus samples for embryo-passage

The virus isolates of FPV grown on CAM was triturated in a pestle and mortar, suspended with PBS followed by treatment with Gentamycin at the ratio of 1:10 (1 ml gentamycin was mixed with 10 ml of virus suspension) and Nystatin at the ration of 10:2 (1 ml nystatin was mixed with 2 ml of virus suspension) and then kept at room temperature for 45 minutes. After treatment with antibiotic, the suspension was checked for bacterial sterility. For this, a small amount (1 ml approximately) of sample was inoculated onto blood agar plates and incubated at 37^0C for 24 hours and then observed for any bacterial growth. The bacteriologically sterile inoculum was used for this study (Siddiky *et al.*, 2004).

Standard CAM route of inoculation (Tripathy and Hanson, 1975; Hannseang, 1981) was followed for propagation and titration of FPV in eggs containing viable 10-12 days old embryos. The inoculated eggs were candled twice daily throughout the period of incubation. Any abnormalities or death of embryos were recorded. The embryos that died within 24 hrs of inoculation were discarded considering the death due to non specific cause.

Collection of Chorio-allantoic Membrane (CAM)

After 5-6 days of inoculation, the embryos which either died or remained alive were chilled in refrigerator at 4^0C to 8^0C for 1-2 hour. After chilling, the eggs were removed from the refrigerator and were kept at room temperature for drying off the moisture of the shell. The egg shell was painted with tincture of iodine over the air cell and then cracked with a pair of sterile scissors. The CAM which showed confluent growth of pocks were harvested with a pair of scissors and a fine pointed forceps maintaining strict aseptic measures. The CAM so collected was put on petridishes containing sterilized PBS and washed for three to four times with PBS and changing of PBS and petridish so as to remove blood, blood vessels or any other debris what so ever. The embryos were also examined thoroughly for any embryopathy (Siddique *et al.,* 1997). The virus growth exhibited by distinct pock lesions on chorio-allantoic membranes (CAM) was finally washed with PBS and transferred to screw capped test tubes and stored at 25^0C for future use.

Preparation of fowl pox virus suspension

The collected CAM and embryo of 5^{th} passage were grinded by sterile pestle and mortar. The sterile PBS was added in sufficient quantity to make a 20% suspension of materials by weight and volume (w/v). The suspension then filtered through a double layer and sterilized gauze cheese cloth to remove the coarse particles. The filtrates were then centrifuged at 2500 rpm for 15 minutes to remove tissue particles as sediment at the bottom of the test tube leaving the virus particles in the supernatant fluid. The supernatant clear fluid was treated with antibiotics as described in the preparation of samples and then tested for bacteriological sterility. The bacteriologically sterile preparation of virus suspension was used as stock virus for challenge exposure test and PHA test.

Determination of EID_{50} of the challenge FPV

This was performed following Reed and Muench (1938) method or Sperman and Karber (Buxton and Fraser, 1977). For this 10 fold serial dilutions ranging from 10^{-1} to 10^{-9} were prepared in sterilized nutrient broth. From each dilution, 0.1 ml was inoculated onto each of the five ten-days-old embryonated chicken eggs via chorio-allantoic membrane (CAM) route. One drop of each dilution was put onto nutrient agar plate to test for bacterial contamination and incubated for 24 hours at 37^0C. For each test, five eggs were kept as control. The eggs were afterwards incubated at 37^0C and candled twice daily for five days to check for embryopathy. Embryos, those died within 24 hours of inoculation were discarded and the mortality of the embryos were recorded. The embryos that died or survived during the period of observations were chilled at 4^0C to 6^0C for two to four hours before collection of CAM. The embryos, dead or alive, were also examined for the lesions and the observations were recorded. The chorio-allantoic membranes of the embryos revealing pock lesions were considered infected and recorded for

calculation of EID_{50}. The EID_{50} of the test vaccines were calculated from the data so obtained.

Vaccination of experimental chicks

Vaccination was done with freeze dried live attenuated FPV vaccine of DLS produced at LRI, Bangladesh and Diftosec-CT® imported by Advance Animal Science Co. Ltd. Vaccination was carried out following conventional wing web puncture (WWP) method (Siddiky *et al.,* 2004).

The chicks (n = 80) were divided into six groups namely A, B, C, D, E, and F where group A and F contain 20 chicks each and used to determine persistence of maternally derived antibody and unvaccinated control respectively. Whereas group B, C, D and E contain 10 chicks each and used for vaccination (Table 1).

