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Protection of Khaki Campbell Ducks against Duck Plague Using an Inactivated Duck Plague Vaccine

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ABSTRACT

Duck plague (DP) or duck viral enteritis is a fatal viral disease of ducks that causes huge economic losses in the duck industry. The present study was performed to determine the immune response and protective efficacy of an inactivated DP vaccine prepared from a local virulent DP virus. A virulent DP virus was obtained from the laboratory repository of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh (Bangladesh). The DP virus (EID_{50} $10^{5.3}$/ml) was inactivated using 0.04% formalin. The alum (40 g/L) was added to the inactivated DP virus as an adjuvant. A total of 60 Khaki Campbell male ducks aged 17 weeks were randomly divided into three groups. Ducks of groups A (n = 20) and B (n = 20) were vaccinated intramuscularly in the breast muscle with 1 ml of inactivated DP vaccine and a live attenuated DP vaccine, respectively. Ducks of group C (n = 20) were kept as unvaccinated control. Booster vaccination was administered at 2 weeks after primary vaccination. Antibody titers of vaccinated ducks were measured at 7, 14, 21, and 28 days post-vaccination (DPV) using a passive haemagglutination (PHA) test. Ducks of both vaccinated and unvaccinated groups were challenged with 1 ml virulent DP virus (EID_{50} $10^{4.3}$/ml) at 28 DPV. Clinical signs, morbidity and mortality, and gross pathological lesions of vaccinated and control ducks were observed for 10 days post-challenge to evaluate the protective efficacy of inactivated DP vaccine. The mean PHA antibody titers of vaccinated ducks of group A at 7, 14, 21, and 28 DPV were 5 ± 0.43, 26 ± 1.71, 43 ± 3.4, and 54 ± 3.28, respectively. Ducks in group B had mean serum PHA antibody titers of 21 ± 1.71, 41 ± 3.28, 52 ± 3.41, and 84 ± 7.25 at 7, 14, 21, and 28 DPV, respectively. No mortality or gross pathological lesions were observed in vaccinated ducks after they were subjected to a challenge infection. Additionally, no significant difference was observed between groups A and B in terms of the challenge infection. The mortality rate of the control group of ducks was 70%. Hemorrhage in the trachea and intestine and necrotic foci in the liver were seen in unvaccinated control ducks (group C). Experimentally developed inactivated DP vaccine induced a protective serum antibody titer and conferred 100% protection against virulent challenge infection up to 10 days observation period.

Keywords: Duck plague, Khaki Campbell, Protective efficacy

INTRODUCTION

The duck plague (DP), also known as duck virus enteritis, is a viral disease of ducks worldwide, including Bangladesh, India, China, and Egypt (El-Tholoth et al., 2019; Neher et al., 2019; Khan et al., 2021; Liang et al., 2022). The causal agent of DP is a double-stranded DNA virus that belongs to the family Herpesviridae (Dhama et al., 2017). This viral infection affects both domestic ducks and wild waterfowl and is extremely contagious and fatal in nature (Kaleta et al., 2007). Its impact is significant, leading to economic losses both in broiler and layer duck farms (Islam et al., 2021).

In Bangladesh, the DP virus was first isolated and identified by Sarker (1980). Outbreaks of DP occur almost every year between March and June in Bangladesh (Sarker, 1980; Hoque et al., 2010). Khan et al. (2018) reported 55.86% mortality due to DP outbreaks in Bangladesh. Several investigators isolated and characterized the DP virus from natural disease outbreaks in Bangladesh (Islam and Khan, 1995; Akter et al., 2004; Ahamed et al., 2015).

Two types of vaccines that can be used to immunize ducks against DP include live attenuated and inactive DP vaccines (Shawky and Sandhu, 1997; Kulkarni et al., 1998). The immune system of duck can recognize both live and inactivated viral antigens and mount immune response. Live attenuated DP vaccine is prepared by attenuating a wild type of DP virus. It mainly induces a cell-mediated immune response and confers adequate protection against DP virus infection (Lian et al., 2010; Huang et al., 2014). This vaccine is routinely used in vaccination programs against DP. In order to be effective live attenuated DP vaccine requires a cold chain during its storage and transport (Khan et al., 2018).

