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FACTORS AFFECTING EFFICACY AND IMMUNOLOGICAL RESPONSE OF PESTE DES PETITS RUMINANTS (PPR) VACCINES IN SMALL RUMINANTS

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ABSTRACT

The efficacy of Peste des petits ruminants virus (PPRV) local vaccines available in Pakistan was evaluated on the basis of the humoral immune response measured by haemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) tests in sheep (n=90) and goats (n=90). The effect of storage temperature on HA activity of vaccine virus was measured by holding the vaccine at -20, 4, 27 and 40°C for 24 hours. The titer of freshly prepared vaccine was 1:16 and remained unchanged for 24 hours in the vaccines stored at - 20 and 4°C. However, drop in titer (1:2 HA) was recorded in the vaccine kept at 40°C for 24 hours. The haemagglutination activity of PPR virus constituted in buffer with pH 6.8 and 7.0 was recorded as highest when assay was performed with chicken and human blood group'O' erythrocytes (1%). The lowest titer was recorded when vaccine was reconstituted in buffer at pH 8.0. After 14th day post vaccination, there was a gradual increase in the antibody titer till 56th day. Geometric mean titer (GMT) of antibodies against locally manufactured PPRV vaccine was higher (207.9) in comparison with Pestivec (73.3), a vaccine imported from Jordan at 63rd day post vaccination in sheep; the corresponding values in goats were 147.0 and 48.5, respectively. All animals of control group were negative for antibodies by both of the diagnostic tests. It was concluded that the efficacy and immunological response of local vaccine (GMT 207) is better than the imported Pestivec (GMT 73.3)

Key words: PPRV, Pestivec, AGID, HI, sheep, goats.

INTRODUCTION

Peste des petits ruminants (PPR) or goat plague is highly contagious viral disease of small ruminants such as sheep and goats (Dhar *et al*., 2002; Asim *et al*., 2009). PPR virus is enveloped, pleomorphic particles containing single stranded RNA, approximately 16 kb long with negative polarity as a genome and are classified under Paramyxovirus genus Morbillivirus (Barrett *et al.,* 2005). The virus may survive at 60°C for 60 minutes, remain stable from pH 4.0 to 10.0 and is killed by most of the disinfectants but have long survival time in chilled and frozen tissues (Asim *et al*., 2009). PPR virus is transmitted between animals such as sheep, goats and other small ruminants (Furley *et al.,* 1987) through inhalation of aerosols and direct contact with ocular/nasal secretions, faeces, contaminated water and feed troughs (Saliki *et al.,* 1993). Specific clinical signs of PPR include sudden pyrexia (40-41oC), purulent ocular/nasal discharge with congested conjunctiva, erosions, respiratory distress, sneezing, ulceration of mucous membranes and gastroenteritis (Dhar *et a.,* 2002; Haffar *et al.,* 1999; Ozkul *et al.,* 2002). This disease is immunosuppressive in nature, having high morbidity and mortality rates of 100 and 90%, respectively (Dhar *et al.,* 2002). The disease is considered as one of the main reasons for low productivity of small ruminants in its endemic regions (Stem, 1993). A number of serological and molecular diagnostic tests are in use for the identification of PPR virus, including competitive enzyme-linked immunosorbent assay (cELISA), immunocapture enzyme-linked immunosorbent assay (Ic-ELISA), agar gel immunodiffusion (AGID), polymerase chain reaction (PCR), isolation on cell culture and haemagglutination inhibition (Anonymous, 2004; Khan *et al*., 2007). For control of PPR, the usual practice was the use of heterologous rinderpest (RP) vaccine (Plowright and Ferris, 1962). After rinderpest eradication from Pakistan, the use of rinderpest vaccine for the control of PPR in small ruminants has been restricted in order to avoid complications in RP sero-surveillance. However, a homologous vaccine is being introduced to immunize the susceptible population (sheep and goats) against this highly contagious disease.

In Pakistan, during the last few years, PPR outbreaks have increased to an alarming level involving newer areas and imported vaccine are currently used against this disease (Ali, 2004). An outbreak of Peste Des Petites Ruminants (PPR) was investigated in goat flocks in district Chitral, N.W.F.P., Pakistan in June 2006. 23 serum samples and 09 occular and nasal swabs from the diseased animals were tested for the presence of Peste des Petits Ruminants antibodies and antigen through competitive and immuno-capture ELISA, respectively. Out of 23 serum samples 09 were found positive for PPR antibodies. Out of 09 occular and nasal swabs 03 were found positive for PPR antigen. (Mirza *et al*., 2008). The present study was designed to investigate the effect of different physical factors on efficacy of vaccine, to

monitor the immunological response of, locally available vaccines of PPR in Pakistan and to compare it with imported vaccine.

