INTRODUCTION

Peste des petits ruminants (PPR) is an acute, highly contagious viral disease of sheep and goats caused by morbillivirus of family parapyxoviridae. It is characterized by high fever, erosive lesions in the mouth, muco-purulent discharges from eyes and mouth, diarrhea, pneumonia and death. Morbidity and mortality rates may vary up to 100 and 90% respectively (Wasee Ullah et al., 2016).

Since first outbreak of PPR in Pakistan (Amjad et al., 1996) many PPR outbreaks have been reported from different parts of the country (Abubakar et al., 2018). During last decade the number of PPR outbreaks has increased at alarming rate affecting sheep and goats of areas where disease has never been reported (Abubakar et al., 2015; Abubakar et al., 2018).

Vaccination is considered an effective control measure against PPR in small ruminants. In Pakistan homologous PPR vaccine (Nigerian strain 75/1) which provides protection for at least three years is available for immunization of small ruminants (Zahur et al., 2014). High morbidity and mortality due to PPR has been reported in young animals. Studies have shown that maternal antibodies against PPR persist for six months but fell below protection level four months after birth (Bodjo et al., 2006). Previous studies (Bodjo et al., 2006; Balamurugan et al., 2012) estimated the age of PPR vaccination in lambs/kids having maternal antibodies is 2-3 months with any of three vaccines. However, vaccine C may also be used at the age of < 1 month to get protection against PPR.

MATERIALS AND METHODS

The study was carried out in lambs of Thalli breed kept at Livestock Experimental Station (LES), Rakh Ghulaman. The ethical approval of the study was obtained from Animal Welfare Committee of the National Agricultural Research Center (NARC), Islamabad. All the
lambs (n=94) were sero-screened for PPR virus (PPRV) antibodies using competitive ELISA (c-ELISA) (ID Screen® PPR Competition, ID.vet, Montpellier, France) before vaccination. The PPR vaccine from three different companies A, B and C were used for the experiment. Each dose of PPR vaccine from company A, B and C contained PPR virus Nigerian strain 75/1 not less than 2.5 TCID<sub>50</sub>. The names of the companies are kept anonymous to avoid any trade conflict or competing financial conflict of interest. The lambs were divided into 10 groups. The lambs in Group A1, A2 and A3 were vaccinated with PPR vaccine from company A and the lambs in Group B1, B2 and B3 were vaccinated with PPR vaccine from company B. However, PPR vaccine from company C was given to four groups of lambs (Group C1, C2, C3 and C4). All the lambs were given recommended dose (1 ml each) of PPR vaccine through subcutaneous route (Table 1). Blood samples were collected from experimental animals at monthly interval by jugular vein puncture and transported to the Animal health Laboratories, NARC, Islamabad under cold conditions. Sera were harvested from clotted blood samples by centrifugation (Sigma, Germany) at 1500×g for 10 minutes. Serum samples were stored at -20°C till further analysis. The serum samples were analysed for the presence of PPRV haemagglutinin (H) antibodies using c-ELISA with a kit (ID Screen® PPR Competition, ID.vet, Montpellier, France) according to manufacturer’s instructions. The results of c-ELISA were expressed in terms of percent inhibition (PI). The PI values were calculated using the formula:

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PI = \frac{OD_{\text{sample}}}{OD_{\text{NC}}}
\]

Where \(OD_{\text{sample}}\) = Optical Density of sample
\(OD_{\text{NC}}\) = Optical Density of Negative control

Samples showing \(PI\leq50\) were considered as positive and responsive to PPR vaccine while the sample showing \(PI>50\) were considered as negative and non-responsive to PPR vaccine.

**RESULTS AND DISCUSSION**

The sero-screening of lambs before vaccination indicated that 13 lambs were positive for PPRV antibodies. Five of these lambs were present in Group A1, 2 in Group A2, 3 in Group B1 and one each in Groups B2, C1 and C2. The lambs positive for maternal antibodies against PPRV were differentiated into two groups on the basis of age of vaccination for analysis purpose. The PI values of lambs with maternal antibodies against PPRV vaccinated at 1-<2 months of age reduced from 25 to 55 one month after vaccination. These PI values remain negative (PI=82) for PPRV till the end of experiment. The PI values of lambs with maternal antibodies against PPRV vaccinated at 2-3 months of age also became negative (PI=52) after two month of vaccination and remain negative (PI=70) till the end of the experiment (Fig. 1). Maternal immunity plays a significant role in protecting young animals against infectious diseases (Pravieux et al., 2007). However, studies carried out in human and animal models showed negative impact of maternal immunity on vaccine specific humoral response (Rowe et al., 2004).

This study also showed that PI values in the lambs born with maternal antibodies against PPR decreased rapidly when vaccinated against PPR at 1-<2 months or 2-3 months of age making lambs susceptible to PPR. Previous studies have indicated that maternal antibodies against PPR persist for 3-4 months of age (Bodjo et al., 2006) therefore, the suitable age of vaccination for young animals having maternal antibodies is 3-4 months after birth.

