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In ovo Vaccination in Ducks against Duck Virus Enteritis

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ABSTRACT

Background: One of the major diseases affecting ducks is Duck Virus Enteritis causing heavy mortality and high economic losses in duck farming.

Methods: In this study, *in ovo* vaccination technology was adopted using a Duck Embryo Fibroblast passaged Duck Virus Enteritis vaccine virus. This would be the first reported use of *in ovo* vaccination in ducks against a viral disease. Virus Neutralisation Test was used to measure the immune response after standardisation of the technique using hyperimmunised Barred Plymouth Rock rooster. An optimised dose of the Duck Virus Enteritis vaccine virus was computed using Embryo Lethal Dose50 assessment of hatchability. *In ovo* vaccination was done in 2 groups of 40 embryos each, one group acting as negative control and the other group as virus treatment group. A booster dose was given to the treatment group on 14th day post hatch.

Result: Virus Neutralisation test using a beta method was carried out to measure the immune response. The treatment group showed an immune response of 3.33±0.21 Log_2 Antibody titre. This opens up a plethora of options of application of *in ovo* vaccination in ducks against viral diseases which would be a boon to the duck farming industry.

Key words: Duck Virus Enteritis, Ducks, *In Ovo* Vaccination.

INTRODUCTION

Ducks are an important component of poultry industry being more resistant to most infectious diseases. They are more productive as they lay eggs even during second and third year. As they are also foragers, feed cost is reduced in semi-intensive rearing (Rajput *et al.,* 2014). However, Duck Virus Enteritis (DVE) or duck plague as it is popularly known due to the high mortality caused, is a serious threat to duck farming. Sudden death and haemorrhages and necrosis on post mortem examination are typical (Neher *et al.,* 2018). Young ducklings are highly susceptible. It is caused by Anatid alphaherpesvirus-1 from the genus *Mardivirus* under *Herpesviridae* family (Li *et al.,* 2009). Liver and kidney specific biomarkers were reported to be elevated due to the viral infection (Chabukdhara *et al.,* 2023). Vaccination in India is carried out using chicken embryo adapted live virus which has drawbacks (Konwar *et al.,* 2019). There is latency of the virus in the Trigeminal nerve which can cause an outbreak due to reactivation (Shawky and Schat, 2002). This also causes a problem in vaccination in live vaccines while inactivated vaccines are not efficient in generating an immune response (Aravind *et al.,* 2015; Samia and Sandhu, 1997).

In ovo vaccination provides early immunity, uniform and fast delivery as well as reduced stress and labour costs (Souza, 2008). *In ovo* vaccination in ducks against aflatoxicosis was done in our previous research (Sarmah *et al.,* 2023). Other than that, there are no reports of *in ovo* vaccination in ducks, however *in ovo* feeding of amino acids and vitamins has been carried out (Hussian *et al.,* 2019; Syahruddin *et al.,* 2019). In this study, *in ovo* vaccination was carried out on 25th day of embryonation in Khaki Campbell ducks using a duck embryo fibroblast adapted vaccine at an optimised dose to generate immune response against DVE. The vaccine virus was passaged 4 times in chicken embryo fibroblast (CEF) cells to observe whether the virus was replication competent. Then, it was passaged 4 times in duck embryo fibroblast (DEF) cells as well. Titration of the virus was done as previously described (Reed and Muench, 1938). Virus Neutralisation Test (VNT) was standardised using serum of a hyperimmunised Barred Plymouth Rock rooster. The immune response was checked by Virus Neutralisation Test. The results revealed that immune response was generated which could be a major breakthrough as there are no reports of *in ovo* vaccination in ducks against DEV.

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MATERIALS AND METHODS

This study was conducted from 2018 to 2020 at the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram.

Passage of vaccine virus in CEF

The vaccine virus was passaged in CEF cell culture 4 times using 5% Foetal Bovine Serum (FBS) concentration in

Dulbecco's Minimum Essential Medium (DMEM) as growth media and 1% FBS concentration in DMEM as maintenance media. of virus (10 µl). The virus inoculated media were collected after 3 freeze thaw cycles and centrifuged at 10000G at 4°C for 15 minutes and the viral supernatants collected and kept at -20°C.

Passage of vaccine virus in DEF

The CEF passaged virus was diluted 1:100 times and then passaged 4 times in DEF and the viral inoculated media were collected using same conditions as before after appearance of cytopathic effects (CPE) after 96 hours post inoculation.

Titration of DEF passaged virus

This was done as per Reed and Muench (1938) in 96 well cell culture plates (Tarsons). Fixing was done with 4% paraformaldehyde (Merck) and staining by 0.1% crystal violet (Loba Chemie).

Hyperimmunization of Barred Plymouth Rock rooster

Hyperimmunization of a Barred Plymouth Rock rooster was done as per Table 1.

Standardisation of VNT

A beta method using $10^{2.43}$ Tissue Culture Infective Dose ($TCID^{50}$) CEF passaged virus was used with two-fold dilutions of decomplemented hyperimmune sera raised from the Barred Plymouth Rock rooster which remained apparently healthy even after intravenous virus inoculation.

Embryo Lethal Dose 50 (ELD50) assessment of DEF passaged virus

ELD50 was assessed by hatchability of duck eggs after viral inoculation by in ovo route on 25th day of embryonation at different dilutions from 10^{-2} to 10^{-5} of the DEF passaged virus.