Table 1: Vaccination schedule used for experimentally reared chicks by DLS-FPV and Diftosec-CT® FPV vaccine.

Types of vaccine	Group/Subgroup of chicks	Route of vaccination	No. of chicks	Age of vaccination (day)		Dose of vaccine/ chicks
				Usual	Suggested	
DLS-FPV	B	WWP	10	22	-	0.1 ml
	C	WWP	10	-	18	0.1 ml
Diftosec-CT®	D	WWP	10	42	-	0.1 ml
	E	WWP	10	-	22	0.1 ml
	NVC	-	20	-	-	-

DLS-FPVV : Department of Livestock Service-Fowl pox virus vaccine
WWP : Wing web puncture; NVC = Non vaccinated control

Vaccinated chicks were observed for the 'take' reaction. A 'take' consists of swelling of the skin or a scab, the typical lesions of FP at the site of vaccine administration (Tripathy and Reed, 1997). All chicks were examined for 6-9 days to observe any 'take' reaction.

Chicken serum

Chicken sera were collected and stored at -20^0C in screw capped vials until used. The stored serum samples were inactivated at 56^0C for half an hour in hot water bath during the test.

Protective efficacy study

The chicken of both vaccinated and unvaccinated control groups were given challenge exposure with 10^{-6} EID_{50}/0.1 ml containing field isolates of FPV for the protective efficacy study at three weeks of post vaccination following wing web puncture method as described by Winter field and Reed, 1985 and Boosinger *et al.,* 1982. It was done by puncturing the wing web. Then the pox virus containing PBS was applied on the area with cotton bud. A virus content of 10^{-6} EID_{50}/0.1 ml was considered as one chick dose and was used as challenge inoculum for an individual test. The presence or absence of challenge virus 'take' was assessed 6-9 days post challenge (Tripathy and Reed, 1997; Winterfield and Hitchner, 1965).

The challenge chicken were observed for any change (Gross typical pox lesions, diffuse thickening of skin and feather follicle frequently covered with thick scab) up to 7-10 days post challenge (Olufemi and Reed, 1996). Chickens with mild and no reaction were considered as protective (Sarma and Sharma, 1988). The rate of pox lesions of chickens from each vaccinated group indicated the efficacy of vaccine which revealed a relationship with the rate of protection of chickens against virulent challenge.

Passive haemagglutination test

The test was used to determine the antibody titres from the chicks of vaccinated and non vaccinated control group to FPV vaccine as per the method described by Tripathy et al., (1970) with some modifications of the method. In brief 25 μl of test serum was taken in each well from 2^{nd} to 10^{th} well of horizontal row of V-shaped microtitre plate and 25 μl of test serum was taken in the 2^{nd} well. Two fold dilution of serum ranging from 1:2 to 1:512 were made by transferring 25 μl of mixture from 2^{nd} well to 3^{rd} well and thus containing successively up to the 10^{th} well from where an excess amount of 25 μl of the mixture was poured off. Then 25 μl of 0.5% FPV sensitized tanned SRBC was added in each of the nine well. The controls include: 1^{st} well contained equal volume of 25 μl of sensitized SRBC and normal serum; 11^{th} well contained equal volume of 25 μl of 1:20 fowl pox virus antigen suspension and tanned SRBC; 12^{th} well contained 50 μl of virus sensitized tanned SRBC. The contents of the wells were mixed by gentle agitation and incubated at room temperature for 3-4 hours. Deposition of a different thin layer of clumping of SRBC at the bottom of the plate indicates the positive result. Buttoning of SRBC was at the bottom of the plate indicated the negative result. The end point was determined by observing the highest dilution at which cells agglutinated. Agglutination was indicated by a flat or deposition of a diffuse thin layer of clumping sensitizes SRBC observed at the bottom of the well. The result of the test that calculated on the basis of the reciprocal of the highest dilution of serum causing agglutination of sensitized SRBC was considered as the titer of the serum (Siddique et al*.,* 1997).

Statistical analysis

Data obtained were analyzed statistically for difference in the PHA titers using mean and standard error of Mean. The analysis was performed according to procedures described by Shil and Debnath (1985).

