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Evaluation of shelf life of live attenuated lentogenic strain PPMV-1 vaccine

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Abstract

The present study was conducted to evaluate the shelf life of live attenuated lentogenic strain PPMV-1 vaccine. Twenty four, 1 ml freeze dried vaccine vials were kept in the refrigerator at -120°C temperature. Each freeze dried vial contained 0.5 ml allantoic fluid containing live attenuated lentogenic strain PPMV-1 vaccine virus (local isolate) with addition of 0.5 ml cryoprotectant. HA titer as and EID_{50} were estimated just before keeping in the storage (in refrigerator at -120°C temperature) and thereafter, every 15 days interval until the viability of the vaccine virus became nil. before freeze drying of the live attenuated lentogenic strain PPMV-1 vaccine (local isolate) the HA titer, EID_{50} and number of dose of the vaccine per vial were 2^{10} , $10^{10.57}$ and 5874 respectively. After freeze drying, those values reduced to 2^1 , $10^{10.37}$ and 3706.5 accordingly to HA titer, EID_{50} and number of dose of vaccine per vial respectively. After keeping of the vaccine at -120°C temperature, the HA titer reduced to 2^9 that had remained unchanged, up to 90 days of post storage study period. On the other hand, the EID_{50} remained unchanged from the day of storage up to 90 days post storage study course at satisfied level. And, subsequently, the dose of the vaccine per vial also remained unchanged i.e. 3706.5 which was satisfactory up to the end of the study period. From the study it was concluded that freeze dried live attenuated lentogenic strain PPMV-1 vaccine (local isolate) had not lost its infectivity at -120°C within 3 months.

Keywords: Shelf life, live attenuated, lentogenic strain, PPMV-1 vaccine

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) [1]. 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) [8]. 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) [9]. 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) [8]. 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) [6, 5]. The present study was conducted to evaluate the shelf life of live attenuated lentogenic strain PPMV-1 vaccine.

Materials and methods

One millilitre (n=24) of freeze dried vaccine vials were kept in the refrigerator at -120°C temperature. Each freeze dried vial contained 0.5 ml allantoic fluid containing live attenuated lentogenic strain PPMV-1 vaccine virus (local isolate) with addition of 0.5 ml cryoprotectant. HA titer as per OIE (2009) [4] and EID_{50} as per FAO (2002) [2] and Reed and Munch (1938) [7] were estimated just before keeping the vaccine in the storage (in refrigerator at -120°C temperature) and at every 15 days interval until the viability of the vaccine virus became nil.

Determination of HA titer and EID_{50} of the vaccine just before lyophilization

The HA titer and EID_{50} of the live attenuated lentogenic strain PPMV-1 vaccine (local isolate)

were estimated just before lyophilization as per OIE (2009)⁽⁴⁾ and FAO (2002)^[22] and Reed and Munch (1938)^[7] respectively.

Determination of HA titer and EID₅₀ of the lyophilized vaccine

The HA titer and EID₅₀ of the live attenuated lentogenic strain PPMV-1 vaccine were determined just after lyophilization and every 15 days interval keeping the vaccine in refrigerator at -120 °C temperature as per OIE (2009)^[4] and FAO (2002)^[2] and Reed and Munch (1938)^[7].

Statistical analysis

General linear model was used to analysis the experimental data. Logarithmic transformation was done before analysis. Comparisons of different groups were performed by least significant difference (LSD) method. Pair t-test was done in case of small sample. The entire analysis was performed using SPSS-23 software package.

Results and Discussion

HA titre, EID₅₀ and number of doses per vial of live attenuated lentogenic strain PPMV-1 vaccine (local isolate) kept at -120 °C in freeze dried form are depicted in Table 1, Fig. 1, Fig.2, Fig. 3 and Fig. 4.



Fig 1: Inoculation of live attenuated lentogenic strain PPMV-1 vaccine virus (local isolate) in SPF eggs for estimation of EID₅₀

Table 1: Showing HA titre, EID₅₀ and number of doses per vial of live attenuated lentogenic strain PPMV-1 vaccine (local isolate) kept at -120⁰ C in freeze dried form

Sl. no.	Period of storage(days)		HA titre	EID ₅₀	Doses /ml		Doses /vial
1.	Before freeze drying		2 ¹⁰	10 ^{10.57}	10 ^{4.07}	11,748	5874
	After Freed drying						
2	0		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
3	15		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
4	30		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
5	45		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
6	60		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
7	75		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5
8	90		2 ⁹	10 ^{10.37}	10 ^{3.87}	7413	3706.5

