RESEARCH ARTICLE

EVALUATION OF EFFICACY OF PPR LIVE ATTENUATED VACCINE

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ABSTRACT

Peste des Petits Ruminants (PPR) is a disease of major economic importance and imposes a significant constraint upon sheep and goats production owing to its high mortality rate. A study was undertaken to find out the difference in age wise antibody response following PPR vaccination in sheep of 3 different age groups at Livestock Research Station, Palamaner, Chittoor, Andhra Pradesh state, India. For this purpose three different age groups viz., Group I sheep of 6-12 months, Group II sheep of 1-2 years and Group III sheep of above 2 years age has chosen for the study. These sheep were given 1ml reconstituted PPR vaccine subcutaneously. Six apparently healthy sheep belonging to the same farm were kept as control group (Group IV). Blood samples were collected before and after vaccination, sera were obtained and analyzed on day zero, 30, 60 and 90 for antibodies against PPR by competitive ELISA (c-ELISA). Results showed that mean Percent inhibition values in all age groups (G-I, G-II and G-III) gradually increased until 90 days (maximum period of observation) of post immunization, indicating initiation of antibody response to vaccine under field condition. No untoward reactions were observed following vaccination and also the immune response was uniform in all the age group of sheep under study. All vaccinated animals developed high titre of antibodies. Further studies are required to determine the duration of immunity of PPR live attenuated vaccine.

INTRODUCTION

Peste des Petits Ruminants (PPR) is a disease of major economic importance and imposes a significant constraint upon sheep and goats production owing to its high mortality rate. The disease is characterized by fever, necrotic-stomatitis, gastroenteritis and pneumonia (Khan *et al*., 2007). Infection rates in enzootic areas are generally high (above 50%) and can be up to 90% during an outbreak (Radostits *et al.,* 2007). The existence of PPR has been recognized in India since 1987 (Shaila *et al*., 1989), After the ban on the use of rinderpest vaccine under global rinderpest eradication programme (GREP), there was an urgent need for a safe and efficacious vaccine to combat the menace of PPR disease in India. Then the PPR live attenuated vaccine was developed. The objective of this study was to evaluate a live attenuated vaccine for providing protection against PPR disease to small ruminants which are the species most susceptible to PPR virus.

MATERIAL AND METHOD

A total of 18 sero-negative sheep were selected to study the efficacy of PPR vaccine in different age groups at Live stock research station, Palamaner. They were grouped into 3 groups of six animals each and a group of six apparently healthy sero negative sheep were kept as controls.

Group - I Sheep in the age group of 6- 12 months were placed in this group.
Group -II This group consisted of 1-2 years old sheep.
Group -III Above 2 years old sheep were placed in this group.
Group -IV Apparently healthy sheep (2 from each group) were kept as controls.

All the animals were dewormed and maintained under same standard managemental conditions.

Sheep in 3 different age groups (Group-I, II and III) were vaccinated with PPR live attenuated vaccine to evaluate the efficacy of vaccine in different age groups. Sheep in Group-IV did not receive vaccine and served as controls.

Sera samples were collected from Group-I, II and III at zero day (pre vaccination), 30, 60 and 90 days of post vaccination and the antibody titres were estimated by using c-ELISA. Similarly sera samples were also collected from Group-IV at zero, 30, 60 and 90 day and antibody titres were estimated. c-ELISA kit was procured from IVRI, Mukteswar and the standard protocol given along with kit was followed. Percent

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Inhibition (PI) values were calculated from the OD values obtained.

Competitive ELISA: Competitive ELISA as previously described by Libeau *et al.*, 1994 and modified by Singh *et al*. (2004) for PPRV was used. This c-ELISA is reported to have the efficacy similar to that of Virus Neutralisation test (VNT) at detecting PPR antibodies.

The reconstituted stock antigen of PPRV was diluted at the ratio of 1:50 in 1X PBS, mixed well and added (50 µl) to all the wells of a 96-well ELISA plate (Nunc,Maxisorp). The plate was covered with a lid and incubated at 37°C for one hour on an orbital-shaker with continuous shaking at moderate speed. At the end of the incubation period, the antigen was discarded from the plate. The plate was washed three times by filling up the wells with the washing buffer and then discarding the buffer. Following reagents were then added very carefully step by step: 40 µl of blocking buffer in all the wells, 20 µl of additional blocking buffer to monoclonal antibody control (Cm) wells. 60 µl of additional blocking buffer to each of the conjugate control (Cc) wells. 20 µl per well of each test serum sample in a set of two wells using a separate tip for each sample (vertical duplicates as per the template provided). 20 µl of strong positive serum control (C++) in each of the four designated wells in the plate. 20 µl of weak positive serum control (C+) in each of the four designated wells in the plate. 20 µl of negative serum control (C-) in each of the two designated wells in the plate. 40 µl of diluted monoclonal antibody in each well of the plate except the conjugate control wells (Cc). Contents of the wells were mixed by gently tapping the sides of the plate. The plate was covered with a lid and incubated at 37° C for one hour on an orbital-shaker with continuous shaking at moderate speed. At the end of the incubation, the plate was taken out of the incubator and repeated the discard and washing procedures as given in previous step. Diluted (1:600) anti-mouse conjugate (50 µl) was added in all the wells of the plate. Contents of the wells were mixed by gently tapping the sides of the plate. The plate was covered with a lid and incubated at 37° C for one hour on an orbital- shaker with continuous shaking at moderate speed. At the end of the incubation, the plate was taken out of the incubator and repeated the discard and washing procedures as given in previous step. Freshly prepared OPD-substrate mixture (50 µl) was added in each well of the plate. Also added 50 µl of the same in each well of the blank 8-well module supplied with the kit.

