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Serological evaluation of velogenic strain of ppmv-1 in vaccinated birds

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Abstract
The present study was conducted to know the HI titres in pigeons challenged with velogenic strain of PPMV -1 (local strain). Twenty four healthy pigeons were vaccinated with live attenuated lentogenic strain PPMV-1 vaccine (local isolate) and 12 non-vaccinated healthy pigeons were randomly selected and taken to the Laboratory Animal House in isolated condition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata for the challenge study. Vaccinated pigeons were divided into two groups i.e. vaccinated challenge group and vaccinated control group, comprised of 12 pigeons in each group. All the pigeons were inoculated orally by velogenic strain PPMV-1 @ 10^6 EID_{50} to each bird. Serum samples were collected from all the birds of all the 3 groups weekly for 3 weeks for the estimation of HI titres. HI log mean titre in the non-vaccinated challenge group was below detectible level (0.00 ± 0.00) before challenge. After challenge, the mean titre non-significantly increased (i.e. 0.301 ± 0.00) in the survived birds (4 numbers) at the end of 1st week of post challenge whereas, in the vaccinated control group, the log HI mean antibody titre before immunization was 1.982 ± 0.044 which was also above protective level. With the advancement of time, there was non-significant alteration of log HI mean antibody titres i.e. 2.007 ± 0.085, 1.957 ± 0.094 and 2.007 ± 0.1393 on the day of 7th, 14th and 21st respectively which were always persisted above protective level. In the vaccinated challenge group the log HI mean titre values before vaccination was 2.258 ± 0.045 which was above protective level (i.e. 1.50 ± 0.00). After challenge, the mean HI log titre significantly (≤0.05) decreased to 2.025 ± 0.091 and 2.025 ± 0.082 at the end of 1st week and 2nd week of post challenge respectively but never became below protective level. Thus, the live attenuated lentogenic strain PPMV-1 vaccine (local isolate) was potent and efficacious, gave adequate protective antibody response and withstood challenge with the velogenic strain of PPMV-1.

Keywords: PPMV -1 vaccine, live attenuated lentogenic strain, pigeons

Introduction
Pigeon paramyxovirus type 1 (PPMV-1) isolates are antigenic variants of avian paramyxovirus type 1 (APMV-1) is a member of the genus Rubulavirus within the virus family Paramyxoviridae (Van Regenmortel *et al.*, 2000) [7]. As per the report of FAO, about 50% countries of Asia, there is prevalence of velogenic strains of paramyxovirus. On world scale, about 20% of countries having velogenic strains of PMV. Velogenic form is an extremely important problem in tropical countries. Where PMV is enzootic, outbreak of the disease regularly result in mortality of 50-100% and it causes highest economic losses by death and loss of production (Roy *et al.* 2000) [4]. Infection with virulent strains has resulted in several panzootics since 1926 (Alexander *et al.*, 2004) [1]. Clinical signs in poultry birds include drop in egg production, respiratory distress, listlessness, weakness and central nervous system signs (Saif *et al.*, 2008) [6]. Challenge study of the vaccinated birds with velogenic strain of PPMV -1 (local strain) are scanty in India. Hence, the present study was conducted to know the HI titres in pigeons challenged with velogenic strain of PPMV -1 (local strain).

Materials and Methods
Source of vaccine
Vaccine used in the present study was previously developed with the lentogenic strain PPMV-1 virus isolated from local field outbreak. Department of Veterinary Epidemiology and Preventive Medicine, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal Fishery Sciences, Kolkata previously isolated and identified PPMV- 1 from local field outbreak.

Source of birds

Twenty four vaccinated pigeons were randomly selected and 12 non vaccinated pigeons were taken to the Laboratory Animal House in isolated condition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata for challenge study.

Source of challenge virus

The Velogenic strain of PPMV-1 which was used in the study was previously isolated from local field outbreak. The virus was previously pathotyped as velogenic one in the Department of Veterinary Epidemiology and Preventive Medicine, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata. The virus was further characterized and confirmed by OIE, FAO and National Reference Laboratory for Newcastle disease and Avian Influenza, Italy.

Design of work for challenge study

Twenty four healthy pigeons which were previously vaccinated with live attenuated lentogenic strain PPMV-1 vaccine (local isolate) and 12 non-vaccinated healthy pigeons were randomly selected and taken to the Laboratory Animal House in isolated condition, Faculty of Veterinary and Animal Sciences,West Bengal University of Animal and Fishery Sciences, Kolkata for the challenge study. The total numbers of vaccinated pigeons were divided in to two groups i.e. vaccinated challenge group and vaccinated control group, comprised of 12 pigeons in each group. The 12 number of non-vaccinated pigeons were kept as non-vaccinated challenge group. All the groups of pigeons were housed / isolated separately in cases and provided food and water ad lib. After passing of one week for aquestamised, the serum antibody titres were estimated from all the pigeons of all the three groups. The all the pigeons of vaccinated challenge group and non-vaccinated challenge group were inoculated orally by velogenic strain PPMV-1 @ 10^6 EID_{50} to each bird and observed for any abnormality / mortality after next 3 weeks. And the 12 pigeons of vaccinated control group were kept as untreated /non-challenged control received only equal volume of PBS. Serum samples were collected from all the birds of all the 3 groups weekly for 3 weeks and HItitres were estimated as per standard protocol of OIE, 2009.