An inactivated DP vaccine contains killed viruses which may still have pathogen-recognition patterns and can induce an antibody-mediated immune response. This vaccine provides shorter-term protection and requires booster doses for long-term immunity (Plotkin, 2008). The killed vaccine does not require a cold chain and has the advantage of using

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developing countries (Melnick, 1978). It can be administered during disease outbreaks. The adjuvant is used in the inactivated vaccine to increase immunogenicity, facilitating higher and longer-lasting immunity. Inactivated DP vaccine conferred 100% protection against the virulent challenge of the DP virus in vaccinated ducks (Shawky and Sandhu, 1997). Soma et al. (2018) prepared an inactivated DP vaccine and tested its antibody response in Khaki Campbell ducklings. The protective efficacy of this inactivated DP vaccine has not been studied in ducks following a challenge infection with the virulent DP virus.

Most European countries and the USA use both live attenuated and killed DP vaccines to prevent DP in broiler ducks and swans (Shawky and Sandhu, 1997; Shawky et al., 2000). Live attenuated DP vaccines produced by the Livestock Research Institute (LRI) or imported from foreign countries are used to vaccinate ducks against DP in Bangladesh. Many commercial DP vaccines yielded an inadequate immune response (Kulkarni et al., 1998). Vaccination failure may result if the seed virus used for vaccine preparation is not antigenically matched with the circulating virus. Although the DP virus is a single antigenic type, vaccination failure is reported (Das et al., 2009; Khan et al., 2018). The inability to maintain a cold chain for live attenuated DP vaccine during its storage and transport might be one reason for vaccination failures in Bangladesh (Khan et al., 2018). There is a need to develop an inactivated DP vaccine since it does not require a cold chain. In some countries where maintaining the cold chain for live DP vaccine during transportation and storage is not feasible, the inactivated DP vaccine may be a suitable replacement for the attenuated live DP vaccine. This present research aimed to develop an inactivated DP vaccine using a virulent local DP virus isolates and to determine antibody response and the protective efficacy of inactivated DP vaccine in the Khaki Campbell duck.

MATERIALS AND METHODS

Ethical approval

The experiments related to the efficacy trial of the DP vaccine and challenge infection with virulent DP virus in Khaki Campbell duck were conducted according to the guidelines of the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh(protocol approval number: AWEEC/BAU/2020/08) and WOAH (2008).

Ducks

Day-old Khaki Campbell (*Anas platyrhynchos domesticus*) male ducklings (n=60) were obtained from a commercial duck farm at Mymensingh, Bangladesh. Parent flocks were vaccinated against DP and duck cholera vaccines. The DP vaccine was made from an attenuated strain of DP virus manufactured by the LRI, Dhaka, Bangladesh. The duck cholera vaccine was produced from the inactivated virulent strain of *Pasteurellamultocida*manufactured by the Livestock and Poultry Vaccine Research and Production Center (LPVRPC), BAU, Mymensingh, Bangladesh. The ducklings were free from diseases confirmed by a veterinarian's physical examination. Ducks were reared for six months from August 2019 to February 2020 in an isolated experimental animal shed at the Department of Microbiology and Hygiene, BAU, Mymensingh, Bangladesh, and supplied with commercial feed (Nourish poultry feed, Dhaka, Bangladesh) three times daily and water *ad libitum*. A veterinarian regularly evaluated the health conditions of ducks.