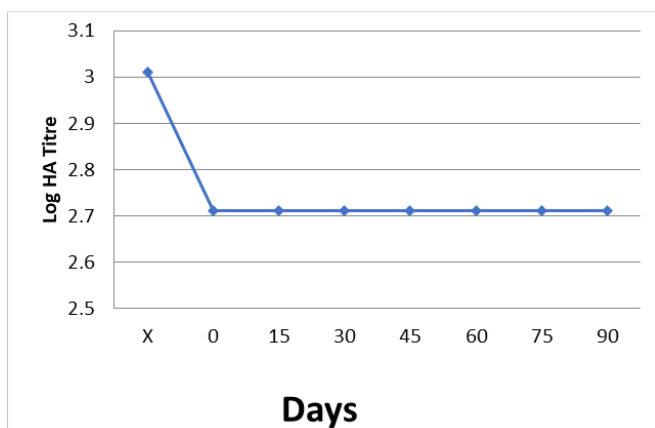


Fig. 2: Showing Log HA titer of live attenuated lentogenic strain PPMV-1 vaccine (local isolate) kept at -120⁰ C in freeze dried form

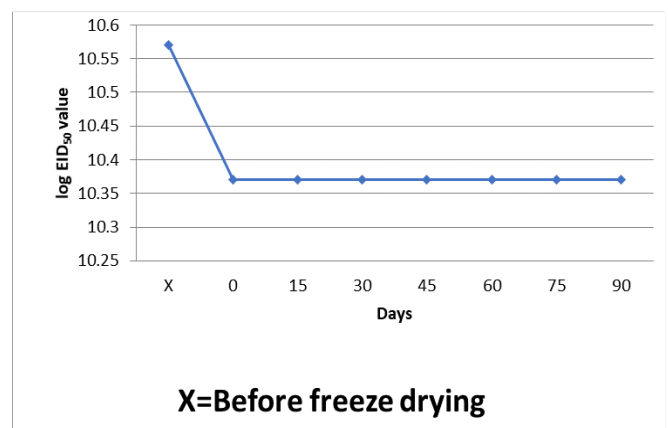


Fig. 3: Showing virus inoculation in embryonated SPF Eggs for calculation of EID₅₀

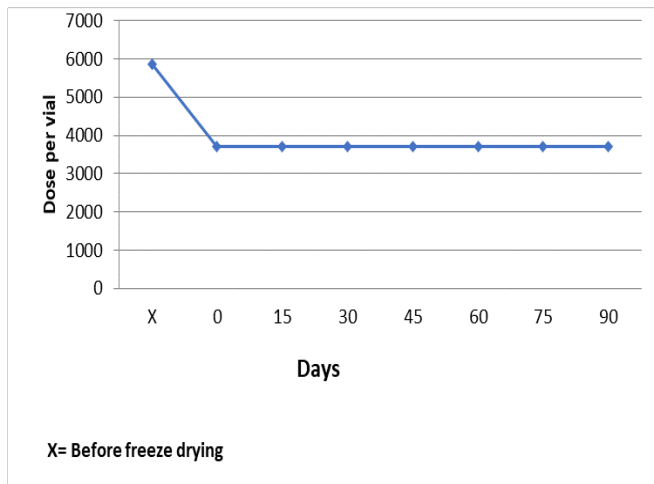


Fig. 4: Showing number of doses per vial of live attenuated lentogenic strain PPMV-1 vaccine (local isolate) kept at -120°C in freeze dried form

From the table 1 it was evident that before freeze drying of the live attenuated lentogenic strain PPMV-1 vaccine (local isolate) the HA titer, EID_{50} and number of dose of the vaccine per vial were 2^{10} , $10^{10.57}$ and 5874 respectively. After freeze drying, those values reduced to 2^{10} , $10^{10.37}$ and 3706.5 accordingly to HA titer, EID_{50} and number of dose of vaccine per vial respectively. After keeping of the vaccine at -120°C temperature, the HA titer reduced to 2^9 that had remained unchanged, up to 90 days of post storage study period. On the other hand, the EID_{50} remained unchanged from the day of storage up to 90 days post storage study course at satisfied level. And, subsequently, the dose of the vaccine per vial also remained unchanged i.e. 3706.5 which was satisfactory up to the end of the study period. Similar finding was also reported by Health *et al.* (1991) [3] who reported that commercial vaccine was stable in freeze dried form for 3 months at $18-22^{\circ}\text{C}$ and lost only $0.3\log_{10}$ on storage for a further 3 months. Spradbrow (1992) [10] reported that NDV vaccines are less heat stable in liquid forms than in freeze dried form. Additives protected virus infectivity during free drying and subsequently storage.

Conclusion

From the study it was concluded that freeze dried live attenuated lentogenic strain PPMV-1 vaccine (local isolate) had not lost its infectivity at -120°C within 3 months.

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