

Original Research Article

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Studies on Seroconversion of the Live Attenuated Lentogenic Strain of PPMV -1 Vaccine

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ABSTRACT

The present study was carried out to know the seroconversion of the live attenuated lentogenic strain of PPMV -1 vaccine in field level. Seroconversion study was undertaken to assess the antibody status of the pigeons which are vaccinated with pigeon paramyxovirus type 1 (PPMV- 1) vaccine (local isolate) in different areas of the districts Nadia, 24 Parganas (North), and Kolkata in West Bengal, India during the period from June 2016 to May 2017. One thousand pigeons were selected from the different villages/ areas of the Nadia district, 24 Parganas (North) and Kolkata for the study. All the pigeons were vaccinated with live attenuated lentogenic strain PPMV-1 vaccine (local isolate) @ $10^{6.5}$ EID₅₀ per dose to each bird intranasally ignoring the age of the birds and booster dose was given after 21 days of 1st vaccination and further, revaccination was done every 2 months interval. Serum samples were separated aseptically and individually. Antibody titres were assessed from individual serum sample by haemagglutination inhibition (HI). The mean antibody titre before first vaccination was very negligible (0.1195 ± 0.0379) which was below protective level (2^5 or 1.50 ± 0.00). After first vaccination the mean antibody titre increased significantly ($P \leq 0.01$) and reached to 1.4822 ± 0.0278 which was also considered below protective level before boosting i.e. 21 days of post first vaccination. Again the mean antibody titre increased significantly ($P \leq 0.01$) after boosting and persisted above protective level i.e. reached to 1.9599 ± 0.0351 on 2 (two) months of post boosting (i.e. 81st days study). That higher level of titres always persisted significantly ($0 \leq 0.01$) above protective level up to 8 (eight) months of post boosting, (i.e. 261st days study). Thus, after first vaccination the mean antibody titre increased significantly and reached just below protective level. After boosting and regular revaccination every 2 months interval the antibody titre always persisted above protective level throughout the study period.

Keywords

PPMV -1 vaccine,
Live attenuated
lentogenic strain,
Pigeons

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Introduction

Pigeon keeping is the art and science of breeding domestic pigeons. People have practiced pigeon keeping for about 10,000 years in almost every part of the world. Like poultry birds and canaries, pigeons are also susceptible to various health related problems i.e. bacterial, viral, fungal infections and parasitic infestation. Among all these problems, paramyxovirus (PMV) infection is the most dreadful disease in pigeon. It is one of the OIE listed diseases, highly fatal and is endemic in tropical and subtropical countries of the world. 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). 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). Vaccination is the most effective means of controlling PMV infection. A few live attenuated PPMV vaccines are being used in some countries (Peters, 2017 and Miller, 2017). But in India now-a-days, there is commercially no availability of such type of vaccine for using at field level against PPMV infection in pigeon. Therefore, production of a good quality, absolutely efficacious, live attenuated vaccine from field isolate of PPMV -1 is a must. Considering the above facts the present study was carried out to know the seroconversion of the live attenuated lentogenic strain of PPMV -1 vaccine in field level.

Materials and Methods

Seroconversion study was undertaken to assess the antibody status of the pigeons which are vaccinated with pigeon paramyxovirus type 1 (PPMV- 1) vaccine (local isolate) in different areas of the districts Nadia, 24 Parganas (North), and Kolkata in West Bengal, India during the period from June 2016 to May 2017. One thousand pigeons were selected from the different villages/ areas of the Nadia district, 24 Parganas (North) and Kolkata for the study. All the pigeons were vaccinated with live attenuated lentogenic strain PPMV-1 vaccine (local isolate) @ $10^{6.5}$ EID₅₀ per dose to each bird intranasally ignoring the age of the birds. Boostering was performed after 21 days of 1st vaccination and further, revaccination was done every 2 months interval. Blood samples were collected randomly from 10% of the vaccinated pigeons just prior of each vaccination. Serum was separated aseptically and individually. Antibody titres were assessed from individual serum sample in the laboratory by haemagglutination inhibition (HI) test as per OIE, 2009.

Preparations of 1% chicken wash RBC

Around two ml of blood was collected from healthy chicken which was free from antibody of Newcastle disease virus (NDV) in equal volume of Alsever's solution and mixed well. The blood was centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded. The cells were washed with double volume of PBS (pH 7.2) and centrifuged 3 times at 1500 Rpm for 10 minutes.

The packed RBCs were resuspended in PBS to give a 10% (V/V) suspension as stock solution and stored in refrigerator. The working solution as 1% (V/V) suspension was prepared from stock solution in PBS while HI tests were performed.

Haemagglutination inhibition (HI) test

The Haemagglutination test protocol was as followed as per the standard protocol of OIE, 2009 and is as follows:

25 µl of PBS was dispensed into each of 12 well of a v-bottomed microtitre plate. 25µl of serum was placed into the first well and mixed thoroughly. Two fold dilution of 25 µl volumes of the serum suspension were made across the plate up to 12th well and from that well 25 µl was discarded. 25 µl of 4HAU of antigen (virus) was added to each well, after tapping the plate gently mixed and allowed the plate for 20 minutes. 25 µl of 1% (v/v) chicken washed RBC was dispensed into each well and mixed by gentle shaking. Allowed the plate at room temperature for 30 minutes, when control RBCs settled to a distinct button. The agglutination was assessed by tilting the plate and observed the presence or absence of tear shaped steaming of RBCs. The HI titre was expressed as reciprocal of the highest dilution of serum, inhibited agglutination of RBCs by 4HAU of antigen (virus).