Virus

A local virulent DP virus was obtained from the laboratory repository of the Department of Microbiology and Hygiene, BAU, Mymensingh (Bangladesh). The virus was revived into 10 days-old embryonated duck eggs through the chorioallantoic membrane (CAM) route for six passages (Ahamed et al., 2015). The stock DP virus was stored in a small aliquot in a 5 ml screw-capped vial at -86°C in the lab repository. The class II A2 biosafety cabinet (Thermo Scientific, Waltham, MA, USA) was used to inoculate DP virus into the embryonated duck eggs. The stock DP virus was previously isolated from a natural outbreak of DP (Islam et al., 2021) and was used to prepare inactivated DP vaccine and challenge infection. The EID_{50} of the DP virus was determined by the standard procedure (Kulkarni et al., 1998).

Vaccines

The DP virus (EID_{50} $10^{5.3}$/ml) was inactivated by 0.04% formalin on a shaker incubator at 37℃ for 24 hours. Virus inactivation was confirmed by three successive blind passages in the 10-day-old embryonated duck eggs. The sterility of inactivated DP vaccine was checked according to the method described by Igomu et al. (2020). Alum adjuvant (0.04 g/ml) was added to the inactivated DP virus suspension and mixed properly on a shaker incubator at 37℃ for 2 hours (Gupta and Rost, 2000; Aguilar and Rodriguez, 2007). A live attenuated DP vaccine(batch no. 04/2019) manufactured by LRI, Dhaka, Bangladesh, was used as a positive control.

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Experiment design

A total of 60 healthy Khaki Campbell ducks aged 17 weeks were randomly divided into three groups (A, B, and C) and reared in three separate houses for 8 weeks from January to February 2020. Serum samples were tested by passive haemagglutination (PHA) test to verify that ducks were free of antibodies against the DP virus. Ducks were adopted in the animal house facilities for one week prior to the experiment. Ducks of group A (n=20) and B (n=20) were vaccinated intramuscularly (IM) at the breast muscle with 1 ml of inactivated DP vaccine and 1 ml of live attenuated DP vaccine, respectively, at 17 weeks of age. A booster dose of the same vaccine was administered at 19 weeks of age (Table 1). Ducks of group C (n=20) were kept as unvaccinated control. Blood samples were collected from the wing vein of all vaccinated and control ducks at 0, 7, 14, 21, and 28 days post-vaccination (DPV) using a 5 ml disposable plastic syringe (JMI Syringe and Medical device, Cummilla, Bangladesh). Sera were separated from blood samples, and antibody titers in sera were determined using the PHA test (Soma et al., 2018). Ducks of all groups (A, B, and C) were challenged by injecting 1 ml of virulent DP virus (EID_{50} $10^{4.3}$/ml) through IM route at 21 weeks of age (Table 1). The challenged ducks were observed twice daily for clinical signs of DP, such as abrupt death, extreme thirst, partial paralysis, and watery, greenish diarrhea (Dhama et al., 2017). Clinical statuses were indicated on the lines of intravenous /intracerebral pathogenicity index (PI) as described in Poultry Biologics National Research Council (NRC, 1963). The clinical manifestation of DP was recorded to calculate PI (Kulkarni et al., 1998). The scores of clinical manifestations of DP are shown in Table 2. The postmortem examination was done for vaccinated and controlled ducks. The live ducks were killed by disarticulation of the head at the atlantooccipital joint without anesthesia (Charlton et al., 2000). Dead ducks were placed on a surgical tray. A longitudinal incision was made through the skin of the neck to the thoracic inlet. Trachea was removed and examined after giving a longitudinal incision. A transverse incision was made through the posterior part of the abdominal muscles. On each side, the incision was given through the costochondral junction. The ventral abdominal wall and breast were removed as one piece. Visceral organs such as the liver, spleen, and intestine were removed. Gross pathological lesions such as hemorrhage in the trachea, intestine, focal necrosis in the liver, and splenomegaly were recorded during postmortem examination.