The PI values of the lambs in Group A1 (PI values ranges from 72-59) and B1 (PI values ranges from 83-85) remained above 50 even after vaccination indicating that vaccination of lambs at the age of 1-<2 months with vaccine from company A or B did not give protective titres against PPR. In contrast to that PI values of lambs in Group C1 (PI values ranges from 90-33) and C2 (PI values ranges from 79-42) which were vaccinated with vaccine from company C at the age of <1 month and 1-<2 months respectively decreased below 50 two to three months post vaccination. These values remained below 50 till the end of the experiment indicating that vaccine C provided protective titres against PPR. The vaccines used in this experiment from company A, B or C were prepared using Nigerian strain but the type of stabilizers was different in all the vaccines. Studies have indicated that immune response of the vaccine also varies with change of stabilizer and number of viruses in the vaccine (Riyesh et al., 2011). The PI values of the lambs without maternal antibodies vaccinated at the age of 2-3 months and >3 months with vaccine A, B or C decreased below 50 one month post-vaccination and remained below 50 till the end of the experiment (Fig. 1). Vaccine safety and efficacy are biggest challenges in providing protective immunity against infections at early age. Our study indicated that none of three PPR vaccines produced any negative effect on health of lambs even if these vaccines were administered at the age of <1 month. The results also indicated that lambs without maternal antibodies weather vaccinated with vaccine A, B or C gave PI values below 50 if vaccinated at the age of 2-3 months or >3 months. Based on these findings the most appropriate age for vaccination against PPR is 2-3 months. The study also indicated that lambs without maternal antibodies against PPR may also be vaccinated at the age of <1 month with vaccine C to get protection against PPR. However, this experiment was carried out in limited number of lambs of a single breed on single experimental farm therefore further studies are required in large number of animals from different breeds to get better understanding of appropriate age of PPR vaccination in small ruminants of Pakistan.

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Fig. 1: Mean percent inhibition (PI) values of Peste des petits ruminants (PPR) c-ELISA in lambs vaccinated with commercially available PPR vaccines from three different companies A, B and C. Each dose of PPR vaccine from company A, B and C contained PPR virus Nigerian strain 75/1 not less than 2.5 TCID50.

Table 1: Lambs (n=94) kept at Livestock Experimental Station (LES), Rakh Ghulaman were vaccinated with Peste des petits ruminants (PPR) vaccine from three different companies A, B and C. Each dose of PPR vaccine from company A, B and C contained PPR virus Nigerian strain 75/1 not less than 2.5 TCID50. Serum samples collected from experimental animals were analysed using c-ELISA for presence of PPR virus antibodies.

| S. No. | Group | PPR vaccine | Age at vaccination | No of lambs |
|--------|-------|-------------|--------------------|-------------|
| 1      | A1    | A           | <2 month           | 9           |
| 2      | A2    | A           | 2-3 months         | 9           |
| 3      | A3    | A           | >3 months          | 9           |
| 4      | B1    | B           | <2 month           | 10          |
| 5      | B2    | B           | 2-3 months         | 10          |
| 6      | B3    | B           | >3 months          | 10          |
| 7      | C1    | C           | <1 month           | 8           |
| 8      | C2    | C           | <2 month           | 11          |
| 9      | C3    | C           | 2-3 months         | 9           |
| 10     | C4    | C           | >3 months          | 9           |

Authors contribution: HI: Planning of study, collection of blood samples and data, analysis of data and writing of manuscript. AU: Collection of blood samples and data. AA: Collection of blood samples, analysis of samples and assistance in write up of manuscript. MJ: Collection of blood samples and analysis of samples. ABZ: Planning of study and analysis of data. MJB: Maintenance of experimental animals and collection of blood samples from experimental animals. MSA: Maintenance of experimental animals and collection of blood samples from experimental animals. MA: Assisted in planning of study and write up of manuscript.

REFERENCES
Abubakar M, Zahur AB, Naeem K, et al., 2018. Field and molecular epidemiology of peste des petits ruminants in Pakistan. Pak J Zool 50:559-66.
Abubakar M, Irfan M, Manzoor S, et al., 2015. Peste des petits ruminants in Pakistan; past, present and future perspectives. J Anim Sci Technol 57:1-8.
Amjad H, Forsyth M, Barrett T, et al., 1996. Peste des petits ruminants in goats in Pakistan. Vet Rec139:118-9.
Balamurugan V, Sen A, Venkatesan G, et al., 2012. Study on passive immunity: time of vaccination in kids born to goats vaccinated against peste des petits ruminants. Virol Sin 27:228-33.
Bojdjo SC, Couacy-Hymann E, Koffi MY, et al., 2006. Assessment of the duration of maternal antibodies specific to the homologous peste des petits ruminant vaccine “Nigeria 75/1” in Djallonké lambs. Biokemistri 18:99-103.
Pravieux J, Poulet H, Charreyre C, et al., 2007. Protection of newborn animals through maternal immunization. J Comp Path 137:32-4.
Riyesh T, Balamurugan V, Sen A, et al., 2011. Evaluation of efficacy of stabilizers on the thermostability of live attenuated thermo-adapted Peste des petits ruminants vaccines. Virol Sin 26:324.
Rowe J, Poolman J, Macaubas C, et al., 2004. Enhancement of vaccine-specific cellular immunity in infants by passively acquired maternal antibody. Vaccine 22:3986-92.
Wasee Ullah R, Zahur AB, Latif A, et al., 2016. Detection of peste des petits ruminants viral RNA in fecal samples of goats after an outbreak in Punjab Province of Pakistan: a longitudinal study. Biomed Res Int pp:1-8.
Zahur AB, Irshad H, Ullah A, et al., 2014. Peste des Petits Ruminants vaccine (Nigerian Strain 75/1) confers protection for at least 3 years in sheep and goats. J Biosci Med 2:2.