Result and discussion

The study was conducted to evaluate the comparative efficacy study of commercial Diftosec-CT® FPV vaccine imported by Advance Animal Science Co. Ltd., Bangladesh in comparison to locally produced DLS-FPV vaccine.

A total of 80 experimentally reared chicks were divided into six groups namely A, B, C, D, E and F. Among these group, group A was selected to determine the persistence of MDA and group F was kept as unvaccinated control. Other than the control groups, groups B and C were vaccinated with DLS-FPV at day 22 and 18 respectively, whereas groups D and E were vaccinated with Diftosec-CT® at day 42 and 22 respectively. Blood samples were collected to obtain sera on Day 7, 14 and 21 of post vaccination and antibody titres were determined by PHA test.

The vaccinated and unvaccinated control birds were challenged with virulent FPV through WWP route after three weeks of vaccination. The virus used as test antigen and for challenge infection was isolated from field samples after propagation in embryonated chicken eggs through CAM route. The virus was characterized by observing the discrete pock lesions.

Propagation of fowl pox virus in embryonated chicken eggs

Presence of FPV was confirmed by characteristic pock lesions on CAM (Figure 1). Discrete pock lesions along with thickening of CAM were observed in the ECE after harvesting at 5-6 days of post inoculation. This CAM was considered as the source of virulent FPV for the use of challenge virus and test antigen.

Table 2
The optimum growth and average pock counts on CAM with ascending dilution of FPV at different days of post inoculation of FPV in embryonated chicken eggs (ECE).

Days of post inoculation	Growth of FPV	Dilution of virus	Average pock count
1st	Nil	10^{-1}	
2nd	Nil	10^{-2}	
3rd	+	10^{-3}	
4th	++	10^{-4}	4
5th	+++	10^{-5}	5
6th	+++	10^{-6}	4

+ = Indicated growth; ++ = Indicate fine growth; +++ = indicate optimum growth; FPV = Fowl pox virus

CAM = Chorio allantoic membrane; FPV = Fowl pox virus

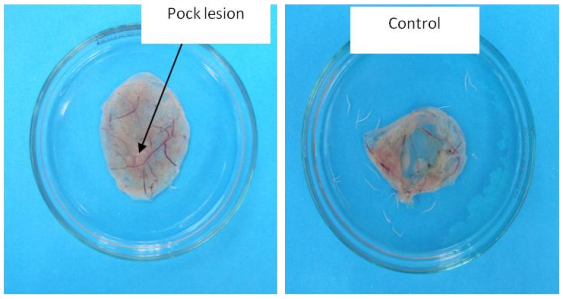

Figure 1
FPV infected chorioallantoic membrane with pock lesions (left) and control (right).

Response to vaccination

Following vaccination 'take' reaction was observed in birds of all the four groups characterized by swelling of the skin, formation of nodules gradually turning to pox lesions which sustained for few days and then subsided.

A 'take' reaction is defined as the formation of pimples turning out to be pock lesions or nodules at the site of inoculation of vaccine.

The results of such responses in all the four groups of chicks such as B, C, D and E is illustrated in figure 2. Following vaccination, 'take' reaction was manifested in 10 (100%) birds, 8 (80%) birds, 9 (90) % birds and 8 (80%) birds of groups B, C, D and E respectively (F).

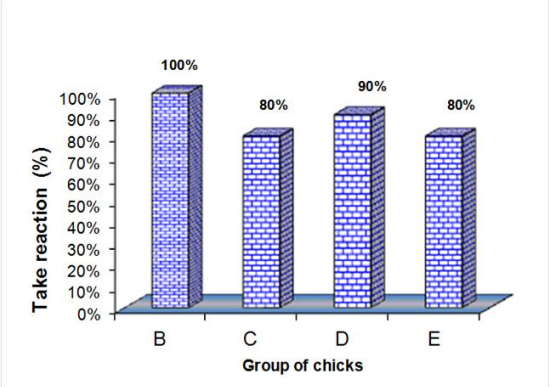

Figure 2:'Take' reaction of chicks at different age groups following vaccination with FPV.

Passive haemagglutination test

Persistence of MDA

The mean ± Standard error of Mean (SE) of PHA titres were 57.60 ± 8.38, 38.40 ± 5.13, 21.87 ± 2.97, 11.73 ± 1.26, 7.47 ± 1.02, ≤ 4 ± 0, ≤ 4 ± 0, ≤ 4 ± 0, ≤ 4 ± 0, ≤ 4 ± 0, ≤ 4 ± 0, ≤ 4 ± 0 and ≤ 4 ± 0, respectively in the experimentally reared chicks. Mean while the highest PHA titre was 57.60 ± 8.38 at day 1 and lowest ≤4 ± 0 at day 16. Moreover the

antibody titre started to decline after day 1 and persisted up to day 13 below the protective level (Figure 3).