Table 1. Experimental design to determine the immune response in Khaki Campbell duck from August 2019 to February 2020 at the Bangladesh Agricultural University, Mymensingh, Bangladesh

Operation	Age of ducks (Weeks)	Dose (ml)	Groups		
			A	B	C
Pre-vaccination serum antibody titre	16		20	20	20
Primary vaccination	17	1 ml	20	20	ND
Booster vaccination	19	1 ml	20	20	ND
Challenge infection	21	1 ml	20	20	20

ND: Not done

Table 2. Clinical manifestation of duck plague with scoring factor in Khaki Campbell ducks aged 21 weeks at the Bangladesh Agricultural University, Mymensingh, Bangladesh

Case	Clinical evidence of duck plague	Scoring factor
1.	Death	3
2.	Acute lesions	2
3.	Chronic lesions	1
4.	Normal	0

Protective efficacy

Vaccinated ducks (group A and group B) and unvaccinated control ducks (group C) were challenged with virulent DP virus, and mortality was evaluated for 10 days post-challenge. The protective efficacy, also known as a preventable fraction (PF) of the vaccine, was calculated using the following method described by Tizard (2004).

PF=(% of control dying-% of vaccinated dying)/% of control dying

Pathogenicity index

The PI was calculated using the following method described in Poultry Biologics (National Research Council, 1963; Kulkarni et al., 1998).

PI = Total score/Total number of observations

Vaccinated ducks (groups A and B) and unvaccinated control ducks (group C) were challenged with 1 ml (IM) of virulent DP virus. Clinical statuses, such as death, severe disease, mild disease, and no disease of challenged ducks were monitored for 10 days post-challenged. The following factors were considered while calculating the PI clinical scores for the ducks in each group. Ducks with a score of 3 were dead, 2 had a serious disease, 1 had a slight disease, and 0 were healthy and active. Data from 10 days were combined to produce a sum multiplied by a scoring factor. The total score obtained was divided by the total number of observations to determine the PI.

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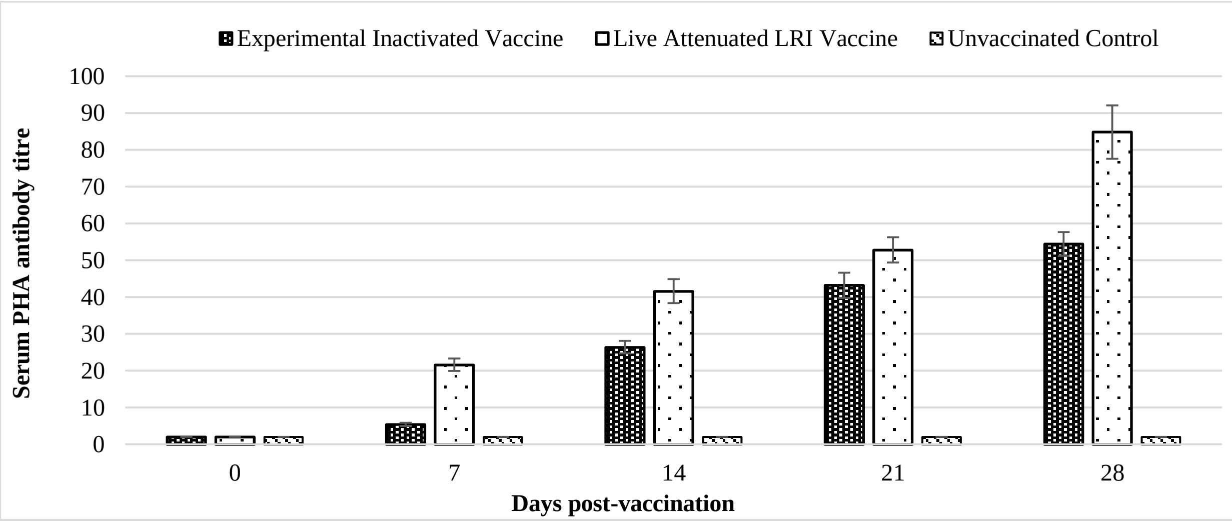
Statistical analysis

Results of the mean PHA serum antibody titer of vaccinated ducks were analyzed using student's t-test and chi-square tests for statistical significance using the statistical package for social science (SPSS) version 25 for Windows 10. A p-value of ≤ 0.05 was considered significant. Serum antibody titters have been presented as mean ± standard error (SE).