Results and Discussion

The Mean antibody titre of different days of immunization wise of the pigeons vaccinated with live attenuated lentogenic strain of PPMV -1 vaccine (local isolate) are depicted in Table 1 and Figure 1.

The mean antibody titre before first vaccination was very negligible (0.1195 ± 0.0379) which was below protective level (2^5 or 1.50 ± 0.00). After first vaccination the mean antibody titre increased significantly ($P \leq 0.01$) and reached to 1.4822 ± 0.0278 which was also considered below protective level before boosting i.e. 21 days of post first vaccination. Again the means antibody titre increased significantly ($P \leq 0.01$) after

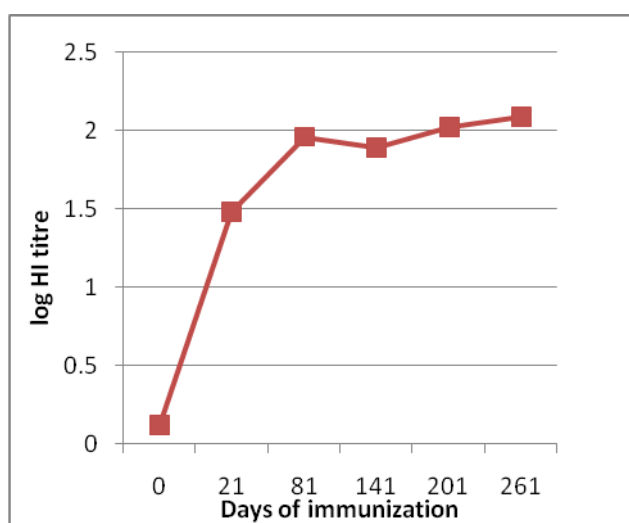
boosting and persisted above protective level i.e. reached to 1.9599 ± 0.0351 on 2 (two) months of post boosting (i.e. 81st days study). That higher level of titres always persisted significantly ($0 \leq 0.01$) above protective level up to 8 (eight) months of post boosting, (i.e. 261st days study). Similar findings was observed by Roy (2009) who reported that pigeons vaccinated with live attenuated PPMV -1 vaccine (local isolate) @ $10^{6.5}$ EID₅₀ intranasally and boosting was performed after 21 days of first vaccination, the antibody titre increased significantly and reached above protection level on 14th days of post first vaccination. The titres persisted above protective level of 40 days observation after boosting. Similar finding was also observed by Adriano *et al.*, (2009) who observed that the antibody response in pigeons vaccinated with LaSota strain vaccine on 0,35 and 86 day. The antibody HI titres were estimated just prior of vaccination and also on the day of 15, 52 and 101 post vaccination. The increasing mean ($\log_2 X$) titres were recorded i.e. 0.93 ± 0.91 (D 0), 2.43 ± 1.55 (D 15), 3.43 ± 2.05 (D 35), 4.36 ± 2.50 (D 32), 4.43 ± 2.28 (D 86) and 3.93 ± 1.49 (D 101). On 141st days of study the above protective level titre was slightly decreased to 1.889 ± 0.051 that was non-significantly in comparison to the titre of 81st days study i.e. 1.959 ± 0.005 . But always it remained above protective level. It might be fact that on 141st days of study, one of the flocks (containing 300 pigeons) was suffering with the clinical signs of anorexia, diarrhoea, depression, respiratory distress and with a few mortality. *Pasteurella multocida* was isolated and identified from that flock. No outbreak of PPMV -1 or no clinical signs of PPMV -1 was seen and even no PPMV -1 virus was isolated from the lesions of the dead pigeons during that period. Therefore, the slight decline of antibody titre might be due to secondary infection of pasteurellosis. Literature regarding this aspect was scanty.

Table.1 Mean antibody titre of different days of immunization wise of the pigeons vaccinated with live attenuated lentogenic strain PPMV -1 vaccine (local isolate)

Sl No	No of pigeons vaccinated	Day of immunization	Log HI titres		
			Before first vaccination	Before boosting	Thereafter every 2 months interval
1.	1000	0	0.1195± .0379 ^a		
2.	1000	21		1.4822± 0.0278 ^b	
3.	1000	81			1.9599± 0.0351 ^c
4.	963	141			1.8898 ± 0.0517 ^{cd}
5.	963	201			2.0249± 0.0344 ^{ce}
6.	963	261			2.0902± 0.0619 ^{cef}

Means bearing different superscripts differ significantly

Fig.1 Mean antibody titre of different days of immunization wise of the pigeon vaccinated with live attenuated lentogenic strain PPMV -1 vaccine (local isolate)



But similar findings were also observed by Sharma (2012) and Sharma (2016) who reported from West Bengal, India that live attenuated lentogenic strains ND vaccine (local isolate) and live attenuated thermostable lentogenic strain ND vaccine (local isolate) were highly potent and generated sufficient immune response in fowls in field condition. But the immune response was may not be optimum when the flocks were concomitantly infected with other diseases like colibacillosis, mycoplasmosis and coccidiosis, tape worms infestation respectively. From this study, it

was concluded that after first vaccination the mean antibody titre increased significantly ($0 < 0.01$) and reached just below protective level. But after boosting and regular revaccination every 2 months interval the antibody titres always persisted above the protective level throughout the study period.

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