Detection of post vaccination PHA titres in chicks

After 7 days, 14 days and 21 day of vaccination the mean ± SE PHA titres obtained from group B were 57.60 ± 8.38, 140.80 ± 31.35, 219.20 ± 18.79 and group C were 51.20 ± 10.61, 128.03 ± 35.05, 204.80 ± 20.90 respectively. The highest antibody titre was 219.20 ± 18.79 obtained from group B on 3rd week of vaccination (Figure 4).

After 7 days, 14 days and 21 days of vaccination the Mean±SE PHA titres obtained from group D were 51.20 ± 10.61, 134.40 ± 22.27, 217.60 ± 19.55 and group E were 46.40 ± 6.05, 121.60 ± 17.72, 198.40 ± 24.23 respectively. The highest antibody titre was 217.60 ± 19.55 obtained from group D on 3rd week of vaccination (Figure 5).

Comparison of post vaccination PHA titres

The post vaccination PHA titre of chicks vaccinated with DLS-FPV and Diftosec-CT® FPV vaccine were 204.80 ± 20.90 and 198.40 ± 24.23 at day 18 and 22 respectively (Figure 6).

Table 5: PHA titres of maternally derived antibody in experimentally reared chicks from day 1 to day 40 using passive haemagglutination test

Age (day)	Chicks of group A (Mean±SE)
Day 1	57.60 ± 8.38
Day 4	38.40 ± 5.13
Day 7	21.87 ± 2.97
Day 10	11.73 ± 1.26
Day 13	7.47 ± 1.02
Day 16	≤ 4 ± 0
Day 19	≤ 4 ± 0
Day 22	≤ 4 ± 0
Day 25	≤ 4 ± 0
Day 28	≤ 4 ± 0
Day 31	≤ 4 ± 0
Day 34	≤ 4 ± 0
Day 40	≤ 4± 0

FPV = Fowl pox virus; PHA = Passive haemaglutination test

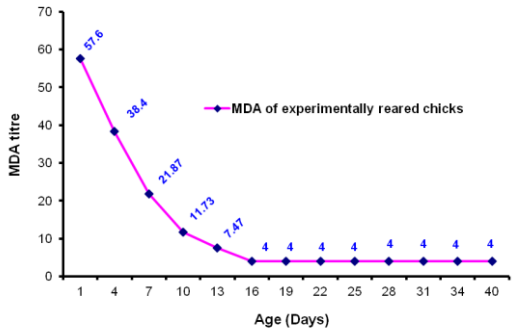

Figure 2: Gradual declination of MDA titre in experimentally reared chicks

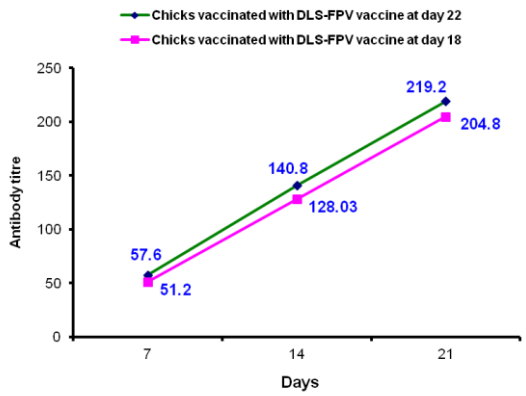

Figure 3: Antibody titre in chicks vaccinated with DLS-FPV vaccine at 22 and 18 days of old birds

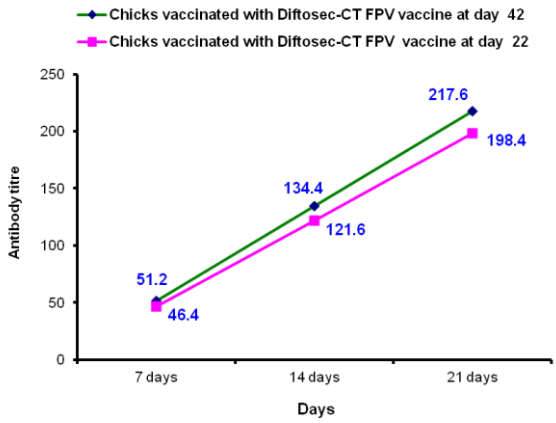

Figure 4: Antibody titre in chicks vaccinated with Diftosec-CT® FPV vaccine at 42 and 22 days of old birds