RESULTS

Serum antibody titer

Pre-vaccination log2 serum PHA test antibody titer of all ducks in groups A, B, and C was 2. The log2 serum PHA test antibody titer (mean ± SE) of experimentally developed inactivated vaccinated ducks (group A) were 5 ± 0.43, 26 ± 1.71, 43 ± 3.4, and 54 ± 3.28 at 7, 14, 21, and 28 DPV. On the other hand, the log2 mean serum PHA test antibody titer for live attenuated vaccinated ducks (group B) were 21 ± 1.71, 41 ± 3.28, 52 ± 3.41, and 84 ± 7.25 at 7, 14, 21, and 28 DPV. The serum PHA antibody titer(mean ± SE) of the unvaccinated control ducks (group C) remained 2 ± 0 before the challenge experiment. A statistically significant difference in serum antibody titers ($p < 0.05$) was observed in vaccinated ducks (groups A and B) at 7, 14, 21, and 28 DPV when compared to unvaccinated control.



Graph 1. The mean passive haemagglutination (PHA) antibody titer of vaccinated and unvaccinated Khaki Campbell ducks at days 0, 7, 14, 21, and 28 post-vaccination at 17, 18, 19, 20, and 21 weeks of age, respectively, from January 2020 to February 2020 at the Bangladesh Agricultural University, Mymensingh, Bangladesh. Antibody titers are reported as mean ± standard error (SE). A statistically significant difference was found in serum antibody titer between the experimental inactivated vaccine and live attenuated vaccine ($p < 0.05$).

Pathogenicity indices

Vaccinated ducks (groups A and B) showed mild clinical signs of DP, such as inappetence and lethargy after challenge infection with local virulent DP virus with PI of 0.15 (Table 3) and 0.15 (Table 4), respectively. On the contrary, control ducks manifested clinical signs at 48 hours post-challenge with PI 2.70 (Table 5). The clinical signs of watery diarrhea, weight loss, depression, and loss of appetite were observed in the sick ducks.

Preventable fractions

Ducks immunized with inactivated DP vaccine (group A) and live attenuated DP vaccines (group B) were 100% protective against challenge infections (Table 6). In the unvaccinated control ducks (group C), 70% mortality was observed following challenge infection. The PF of both inactivated and live attenuated DP vaccines was 100% (Table 6).

Table 3. Calculation of pathogenicity index of 21-week-old Khaki Campbell ducks vaccinated with experimentally developed inactivated duck plague vaccine in February 2020 at the Bangladesh Agricultural University, Mymensingh, Bangladesh

Clinical evidence of DP	Days of observation										Sum × Scoring factor	Total Scores	PI index (total score/total number of observations)
	1	2	3	4	5	6	7	8	9	10			
Death	0	0	0	0	0	0	0	0	0	0	0 × 3	0	
Acute sign	0	0	0	0	0	0	0	0	0	0	0 × 2	0	
Chronic sign	0	0	0	0	0	0	1	2	0	0	3 × 1	3	0.15 (3/20)
Normal	2	2	2	2	2	2	1	0	2	2	17× 0	0	

DP: Duck plague, PI: Pathogenicity Index

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Table 4. Calculation of pathogenicity index of old Khaki Campbell ducks aged 21 weeks vaccinated with live attenuated duck plague vaccine at Bangladesh Agricultural University, Mymensingh, Bangladesh