Results and Discussion

Mean± SE of Log HI titre before and after challenge with Velogenic Pathotype of PPMV-1 (local isolate) are depicted in Table 1 and Fig. 1. In the vaccinated challenge group the log HI mean titre values before vaccination was 2.258 ± 0.045 which was above protective level (i.e. 1.50 ± 0.00). After challenge, the mean HI log titre significantly (≤0.05) decreased to 2.025 ± 0.091 and 2.025 ± 0.082 at the end of 1st week and 2nd week of post challenge respectively but never became below protective level. Again with the advancement of time, the log HI mean titre increased significantly (≤ 0.05) at the end of 3rd week of post challenge and also acquired higher level (i.e. 2.299 ± 0.046) non-significantly than the mean HI log titre of before vaccination (i.e. 2.258 ± 0.045). On the above observation it might be assumed that after challenge to the vaccinated challenge group of pigeons, a little quantity of circulatory antibody titre was neutralized by the inoculated velogenic strain of PPMV-1, resulting significantly decreased (≤0.05) of HI log mean titre on 7th and 14th day of post challenge. As the birds having above protective level circulatory antibody titre there was no causality due to administered virulent PPMV -1. With the advancement of time, the virulent PPMV-1 inoculated pigeons withstood the infection and again restimulation of immune system, the antibody titre increased significantly (<0.05) at the end of observation period i.e. 3rd week of post challenge. Similar findings was reported by by Roy (2009) who observed that the antibody titre was decreased at the initial stage of post challenge due to neutralization of the virus by circulating antibodies.

In the vaccinated control group, the log HI mean antibody titre before immunization was 1.982 ± 0.044 which was also above protective level. With the advancement of time, there was non-significant alteration of log HI mean antibody titres i.e. 2.007 ± 0.085, 1.957 ± 0.094 and 2.007 ± 0.1393 on the day of 7th, 14th and 21st respectively which were always persisted above protective level. The constant persisted above protective level log HI mean titre during the observation period might be due to the continuous stimulation of host immune system by the administered live attenuated PPMV-1 vaccine virus (local isolate) resulting of constant production and release of antibody in the blood circulation. Similar finding was noted by Roy *et al.* (2009) [5], Kapczynski *et al.* (2006) [2] and Wambura and Wilson (2009) [8] who reported that multiage vaccinated pigeons survived the challenge of virulent PMV-1 / PPMV-1.

HI log mean titre in the non-vaccinated challenge group was below detectible level (0.00 ± 0.00) before challenge. After challenge, the mean titre slightly non-significantly increased (i.e. 0.301 ± 0.00) in the survived birds (4 numbers) at the end of 1st week of post challenge. No non-vaccinated challenged bird survived at the end of 2nd week post challenge. In the context, it might be fact that non-vaccinated pigeons could not withstand the virulence challenge virus due to no circulatory protective antibody. At the end of 1st week, the survived challenged birds showed non-significant increase of detectable level mean antibody i.e. 0.031 ± 0.00 which was also below protective titre and could not withstand the challenge virus and ultimately mortality occurred. Similar finding was reported by Roy (2009) [5] who reported that out of 10 non-vaccinated challenged pigeons 8 birds died on 1st week and rest 2 pigeons died on the 2nd week.

Table 1: Mean± SE of Log HI titre before and after challenge with Velogenic Pathotype of PPMV-1 (local isolate)

Groups	No. of pigeon	Log HI Titre (mean ±SE)			
		Before vaccination	After vaccination		
		0 day	7th day	14th day	21st day
Vaccinated challenge Gr.	12	2.258± 0.045 [a]	2.025± 0.091 [b]	2.025[b]± 0.082 [b]	2.299 ± 0.046 [a]
Vaccinated control Gr.	12	1.982± 0.044 [a]	2.007± 0.085 [a]	1.957± 0.094 [a]	2.007± 0.139 [a]
Non- vaccinated challenged Gr.	12	0.00[c]± 0.00	0.301[c]± 0.00	-	-

Means bearing different superscripts differ significantly

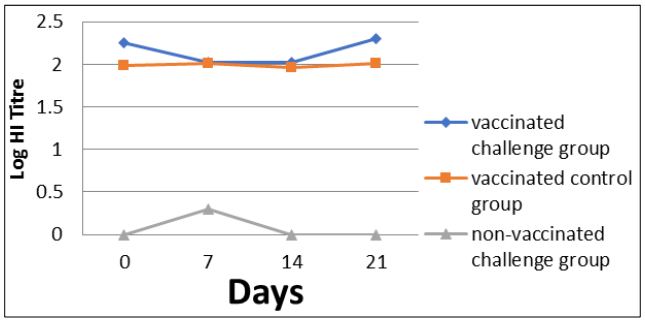

Fig 1: Log HI mean titre before and after challenge with Velogenic Pathotype of PPMV-1 (local isolate)

Conclusion

The live attenuated lentogenic strain PPMV-1 vaccine (local isolate) was potent and efficacious, gave adequate protective antibody response and withstood challenge with the velogenic strain of PPMV-1.

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