In ovo vaccination of duck embryos

In ovo vaccination of duck embryos were carried on 25th day of incubation in 2 different groups of 40 embryos. 0.5 ml was the total amount injected *via in ovo* route. Group 1 was negative control receiving only Phosphate Buffered Saline (PBS) and Group 2 was the treatment group receiving the optimised dose of DEF passaged DEV virus.

Booster dose after 14 days post hatch

A booster dose of 100 $TCID^{50}$ of DEF passaged DEV virus was given subcutaneously on 14th day post hatch to the DEV treatment group.

Virus Neutralisation Test

VNT was done by beta method using constant 1000 $TCID^{50}$ DEF passaged virus with two-fold dilutions of decomplemented sera from six ducklings from each group collected on 10th, 21st and 28th day post hatch.

RESULTS AND DISCUSSION

The present study was aimed with the purpose of introducing a novel technology *i.e.*, *in ovo* vaccination which is the standard for vaccination against Marek's disease in chickens (Sharma and Burmester, 1982), in the duck farming industry to combat a fatal disease like DEV. Firstly, the DEV vaccine virus was passaged 4 times in CEF to assess its replication competence. Following that after 4 passages of the DVE virus in DEF, the virus remained replication competent as observed by the CPE like rounding of cells, blebbing and vacuole formation. The DEV vaccine virus was passaged in DEF instead of CEF prior to vaccination so that there would be no immune response to chicken proteins present in CEF. Also, it was to check the replication competence of the virus in duck cells so that the virus would replicate when given by *in ovo* route. This would help in generating the required immune response specifically against DVE in the ducklings.

Titre of the DEV virus per ml was $10^{5.43}$/ml $TCID^{50}$. Following the ELD^{50} assessment by hatchability of the embryos, 100 $TCID^{50}$ was found to be the safe dose for *in ovo* vaccination as all of the ducklings hatched at this dilution.

The $\log_2$ antibody titre to CEF passaged virus used for standardisation of VNT was observed to be 8.82±0.22. The negative control cells showed a normal monolayer cell architecture with which the monolayers of virus neutralized wells were comparable. CPE was observed 96 hours post inoculation

The hatch of the *in ovo* vaccinated groups were comparable *i.e.,* 32 for the negative control group while it was 34 for the virus treatment group. Therefore, it can be said that there was no mortality due to the virus. Serum collected from the ducklings (6 per group) on 10th, 21st and 28th day post hatch was decomplemented at 56°C for 30 minutes prior to use in VNT. The VNT results are shown in Table 2. The treatment group had a constant immune response of 3.33±0.21 Log_2 Antibody titre even after booster dose of DEV was given.

In ovo vaccination technology is a novel approach to offer protection to diseases in the embryonic stage itself so as to confer protection during the post hatch stage. It

Table 1: Hyperimmunization Schedule.

Day	Inoculation route	Composition
0th	Intradermal	Paraformaldehyde (14.5 mg) + CEF passaged DEV virus (20 µl) + Freund's incomplete Adjuvant
7th	Intradermal	Paraformaldehyde (14.5 mg) + CEF passaged DEV virus (20 µl) + Freund's incomplete Adjuvant
14th	Intramuscular at 2 different sites	Paraformaldehyde (14.5 mg) + CEF passaged DEV virus (20 µl)
21st	Intramuscular at 2 different sites	Paraformaldehyde (14.5 mg) + CEF passaged DEV virus (20 µl)
28th	Intravenous	CEF passaged DEV virus (20 µl)

Table 2: Mean±SE Log_2 Antibody Virus Neutralisation Titre.

Day	Group 1	Group 2
10	0	3.33±0.21
21	0	3.33±0.21
28	0	3.33±0.21

has been reported to result in increased immune response in chickens which was evident by higher lymphoproliferative ability of splenocytes, percentage of CD4 T cells and expression of MHC-II in splenic T cells when herpesvirus of turkey was injected (Boone *et al.,* 2020). Furthermore, inactivated fowl adenovirus 8b given along with poly [di(sodium carboxylatoethylphenoxy)] phosphazene and avian beta defensin as adjuvants stimulated strong immune response in chickens (Sarfraz *et al.,* 2017).

From Table 2, it can be seen that there was no neutralising antibody titre in the negative control while in the virus treatment, an immune response can be observed. The VNT Log_2 antibody titre remained constant in the virus treatment group even after booster dose suggesting the virus was replication competent and that the immune response was skewed towards Th1 system. DEV virus skews the immune response towards Th1 (Rauw *et al.,* 2010). It is important to note that an immune response is generated with a replication competent DVE virus thus creating vast possible avenues of utilisation of *in ovo* vaccination in ducks against DVE. Ducklings are susceptible to DVE prior to the standard vaccination age of 2 - 6 weeks which could be solved by adopting *in ovo* vaccination as industry standard.

CONCLUSION

From the above results, it can be inferred that *in ovo* vaccination can be adopted in ducks against the dangerous disease of DVE which causes high mortality. This would provide protection against the disease during the post-natal stage of the ducklings at which they are most susceptible. Also, there has been no report of *in ovo* vaccination in ducks till the compilation of this article thus this provides a novel approach which is safe, less laborious and cheaper compared to post hatch vaccination. Adoption of the *in ovo* vaccination technology in duck farming would be a boon to the poultry industry.

Conflict of interest

All authors declared that there is no conflict of interest.

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