Clinical evidence of DP	Days of observation										Sum × Scoring factor	Total Scores	PI index (total score/total number of observation)
	1	2	3	4	5	6	7	8	9	10			
Death	0	0	0	0	0	0	0	0	0	0	0 × 3	0	
Acute sign	0	0	0	0	0	0	0	0	0	0	0 × 2	0	
Chronic sign	0	0	0	0	0	0	0	1	1	1	3× 1	3	0.15 (3/20)
Normal	2	2	2	2	2	2	2	2	1	1	18× 0	0	

DP: Duck plague, PI: Pathogenicity Index

Table 5. Calculation of pathogenicity index of unvaccinated Khaki Campbell aged 21 weeks at Bangladesh Agricultural University, Mymensingh, Bangladesh

Clinical evidence of DP	Days of observation										Sum × Scoring factor	Total Scores	PI index (total score/total number of observations)
	1	2	3	4	5	6	7	8	9	10			
Death	0	0	0	4	3	4	2	1	0	0	14 × 3	42	
Acute sign	0	0	2	3	1	0	0	0	0	0	6 × 2	12	
Chronic sign	0	0	0	0	0	0	0	0	0	0	0 × 1	0	2.70 (54/20)
Normal	2	2	2	0	0	0	0	0	0	0	6× 0	0	

DP: Duck plague, PI: Pathogenicity Index

Table 6. The conferred protection in vaccinated Khaki Campbell ducks following challenge infection with the virulent duck plague virus in Bangladesh Agricultural University, Mymensingh, Bangladesh

Experimental group (n)	Number of dead (%)	Number of survived birds (%)	Preventable fraction of vaccine
A (20)	0 (0)	20 (100)	100%
B (20)	0(0)	20 (100)	100%
C (20)	14 (70)	6 (30)	NA

A: Experimentally develop inactivated duck plague vaccine, B: Live attenuated duck plague vaccine, C: Unvaccinated control; NA: Not applicable

Gross lesions

Gross postmortem lesions observed in unvaccinated control ducks were hemorrhagic annular bands in the trachea, hemorrhagic enteritis in the intestine, white foci in the liver, and splenomegaly (Figure 2). No postmortem lesions were found in vaccinated ducks (Figure 2), and they survived against the challenge of infection and conferred 100% protection.