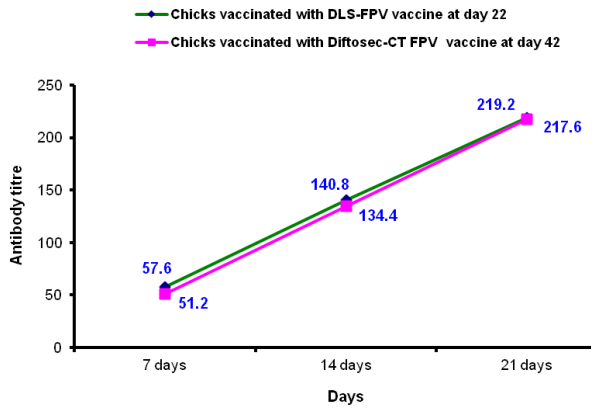

Figure 5: Antibody titre in chicks vaccinated with DLS-FPV and Diftosec-CT® FPV vaccine at 22 and 42 days of old birds.

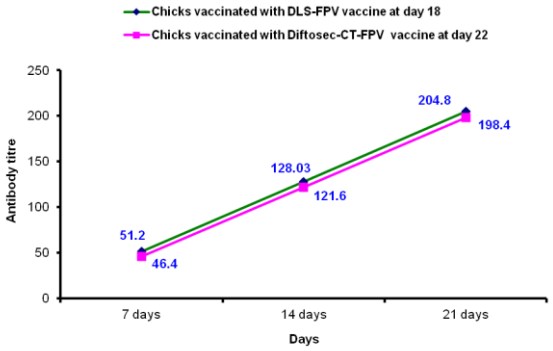

Figure 6. Post vaccination antibody titre in chicks vaccinated with DLS-FPV and Diftosec-CT® FPV vaccine at 18 and 22 days of old birds.

Challenge infection

Challenge infection was conducted with each vaccinated and 10 from unvaccinated control group. Birds of group B, C, D, E and F were subjected to challenge infection with a predetermined chicks dose of virulent FPV after 3 weeks of vaccination. Each bird was exposed to 0.1 ml of 10^{-6} EID_{50} per 0.1 ml administered via WWP route.

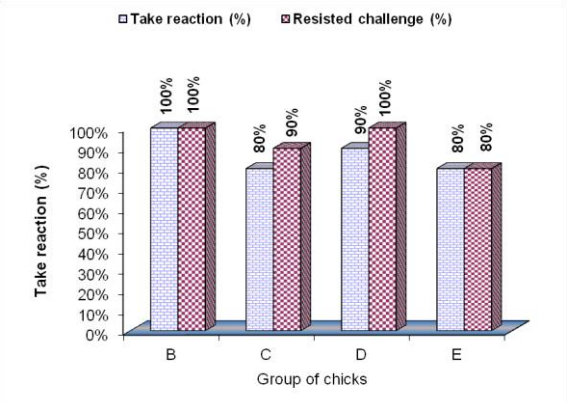

Figure 7: Comparison of 'Take reaction' with percentage of chicks resisted to challenge with virulent Fowl pox virus (FPV)

Challenge exposure indicated that 10 (100%) out of 10 birds of group B and 9 (90%) out of 10 birds of group C resisted such infection. In case of group D, 10 (100%) birds and incase of group E, 8 (80%) birds withstood challenge infection. All the 10 unvaccinated control birds exhibited pox lesions after challenge exposure to virulent FPV.

