

# Evaluation of Mucosal Immunity in Chickens Vaccinated with Thermo Stable Live Oral Pellet Newcastle Disease Vaccine

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## Abstract

The Newcastle disease outbreak in chickens is a continuing threat to the poultry industry. Infection with Newcastle disease is greatly influenced by the immune status of the birds. The mucosal immunity plays a major role in the local immune response in the protection of chickens against diseases. This study was undertaken to find the efficacy of thermo stable live oral pellet vaccine in developing the mucosal immunity in chickens. Samples were collected from Harderian gland, lachrymal fluid, Tracheal fluid, Intestinal washings, Bile and Serum of chickens after administration of oral pellet vaccine to detect presence of NDV specific IgA antibodies. Results showed that there is significant increase in the immune response after one week post vaccination with no significant difference between 14 and 21 days after vaccine. There exists significant difference in Mean OD values between samples of Harderian, Lachrymal, Trachea, Intestine, Bile and serum with bile found to have increased IgA response. Challenge experiment results showed that oral pellet vaccine was able to protect chickens against virus challenge by increasing the mucosal immunity against Newcastle disease.

## Keywords

Mucosal immunity • IgA antibodies • IgA ELISA • Newcastle disease vaccine • Harderian gland • Lachrymal fluid

## Introduction

The poultry industry incurs heavy economic loss due to the outbreak of Newcastle Disease Virus (NDV) throughout the world [1]. The control of NDV is difficult because of the close confinement of chickens. Almost all species of birds are susceptible for the Newcastle disease virus [2]. Moreover, the carrier potential of different species of wild birds was reported by some workers [3]. The involvement of different varieties of birds and also the wild animals are responsible for the NDV outbreak [4]. Therefore, continuous maintenance of immunity is important for the protection of chickens against NDV disease. The local immune response plays a major part of protection against NDV at the site of entry of pathogens [5]. Many researchers stress the importance of the knowledge on the immunological status of the birds against disease is vital for the proper control of the NDV [6-8].

## Vaccine

The thermo stable live Oral Pellet Newcastle Disease Vaccine (OPNDV) developed at the Department of Veterinary microbiology using D58 isolate of NDV was given to chicks at the age of 10th day through oral route. Each oral pellet vaccine grain had 106.5 EID<sub>50</sub>/grain.

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**Received date:** October 28, 2021; **Accepted date:** November 11, 2021; **Published date:** November 18, 2021

## Birds

B1 Broiler Chicks with no history of vaccination from day old were housed in animal house at the Madras Veterinary College. They were provided with unmedicated broiler starter mash and water ad libidum and maintained as per the guidelines provided by the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on animals, Chennai, India). The trial on the birds was approved by CPCSEA (Proposal number: 2491/DR/01).

## Samples

Harderian glands and lachrymal fluid was collected as per the procedure described by [9]. Tracheal fluid was collected by swabbing and the swabs were placed in Tryptose Phosphate Broth (TPB). Intestinal washings were derived by lavage with TPB. Bile was collected from gall bladder using 22 gauge needles [10].

## Experimental Design and Sample Collection

The experimental group comprised of 40 chicks. The birds were divided into 2 groups (20 birds each). The first group was vaccinated with oral pellet Newcastle disease vaccine at a dose of one grain per bird. Control group was not vaccinated. Samples such as Harderian gland, lachrymal fluid, tracheal fluid, intestinal washings, bile and serum were collected from both the groups on 7, 14 and 21 days to assess mucosal immune response.

## Indirect Elisa for Iga

The optimum dilutions of test antigen (infected allantoic fluid and tissue homogenates), samples and conjugate for the test system were determined by checkerboard titration [11].

The reaction volume for the entire assay was 100 µl/well. After each step, the ELISA plate was washed six times in an automatic plate washer (Bio-Rad, Model #1575, USA) utilizing 400 µl of wash solution. The microplate (Immunoplate) was adsorbed with NDV protein in coating buffer to all the wells overnight at 4°C or at 37°C for 60 minutes. After washing, the plate was blocked with blocking buffer and incubated for 60 min at 37°C. After another washing, 1:1000 dilutions of samples were added in duplicate for optimum results and the plate was incubated for 45 min at 37°C. After another washing, goat anti chicken IgA peroxidase conjugate at 1:5000 dilution in blocking buffer was added to the washed plate and incubated for 30 min at 37°C. The enzyme substrate solution (ABTS) was added to the microplate and incubated for 10 min in the dark. The reaction was stopped by adding 1% SDS. Optical density values were measured at 405 nm in an automatic ELISA reader (Bio-Rad, Model #550, CA, USA). Cut-off between positive and negative was kept as 2 times the value of negative.

## Challenge Experiment

Twelve birds were used for challenge studies. Virulent Newcastle disease strain was used for challenge testing.

### Statistical analysis

The data were analysed using Minitab statistical software package. The duplicate Optical Density (OD) values of IgA ELISA were checked for measure of dispersion by calculating the Standard Deviation (SD) and Coefficient of Variation (COV) between OD values. If the COV was more than 20 per cent, the readings were not included for further calculation.

## Opndv Vaccine Experiment

There is significant difference between vaccinated and unvaccinated group ( $P=0.00$ ,  $P<0.05$ ). There is significant difference in the immune response between 7, 14 and 21 days after vaccination ( $P=0.01$ ,  $P<0.05$ ) and shown in Table 1. There exists significant difference in Mean OD values between samples of Harderian, Lachrymal, Trachea, Intestine, Bile and serum ( $P=0.01$ ,  $P<0.05$ ) and shown in Tables 1-3.

**Table 1.** Comparison of vaccinated and unvaccinated group.

Vaccine	N	Mean	Grouping
Vaccinated	36	0.0834722	A
Unvaccinated	36	0.0640278	B

**Table 2.** Comparison of IgA immunity level between different days of vaccination.

Days	N	Mean	Grouping
14	24	0.0825833	A
21	24	0.0809583	A
7	24	0.0577083	B

**Table 3.** Comparison of IgA immunity level between different samples.

Samples	N	Mean	Grouping
Bile	12	0.103167	A
Intestine	12	0.080667	A B
Trachea	12	0.0655	A B
Lachrymal	12	0.0655	A B
Serum	12	0.064417	B
Harderian	12	0.06325	B

## Challenge Experiment

All the 6 birds in vaccinated group survived the challenge and none of the unvaccinated birds survived the challenge with virulent NDV.

## Conclusion

It is concluded that thermostable live OPNDV stimulate the mucosal immune system in the chickens, and it conveyed protection to chickens against the Newcastle disease virus. It is also found that the vaccine maintains the immune status of the birds to protect against the disease.

## Acknowledgement

The authors are thankful to Department of Microbiology, Madras Veterinary College, TANUVAS, Chennai for providing all necessary lab facilities to carry out the work. The corresponding author tribute the contribution made by chickens for this research, which was approved by ethical committee (CPCSEA Proposal number: 2491/DR/01).

## Declaration

The authors declare that there is no conflict of interests.

## Animal Ethics Approval

Committee for the purpose of control and supervision on experiments on animals, Chennai, India (CPCSEA) Proposal number: 2491/DR/01.

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**How to cite this article:** Varalakshmi, S and J John Kirubaharan. "Evaluation of Mucosal Immunity in Chickens Vaccinated with Thermo Stable Live Oral Pellet Newcastle Disease Vaccine." *J Anim Health Behav Sci* 5 (2021): 142.