MATERIALS AND METHODS

Immunization of animals: The present study was conducted at the experimental farm of small ruminants at livestock Research & Development Station, Surezi, Peshawar, Pakistan. The immune response of PPR vaccine was studied on a total of 180 animals (90 sheep and 90 goats randomly selected and divided into three groups A, B and C (30 each) for sheep and goats. In both species, group A was vaccinated with locally manufactured peste des petits ruminants (PPR) virus vaccine prepared by Veterinary Research Institute, Lahore, Pakistan. Group B was vaccinated with commercially available vaccine (Pestivec, Jordan), whereas animals of group C served as unvaccinated controls. Blood sample of each experimental animal was collected prior to the vaccination and on weekly intervals till 63 day post vaccination from jugular vein (Benjamin, 1986), serum was collected and stored at -20ºC for onward use in serological tests.

Source of antigen: PPR virus confirmed by immuno-capture enzyme linked immunosorbant assay (Ic-ELISA) was donated by the Animal Sciences Institute, National Agriculture Research Center (NARC) Islamabad, Pakistan. Known positive serum was raised in rabbits by repeated injections on alternate day and blood was collected at 21st day of 1st injection (Diallo *et al*., 2007)

Haemagglutination test: Haemagglutination test was performed by standard procedure as described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE manual, 2008). This test was performed using 1% (v/v) chicken erythrocytes and four HA units were calculated.

Haemagglutination inhibition (HI) test: Haemagglutination inhibition (HI) test was performed, as described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Anonymous, 2004). Four HA units were used for the titration of serum samples collected from all the groups of both sheep and goats. Geometric mean titers (GMT) were calculated.

Agar gel immunodiffusion test: Agar gel immunodiffusion (AGID) test was performed as described by Pearson and Coggins (1979). The humoral immune response in collected serum samples was also measured by AGID test, using 1% Noble agar (Obi and Patrick, 1984).

Effect of physical factors on vaccine titer: Thermo stability of freeze dried vaccine (local and imported) was evaluated by placing at temperatures of 40, 27, 4 and -20°C for 24 hours using haemagglutination activity of PPR virus. The buffers with different pH values (pH 6.5, 6.8, 7.0, 7.5 and 8.0) were tested to observe the effect on HA activity of vaccine.

RESULTS

Effect of physical factors on vaccine titer: Highest haemagglutination titer of peste des petits ruminants (PPR) vaccine virus (1:16) was recorded by using phosphate buffered saline at pH 6.8 and 7.0, whereas the lowest titer (1:4) was noted at pH 8.0. The HA titer of freshly prepared PPR vaccine was 1:16 and it remained unchanged in vaccines stored at -20 and 4°C for 24 hours. However, only 1:2 HA titer was observed in vaccine stored at 40°C (Table 1).

Humoral immune response: In sheep, group A antibodies were detected in serum samples collected at 14th day post vaccination (GMT value 2.6). The geometric mean titer of 207.9 was detected at the 56th and 63rd day of vaccination (Table 2). In Group A, up to 14th day of vaccination only 14 animals were AGID positive, as compared to 21st day post vaccination where all the animals were shown positive result to AGID (Table 3). In group B, antibodies were detectable at 14th day of vaccination. Till 14th day of vaccination, only 15 animals were AGID positive. GMT value of 73.3 was detected through HI test at 56th and 63rd day of vaccination (Table 2). However, 19 animals were AGID positive from 21st day to 63rd day post vaccination (Table 3). Group C were negative for antibodies by both the serological tests. In goats, antibodies were detectable at 21st day of vaccination in group A, GMT values raised gradually and it was 147 at 56th day of vaccination by HI test (Table 2). AGID was negative at 14th day; however, all animals were AGID positive from 21st to 63rd day post vaccination (Table 3). Same pattern was observed in group B, furthermore, GMT of 48.5 was detected through HI test at 56th day of vaccination. All animals of group C were negative for antibodies by both of the applied tests. It was concluded that the efficacy and immunological response of local vaccine (GMT 207) is better than the imported Pestivec (GMT 73.3).