Incubated the plate and the blank module for about 10 to 20 min at 37°C without shaking or till visible color developed in Cm wells. Once visible color developed in Cm wells, 50 µl of stopping solution (1M sulphuric acid) was added to each well of the plate and the blank module. The plate was tapped by sides and read at 492 nm in ELISA plate reader (Multiskan plus, LabSystem) using EDI software (approved by OIE/IAEA for interpretation of c- ELISA results for the assessment of PPR antibodies and strongly recommended in user's manual of the kit). The blanking plate/module (supplied in the kit) was put in the ELISA plate reader followed by the plate containing the test proper and the instructions as prompted by the computer was followed.

PI =100-(Absorbance of test wells/Absorbance of Mab control wells) X 100

RESULTS AND DISCUSSION

Goats and sheep are considered the mainstay of the livelihood of rural people in India. Viral diseases like PPR cause considerable economic loss through morbidity and mortality and need to be controlled by proper vaccination. The success of RP eradication from India prompted to initiate a national mass vaccination programme for immunizing sheep and goat populations as it is the only option available at present to control PPR. In the past, the rinderpest vaccine has been used. However, this practice is being phased out to avoid confusion during retrospective serologic studies (Singh *et al.*, 2009). To overcome this problem, a homologous Vero cell-based live attenuated PPR vaccine was developed (Sreenivasa *et al.*, 2000) in IVRI, Mukteswar. For effective control of the disease it is necessary to assess the efficacy of vaccine in use, by estimating the post vaccinal antibody titers in experimental groups as well as in field animals.

The PPR live attenuated vaccine used in the present study was prepared at Veterinary Biological Research Institute (VBRI), Hyderabad was used for evaluation of efficacy of vaccine.

In the present study sera samples were collected from all the 3 groups and control group at pre (zero day) and post vaccination (30, 60, 90 days) at Livestock Research Station, Palamaner and were tested for PPR antibody by Competitive Enzyme Linked Immuno sorbent Assay (c-ELISA) (IVRI,Mukteswar).

The PI values gradually increased until 90 days (maximum period of observation) post vaccination in all the animals of 3 Groups (I, II, III) (Fig 1). The present findings corroborate with the studies of Sil and Taimur (2001); Razzaque *et al* (2005) and Asim *et al* (2009) where the authors concluded that following vaccination, the antibody titres gradually increased.

In spite of regular vaccination being done in small ruminant population outbreaks of PPR has been reported. This may be due to intermixing of vaccinated and unvaccinated animals and coverage of population for vaccination may not 100 per cent.

Table 11 Mean PI values of vaccinated and control Groups at different time intervals (pre and post immunization)

Groups	Zero day Mean ± SE	30 day Mean ± SE	60 day Mean ± SE	90 day Mean ± SE
Group-I(6m-12M)	12.27 ± 0.630^a	39.72 ± 4.62	67.41 ± 4.35	75.97 ± 2.96^b
Group- II(1-2y)	18.02 ± 3.050^a	54.99 ± 8.75	63.08 ± 6.18	76.90 ± 3.39^b
Group -III(>2y)	24.70 ± 1.63^a	60.18 ± 3.43	70.55 ± 3.68	77.43 ± 2.55^b
Group-IV (control)	12.27 ± 0.63	15.26 ± 2.14	23.44 ± 2.41	25.51 ± 4.47

Values bearing different superscripts row wise differ significantly.

Table 11a ANOVA

Source of Variation	SS	df	MS	F	F crit
Rows	3708.38	3	1236.127	10.71	3.86
Columns	5132.55	3	1710.85	14.82	3.86
Error	1038.66	9	115.4067	----	----
Total	9879.591	15	----	----	----

Mean PI values in vaccinated Groups and control Groups at different time intervals (pre and post immunization)
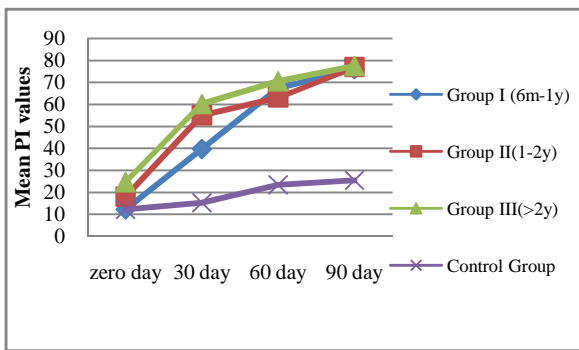
CONCLUSION

In the present study the antibody titres gradually increased until 90 days (maximum period of observation) post vaccination in all the animals of 3 Groups. The literature on duration of immunity to live attenuated vaccine in sheep and goats is scanty. It is proposed that studies are required to determine the total duration of immunity of PPR live attenuated vaccine in sheep and goats.

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