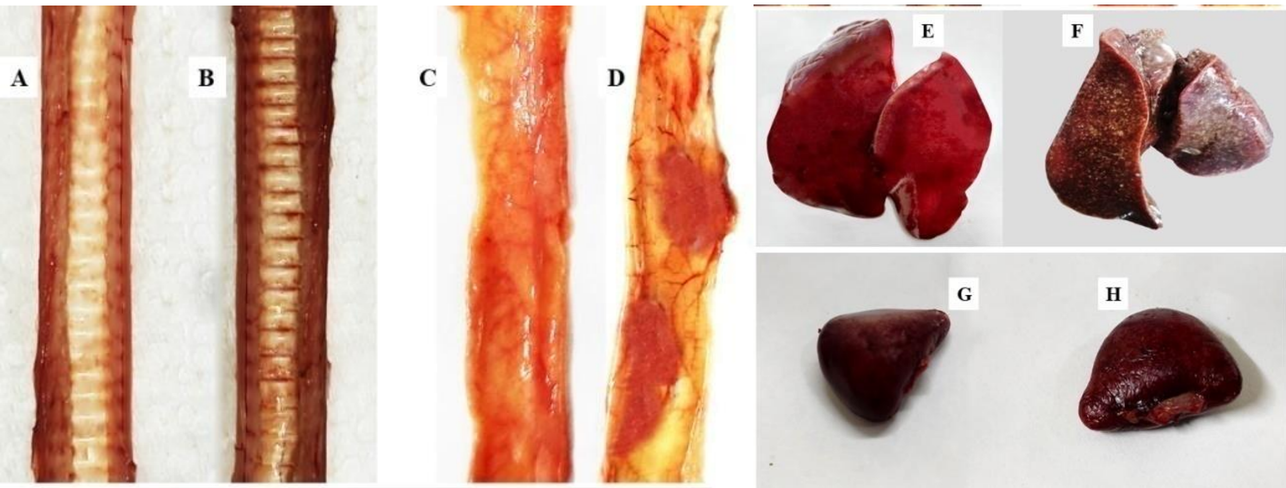

Figure 1. Gross pathological lesions of trachea, intestine, liver, and spleen in vaccinated and unvaccinated control ducks. No lesions were seen in the trachea (A), intestine (C), liver (E), and spleen (G) of experimentally developed inactivated duck plague vaccinated ducks. On the contrary, annular hemorrhagic bands in the trachea (B), hemorrhage in the intestine (D), multiple white necrotic foci in the liver (F), and splenomegaly (H) were observed in unvaccinated control ducks.

DISCUSSION

The duck plague inflicts vast mortality and morbidity in the poultry industry of Bangladesh (Khan et al., 2021). Live attenuated vaccines produced by LRI, Mohakhali, Dhaka, and some private companies are used to vaccinate ducks to control the duck plague in Bangladesh. However, the live attenuated vaccine induces an adequate immune response against the DP virus. Some drawbacks of live vaccines include the reversion of live attenuated viruses into virulent form in the natural host and the lack of heat stability under field conditions (Osman et al., 2021; Ravikumar et al., 2022).

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Thus, it is urgent to develop a DP vaccine that is suitable to use under the field condition of Bangladesh. Several studies indicated that the inactivated duck plague vaccine was protective and advantageous, compared to the live attenuated vaccine (Shawky and Sandhu, 1997). Room temperature is enough to store and can be used in a disease outbreak episode as an emergency vaccination. In Bangladesh, maintaining the cold chain of live vaccines is very difficult, often resulting in vaccination failure (Khan et al., 2018). This problem can be overcome by using an inactivated vaccine. In this study, an attempt was undertaken to evaluate the protective efficacy of an experimentally developed inactivated DP vaccine using local isolate.

It is generally accepted that vaccines produced from local DP virus isolate confer adequate protection against field viral infection (Soma et al., 2018). This study used a well-characterized DP virus isolated from a field outbreak (Islam et al., 2021) to produce the experimentally developed inactivated DP vaccine. The vaccine should have a virus titer of not less than $10^{2.0}EID_{50}$/ dose when tested at any time before the expiry date (ASEAN, 2018). In this study, EID_{50} of the local isolate was fixed to $10^{5.3}$/ml for preparation of inactivated vaccine since the recommended concentration of DPV in the vaccine should be at least EID_{50} 10^3 (Hossain et al., 2005; WOAH, 2008).

Alkylating agents such as formalin and β-propiolactone are widely used in vaccine preparation (Chowdhury et al., 2015). Both can inactivate the virus via the chemical reaction with viral capsid proteins and nucleic acids. However, formalin is a cheaper disinfectant, and a study revealed that a formalin-inactivated vaccine produced a higher serum antibody titer than β-propiolactone inactivated antigen (Chowdhury et al., 2015). Soma et al. (2018) used 0.12% formalin to inactivate the virus for DP vaccine preparation. Viruses inactivated by formalin cannot be reverted into virulent form. Large amounts of antigen are essential to provoke an adequate antibody response. As formalin has a significant disadvantage, uncontrolled use may damage antigens enough to modify immunogenicity to elicit cell-mediated immune responses, resulting in a short-duration immune response (Burrell et al., 2016). In this experiment, 0.04% formalin was used to inactivate the DP virus. Some used 0.04% formalin to inactivate poultry viruses to produce viral antigens (King, 1991; Elveborg et al., 2022).