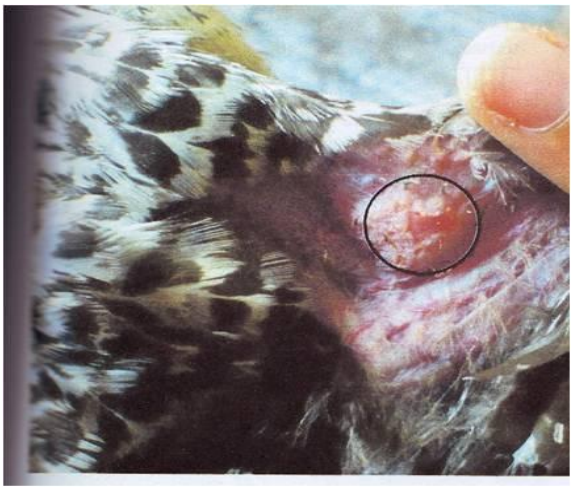

Figure 8. Formation of typical pox lesions after challenge exposure in control chicks

Protective efficacy test

Challenge infection was conducted after 3 weeks of vaccination with each bird vaccinated along with 10 birds from unvaccinated control groups. The challenge infection was performed by WWP method at the predetermined one chick dose of 0.1 ml of virulent FPV having a concentration of 10^{-6} EID_{50}/0.1 ml. Chicks of group B and D exhibited 100% protection whereas, group C and E exhibited 90% and 80% protection respectively (Figure 7).

The present study was entitled as "Comparative Efficacy Study of Commercial Diftosec-CT® Fowl Pox Vaccine in comparison to DLS Fowl Pox Vaccine in layer flock". The parameters of investigation included recording of initial reaction ('take' reaction) on the site of vaccine inoculation, comparison of the level of antibody production following vaccination with Diftosec-CT® and DLS-FPV vaccine, to determine the persistence of MDA using PHA test and resistance of birds to challenge exposure with a virulent field isolates of FPV.

Fowl pox is one of the most important problems in the development of poultry industry in Bangladesh. FP is still a malady of chickens of all age, sexes and breeds either in organized farms or in backyard poultry farming system (Khandaker *et al.,* 1994; Siddique *et al.,* 1997; Siddiky *et al.,* 2004). The disease is appearing as endemic every year in Bangladesh (FAO/OIE/WHO, 1995).

The virus used for PHA test and for giving challenge infection was isolated from field samples. The virus was characterized by observing discrete pock lesions on the CAM of 10-12 days old embryonated

chicken eggs. The characteristic lesions was found on CAM similar to the findings of Cunningham (1972); Minbay and Kreier (1973); Pandey and Mallick (1975); Runrudi *et al.,* (1983) and noted that single pock lesion found on the CAM was considered to be a positive response for that of virus inoculated embryo.

To investigate the persistence of MDA, the chicks (n=20) were reared in group A and blood samples were collected for preparation of sera from the jugular vein of chicks in every three alternate days. Then MDA was measured by PHA test. In the present study it was observed that MDA titre was higher (57.60 ± 8.38) at the age of day 1 and declined to a negligible level (≤4) at the age of day 16. Natour *et al.,* (1998) mentioned that the decay of maternally derived antibody was approximately linear and day old chicks contained high levels of MDA which gradually declined below positive levels within 10-15 days of age. Daroshoke and Belistkaya (1969) reported that PHA titre of 40 was considered to be indicative of an adequate protective immunity. In the present study it was also observed that MDA titre in experimentally reared chicks started to decline after day 1 reached to a negligible level at day 16.

The investigation was carried out to compare the level of antibody production following vaccination with Diftosec-CT® and DLS-FPV vaccine in experimentally reared chicks. For this the chicks (n=80) were divided into five groups namely B, C, D, E and F where groups B, C, D, E each containing 10 chicks which were used for vaccination and group F containing 20 chicks was kept as unvaccinated control. Other than the control group, groups B and C were vaccinated with DLS-FPV at day 22 and 18 respectively. On the other hand, groups D and E were vaccinated with Diftosec-CT® at day 42 and 22 respectively through conventional WWP route. Blood samples were collected to prepare sera at 7, 14 and 21 days of post vaccinations from each bird of vaccinated groups and at the same time blood samples were also collected from 5 birds of unvaccinated control groups. Then the sera samples were subjected to PHA test.

'Take' reaction is the indication of immediate response of vaccination. It is characterized by the formation of pimples that became further swollen and turned to nodular form, considered as typical of pox lesions which sustained for a varying period of 5-7 days. Similar observation were also recorded by Siddiky *et al.,* (2004); Tripathy and Reed (1997); Amin and Siddiky (1993); Winterfield and Reed (1985) and Buxton and Fraser (1977) following FPV vaccine or PPV vaccine inoculation. No adverse reactions were observed in vaccinated bird following appearance of 'take'. This finding supports the earlier report of Sarma and Sharma (1988) and Rao *et al.* (1978).