Table 1: Effect of physical factors on haemagglutination activity of PPR virus vaccines

pH	Mean HA titer	Temperature (°C)	Mean HAtiter
6.8	1:16	-20	1:16
7.0	1:16	4	1:16
7.5	1:8	27	1:8
8.0	1:4	40	Less than 1:2

Table 2: Geometric mean titers by Haemagglutination inhibition test in vaccinated and non vaccinated sheep and goats

Days post vaccination	GMT titers in sheep			GMT in goats		
	Group A	Group B	Group C	Group A	Group B	Group C
0	00	00	00	00	00	00
7	00	00	00	00	00	00
14	2.6	2.0	00	00	00	00
21	12.1	3.7	00	9.2	3.7	00
28	24.3	7.5	00	9.2	5.3	00
35	45.3	18.4	00	26	7.0	00
42	78.8	21.1	00	48.5	10.6	00
49	104	34.3	00	84.4	24.3	00
56	207.9	73.3	00	147	48.5	00
63	207.9	73.3	00	147	48.5	00

Table 3: Detection of antibodies titer against PPR virus vaccines by agar gel immunodifussion (AGID) assay in vaccinated and non vaccinated sheep and goats

Days post vaccination	Sheep (Sera tested)			Goats		
	Group A	Group B	Group C	Group A	Group B	Group C
0	00	00	00	00	00	00
7	00	00	00	00	00	00
14	14	15	00	00	00	00
21	20	19	00	20	02	00
28	20	19	00	20	20	00
35	20	19	00	20	20	00
42	20	19	00	20	20	00
49	20	19	00	20	20	00
56	20	19	00	20	20	00
63	20	19	00	20	20	00

DISCUSSION

PPRV is heat sensitive and this is a serious drawback in the efficient use of the live attenuated vaccine in the endemic areas. In regions having poor infrastructure it is difficult to maintain a cold chain to ensure the preservation of vaccine potency. Worwall *et al.* (2001) developed thermo tolerant freeze dried PPR virus vaccine stable at 45°C for 14 days, which may also be tried in climatic conditions of Pakistan. Haemagglutination activity of PPR virus was the highest using buffer pH 6.8-7.0 and lowest at pH 8.0, which is in agreement with the value (6.8) used by Wosu (1991) in experiments for optimal result of the HA test for PPR virus. HA titer of tissue suspensions at optimum buffer pH was 16 which falls in the titer range (16 to 64) reported by Manoharan *et al*. (2005). Optimum HA activity of PPR virus was observed in vaccines stored at 4°C and -20ºC. Wosu (1985) reported that the PPRV could produce HA both at 4 and 25°C. Results of Ezeibe *et al.* (2003) were better at storage temperature of 4°C in experiment on haemagglutination ability of PPR virus. Thus, to maintain the HA activity of PPR virus, the vaccine may be stored at a temperature range of -20 to 4°C. Two vaccines were evaluated for humoral immune response in sheep and goats for antibodies titration through HI and AGID tests and there was no difference. At 14th day of vaccination, only 15 animals were AGID positive. However, 19 animals were AGID positive at the end of 63rd day post vaccination, whereas highest titers were noticed 45 days after vaccination in sheep (Khan *et al*., 2009). One animal which was still negative might have not received the vaccine properly. On the basis of GMT values, it was concluded that locally manufactured vaccine was little better in comparison with Pestivec (Jordan). Sheep vaccinated with locally manufactured vaccine showed seroconversion one month post vaccination and titer remained well above protection thresh showed level that is 1:10, as reported by Diallo *et al.* (2007) and Awa *et al.* (2002). However, in goats vaccinated with local vaccine, the protective level of antibodies that is more than 1:10 was recorded after 35th day post vaccination and the group received Pestivec had this titer after one and half month, as described by Khan

et al. (2009). Diallo *et al*. (2007) have shown that the PPR vaccination strain did not diffuse from vaccinated animals to unvaccinated animals. This statement is in accordance with our observations that no antibodies were found in non vaccinated control groups which were housed with the vaccinated animals. The immune response in goats was delayed compared with sheep. Taylor (1984) also reported antibodies titer in goats (9%) slightly lower than in sheep (13%). Based on these results, it can be concluded that efficacy of PPR virus vaccines depend on proper storage temperature, pH of buffer and immune response is better in sheep than in goats.

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