An adjuvant enhances the immune response to inactivated vaccine (Edelman, 1980). It enhances phagocytosis, antigen depot, and prolongs immune response by slowly releasing antigens (Wilson et al., 2017). In this study, alum was used as an adjuvant. It is also known as potassium alum or aluminum sulfate, chemically formulated as $Kal(SO_4)_2 \cdot 12H_2O$. Antigens are absorbed into aluminum salts resulting in high concentrations of antigen at the injection site, which are taken up by antigen-presenting cells (HogenEsch, 2002). Alum reacts like a mild irritant, causing the employment of leukocytes required to produce an immune response to the injection site. Aluminum compounds can enhance the immune response by activating complement, stimulating dendritic cells, and releasing chemokines. Several investigators used alum to produce the killed vaccine (Hossain et al., 2004; Wang et al., 2021).

In this study, ducks were vaccinated through the IM route at 17 weeks of age. The muscles of the ducks have abundant blood circulation, making it easier for the body to absorb the drug rapidly. In this study, 1 ml of the vaccine was used to immunize ducks through the IM route. Subcutaneous and IM are the most preferred routes for vaccination of inactivated vaccines. These routes offer a slow release of vaccines from the vaccination site (Kayesh et al., 2008). A booster vaccination is recommended for the inactivated vaccine to prolong the duration as well as increase the antibody titer of the vaccine (Shawky and Sandhu, 1997). In this study, booster vaccination was administrated, which induced statistically significant antibody titer.

Antibody titers of vaccinated ducks were measured by the PHA test. This test is commonly used to measure DP vaccine antibody response (Akter et al., 2004). However, the lack of specificity becomes particularly noticeable at low antibody titters due to the assay's inability to distinguish between biologically active and non-neutralizing antibodies (Roper et al., 2013).

The inactivated vaccine induced the highest antibody titer (54 ± 3.36) at day 28 post-vaccination. Hossain et al. (2005) and Kayesh et al. (2008), respectively, reported protective serum PHA antibody titers of 115.2 ± 12.8 and 57.60 ± 6.40 for the duck plague vaccination. Ducklings with PHA titers 22 ± 0.7 exhibited 100% resistance to the virulent DP virus challenge, according to Konwar et al. (2020). In this study, antibodies present in vaccinated ducks might have neutralized the virulent DP virus following challenge infection, which results in the protection of vaccinated ducks, compared to unvaccinated control.

The protective efficacy of the vaccine was calculated by challenge experiment. The experimentally developed vaccine was 100% protective against virulent DPvirus infection. A vaccine is considered adequate if it protects at least 80% of the challenge infection (Islam et al., 2009). The comparison of the PI of the experimentally developed inactivated DP vaccine and live attenuated DP vaccine indicated that both vaccines induced similar protection against virulent challenge infection. No pathological lesions were recorded in the vaccinated ducks, compared to the unvaccinated control following the challenge. Neutralization of the virus by the antibody of vaccinated ducks might prevent the localization of the DPvirus into the lymphoid tissues of vaccinated ducks.

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CONCLUSION

Data from this study suggest that the inactivated DP vaccine was effective against virulent DP virus infection in the current study condition and could be used as a suitable alternative to the live attenuated vaccine under the field condition of Bangladesh. However, the field trial for developing the administration of inactivated DP vaccine should be carried out on duck farms to evaluate its protective efficacy.

DECLARATIONS

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Availability of data and materials

The datasets generated for the current study are available from the corresponding author upon request.

Ethical consideration

All authors carefully checked the ethical issues such as plagiarism, misconduct, data fabrication, falsification, manuscript redundancy, and duplicate publication or submission.

Competing interests

The authors declare no conflict of interests.

Authors' contributions

Tanvir Ahamed designed and conducted the experiment, analyzed data, and wrote the manuscript. Papia Sultana collected samples and conducted an experiment. Md. Zaminur Rahman interprets the results of the postmortem examination and analyzed the data. Palash Bose conducted laboratory work and analyzed data. Mohammad Rafiqul Islam designed the experiment and wrote the manuscript. Mst. Minara Khatun designed the experiment and edited the manuscript. Md. Ariful Islam conceptualized and designed the experiment, critically analyzed the data, and wrote and revised the manuscript. All authors read and approved the last version of the manuscript.

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