Comparative efficacy of cryoprotectant in the lyophilization of pigeon paramyxovirus-1 vaccine

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ABSTRACT

Preparation of live attenuated freeze dried Pigeon Paramyxovirus-1 (PPMV-1) vaccine selection of proper cryoprotectant which could preserve and protect the virus against injury due to reduction of temperature at the time of lyophilization, was very necessary. PPMV-1 locally isolated lentogenic strain was selected as seed virus which was properly attenuated by serially passaging in 9 to 10 days old embryonated specific pathogen free (SPF) fowl eggs and produced master seed virus and working seed virus. Five per cent lactalbumin hydrolysate with 10% sucrose (LAS) and 1% polyvinyl pyrrolidone (PVP) were used as two different cryoprotectant for lyophilization of working seed virus. After lyophilization value of haemagglutination (HA) titer and embryo infective dose fifty per ml (EID₅₀/ml) were changed and concluded that both LAS and PVP could be used as suitable cryoprotectant where LAS was better than PVP which was first time done in the whole world.

Key words: Cryoprotectant, Lactalbumin hydrolysate, Lyophilization, Polyvinyl pyrrolidone, Sucrose.

Lyophilization which also termed as freeze-drying was a controllable method of dehydrating labile products by vacuum desiccation. In preparation of live attenuated freeze dried vaccine lyophilization is an important step where we stabilize viruses by cooling of the liquid sample and two overlapping drying procedure (Adams, 2007). Cryoprotectant was needed for virus stabilization which preserves and protects the virus against injury due to reduction of temperature at the time of lyophilization (Pegg, 2007). In West Bengal, particularly in Kolkata, more than 4 lakhs and 3 thousand homing pigeons participate in the race every year according to Kolkata Racing Pigeon Organization (KRPO). Domesticated pigeons have an opportunity to interact with feral pigeons and they contact with many acute, chronic and subclinical diseases of which paramyxovirus infection is very important one. There is no specific treatment for PPMV-1 infection. Prevention through vaccination is the only way to control this disease. It is necessary to know about the cryoprotectant for a new vaccine where it can preserves and protects maximum number of virus. In the present study for the production of live attenuated freeze dried vaccine of Pigeon Paramyxovirus-1 (PPMV-1) it was necessary to use a suitable cryoprotectant.

Lentogenic seed virus of PPMV-1 was isolated and confirmed in ‘National Reference Laboratory for New Castle Disease and Avian Influenza, Instituto Zootopifattico Sperimentale delle Venezie, Viale dell’ Università’, 10-35020 Legnaro (PD), Italy. Seed virus was serially passaged in 9 to 10 days old embryonated specific pathogen free (SPF) fowl eggs through allantoic cavity route for proper attenuation of the virus i.e. after virus inoculation the embryo was live up to 120 hours or 5 days with a good haemagglutination (HA) titer. Master seed virus and working seed virus were prepared from the live attenuated virus. Infectivity of the master and working seed virus was evaluated by HA titer and value of embryo infective dose fifty per ml (EID₅₀/ml). Haemagglutination titer was evaluated by standard plate haemagglutination test (OIE, 2012) and EID₅₀/ml was performed to measure the concentration of the live virus in the suspension (Reed and Muench, 1938). One ml aliquot in sterile glass vials was prepared which contained 0.5 ml of allantoic fluid (working seed virus) and 0.5 ml cryoprotectant. Solution of equal volume of five percent lactalbumin hydrolysate and 10% sucrose (Himedia Laboratories Pvt. Ltd., India) and solution of 1% polyvinyl pyrrolidone (Sigma Aldrich Co., USA) were used as two different types of cryoprotectant. Then processing within freeze dryer machine was started (Adams, 2007). After completing the lyophilization or freeze drying vacuum test of the glass vials was done with vacuum torch in dark room and a bluish light was seen within the vaccine vials in positive cases. After lyophilization of working seed virus it was reconstituted in 0.5 ml of 0.85% Normal Saline Solution (NSS) for evaluation of HA titer and EID₅₀/ml. The standard vaccine dose of live attenuated lentogenic strain Newcastle disease vaccine was 10⁶.⁵ EID₅₀/ml (OIE, 2012).

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The doses of vaccine per vial (Reed and Muench, 1938) and comparison was made between two cryoprotectants.

Lentogenic seed virus of PPMV-1 was properly attenuated after 26 serial passages with 2% HA titer of harvested allantoic fluid. Haemagglutination titer and value of EID$_{50}$/ml of working seed virus was $2^5$ and $10^{5.42}$/ml respectively. Macroscopically the structure of vaccine after lyophilization for different types of cryoprotectant was a dry, porous and whitish button. Haemagglutination titer and EID$_{50}$/ml of working seed virus after lyophilization or freeze drying was $2^5$ and $10^{5.33}$/ml respectively for lactalbumin hydrolysate-sucrose (LAS) and $2^6$ and $10^{5.33}$/ml respectively for polyvinyl pyrrolidone (PVP). Doses of vaccine per vial were 338 and 322 for LAS and PVP cryoprotectant used vaccine vials respectively.

There was no published data available in respect to use of different stabilizers in the preparation of PPMV-1 vaccine. So, cryoprotectant was chosen on the basis of freeze dried vaccine preparation and lentogenic strain of paramyxovirus vaccine preparation. Five percent lactalbumin hydrolysate and 10% sucrose used as a good stabilizer in preparation of live attenuated freeze dried Peste des Petits Ruminants (PPR) vaccine (Sarkar et al., 2003) and Rinder Pest (RP) vaccine (OIE, 2015). Sucrose with other additive acted as good stabilizer for LaSota vaccine production in poultry (Pisal et al., 2006). One percent PVP was used as a potential stabilizer in live attenuated Newcastle disease vaccine preparation for poultry (Corbanie et al., 2007). Liquid working seed virus turned to open, porous and dry cake after lyophilization due to primary drying by sublimation of the ice crystal from frozen material and secondary drying or desorption by evaporation of the free water adsorbed into the dried product (Asim et al., 2008). Haemagglutination titer and EID$_{50}$/ml of working seed virus after lyophilization decreased due to the death and autolysis of some virus during lyophilization for cold shock (Adams, 2007). LAS and PVP cryoprotectant in preparation of vaccine the losses of EID$_{50}$/ml after lyophilization was 0.51 and 0.53 respectively. Five percent lactalbumin hydrolysate and 2.5% sucrose in equal volume was used as cryoprotectant for preparation of live attenuated pigeon pox vaccine where EID$_{50}$/ml before and after lyophilization was $10^5$/ml and $10^{4.9}$/ml respectively. So, loss of titre in log 10 (EID$_{50}$/ml) was 0.6 (Mikhael, 2013). But LAS is comparatively better stabilizer than PVP for preparation of this vaccine as LAS protected more live virus during lyophilization and doses of vaccine per vial was more in LAS cryoprotectant used vaccine vials than PVP cryoprotectant used vaccine vials. Sucrose acted as cryoprotectant and polyvinyl pyrrolidone acted as binder. Five percent lactalbumin hydrolysate and 10% sucrose maintained the vaccine virus titre more stable for longer time (Asim et al., 2008).

Cryoprotectant five percent lactalbumin hydrolysate with 10% sucrose (LAS) and 1% polyvinyl pyrrolidone (PVP) both could be used for preparation of live attenuated freeze dried PPMV-1 vaccine. But LAS was more economic compared to PVP in respect of doses of vaccine per vial and cost of cryoprotectant.

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