The average percentage of 'take' reaction following vaccination was manifested in 100% birds, 80% birds, 90% birds and 80% birds of groups B, C, D and E respectively. These findings are similar to the findings of Fatunmbi and Reed (1996); Sarma and Sharma (1988) and Venkatasubba *et al.,* (1978) who vaccinated the birds with FP vaccine and 100% 'take' reaction recorded. Winterfield and Reed (1985) vaccinated the birds primarily with FPV vaccine or PPV vaccine and observed more than 80% 'take' reaction in the vaccinated birds.

The mean PHA titres obtained from group B were 57.60 ± 8.38, 140.80 ± 31.35, 219.20 ± 18.79 and group C were 51.20 ± 10.61, 128.03 ± 35.05, 204.80 ± 20.90 group D were 51.20 ± 10.61, 134.40 ± 22.27, 217.60 ± 19.55 and group E were 46.40 ± 6.05, 121.60 ± 17.72, 198.40 ± 24.23 respectively after 7 days, 14 days and 21 days of vaccination. The highest Mean ± standard error of Mean of groups B and C were 219.20 ± 18.79 and 204.80 ± 20.90 respectively after 21 days of vaccination. The highest Mean ± Standard error of Mean of groups D and E were 217.60 ± 19.55 and 198.40 ± 24.23 respectively after 21 days of vaccination.

The highest Mean PHA titre was 219.20 ± 18.79 obtained from the chicks of group B vaccinated with DLS-FPV vaccine at 22 days of age.

In maximum cases the recorded antibody titre at different stages by PHA micro-plate method in this study observed more than the findings of Amin and Siddiky (2003); Amin and Siddique (1993); Saini *et al.,* (1990); Sarma and Sharma (1988). The variation in the PHA titre value might be due to use of different vaccine virus strain, vaccination method, age of vaccination and strain of challenge virus, which coincide the advocacy of Siddiky *et al.,* (2004); Siddique (1997); Sarma and Sharma (1988) and Ursache (1974). They reported that the PHA test was highly sensitive in detecting specific antibodies following inoculation of birds with FPV.

From the above study, it was observed that chicks were vaccinated at 22 days of age with DLS-FPV vaccine exhibited higher PHA titre than vaccinated at 18 or 42 days of age and DLS-FPV vaccine give slightly highest protection than Diftosec-CT® challenge infection after 3 weeks of vaccination in randomly selected 10 birds from each vaccinated along with 10 birds from unvaccinated control groups with 10^{-6} EID_{50}/0.1ml containing field isolates of FPV for the protective efficacy study following WWP method as described by Winterfield and Reed (1985) and Boosinger *et al.,* (1982). The presence or absence of challenge virus 'take' was assessed 6-9 days post challenge (Winterfield and Hitchner, 1965; Tripathy and Reed, 1997). The challenge chicken were observed for any change (Gross typical pox lesions, diffuse thickening of skin and feather follicle frequently covered with thick scab) up to 7-10 days post challenge (Olufemi and Reed, 1996). Chickens with mild and no reaction were considered as protective (Sarma and Sharma, 1988a) at the rate of survivability of chickens from each vaccinated group indicated the efficacy of vaccine (Figure 8).

Conclusion

The maternally derived antibody titre in experimentally reared chicks was 57.60 ± 8.38 at day 1 and gradually declined to its lower level ≤ 4 ± 0 at day 16. Both Diftosec-CT® and the DLS fowl pox virus vaccines produced almost equal level of antibody titre but chicks vaccinated at day 22 with DLS fowl pox virus vaccine exhibited higher passive haemagglutination titre than vaccinated at day 18 or 42.

In case of usual schedule, DLS fowl pox virus vaccine gave more or less similar protection in comparison with Diftosec- CT® Fowl pox virus vaccine

Suggested schedule might be at days 18 or 22 instead of usual schedule of vaccination at days 21 or 42 in case of DLS-fowl pox virus vaccine and Diftosec- CT® fowl pox virus vaccine respectively. But DLS-FPV vaccine gave 90% and Diftosec-CT® fowl pox virus vaccine gave 80% protection after challenge exposure at 21 days of post vaccination.

Diftosec-CT® fowl pox virus vaccine could be used in chicks to control Fowl pox in Bangladesh as an alternate vaccine against fowl pox.

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