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Evaluation of variability in antibody response induced by vaccination against Peste des petits ruminants (PPR) in Malpura and Avikalin sheep

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ABSTRACT

Amongst several trans-boundary diseases of small ruminants in India, Peste des petits ruminants (PPR) is a devastating disease due to its spread and economic importance. The main strategy for prevention of this disease is the vaccination with attenuated whole virus vaccine. Present study was carried out to identify the sources of variation and also to unravel the genetic variance in the PPR virus (PPR) vaccine elicited immune response in 231 Avikalin and 398 Malpura sheep lambs maintained at organized institute flocks in the semi-arid region of India. Average age at vaccination was 108.5 days. Sera were tested by competitive ELISA (C-ELISA), an attenuated PPR (*Sungri*) was used as the coating antigen. Results revealed significant variability for response to vaccination. Per cent inhibition (PI) values at 0 day of vaccination (0DPrV) was 33.9% and 29.8% in Avikalin and Malpura lambs, respectively. At 28 days post vaccination (28DPV) PI was 62.7% in Avikalin and 58.7% in Malpura lambs. On 28DPV, the protective titre (PI > 50%) was shown by 82.4% Avikalin lambs and 76.2% Malpura lambs. Among environmental determinants, breed, cohort, season and age at vaccination proved to be significant sources of variation ($P < 0.05$). Factors determining the better odds of protection need to be exploited properly to assure protection. The estimate of heritability ($h^2 \pm SE$) at 28DPV was 0.02 ± 0.12 in Malpura sheep, however it was 0.54 ± 0.28 in Avikalin sheep.

1. Introduction

India has a vast population of sheep and ranks third in the world with 65.07 million sheep population (19th Livestock census, 2012; http://dahd.nic.in/dahd/WriteReadData/Livestock.pdf). Sheep husbandry is a backbone of a number of marginal and small farmers who have been rearing the sheep since ages as their traditional business. The Peste des petits ruminant (PPR) being the plague of small ruminants pose heavy threat to the rural economy of India. It is caused by a PPR virus that belongs to the genus *Morbillivirus* within the family Paramyxoviridae. The economic consequences of PPR outbreak can be devastating, given the rural livelihood dependency upon sheep production and utilization. PPR is estimated to cause economic losses of 1800 million Indian Rupees (US$ 39 million) every year in India (Singh, 2011), however, due to increasing inflation, this estimate is likely to have increased by at least 40%. Baseline data on the prevalence of PPR indicate that one third (33%) of small ruminants in India are seropositive (Singh, 2011). Animals excrete the virus prior to showing signs of the disease resulting in to faster pace of spread. PPR adversely affects the export of livestock and their products to countries free from this disease due to its trans-boundary spread and trade sanctions. In PPR endemic regions including India, control measures involve regular vaccination with live attenuated PPR virus vaccine of lineage IV, which has high antigenic stability and induce long term immune response (Venkataramanan et al., 2005).

Currently, three live attenuated PPR vaccines (Sungri/96, Arasur/87 and CBE/97 stains) are available in India for prevention of this disease, of which, Sungri/96, developed by Indian Veterinary Research Institute (IVRI), Mukteswar has undergone extensive field trial (Saravanan et al., 2010; Singh et al., 2010; Singh, 2011). It is possible that the vaccine induced protection across individuals is not homogenous, wherein, vaccine gives a complete protection for a proportion of individuals while rest acquire only incomplete (leaky) protection of varying magnitude (Mehtala et al., 2016). Vaccine response in the population has been reported to be variable for

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several diseases in humans as well as animals (Newman et al., 1996; Raadsma et al., 1999; Poland and Jacobson, 1998; Tan et al., 2001; Sitte et al., 2002; Moreno et al., 2003; O'Neill et al., 2006; Gowane et al., 2013, 2016). Mammalian antibody responses to vaccination are complex polygenic traits modified by environmental and host genetic factors (O'Neill et al., 2006). Halliday (1978) observed variation in the IgGl concentrations in lambs and found that the differences were due to several factors such as number of offspring, breed and production potential. Role of host genetics and other non-genetic factors in variation to vaccine response especially for PPR vaccine has not been studied till today. The importance of host genetics in vaccine response studies is important as genetic variability may influence vaccine response and hence confound vaccine efficacy studies.

Vaccination being the mainstay for the control of PPR for sheep in India, the present study was conceived with an idea to quantify and partition the variability in antibody response to live attenuated PPR virus ('*Sungri* 96' strain) vaccine in Malpura and Avikalin sheep lambs under farm condition. The advantage of using vaccine study as a model is that it also recruits antibody response to PPR vaccine, mimicking the PPR virus and host immune system interaction. Apart from this, in the vaccine study, animals can be immunized as per schedule under well-defined conditions. Objective of this study was to assess the variability in the vaccine response in Malpura and Avikalin lambs for PPR vaccine and to estimate the genetic parameters for variation in antibody titre.

2. Materials and methods

2.1. Animals

The study population was a flock of purebred Malpura sheep and crossbred Avikalin sheep. The flocks were reared at ICAR-Central Sheep & Wool Research Institute, Avikanagar in the semi-arid region of Rajasthan, India at 75°25′E, 26°18′N, at an altitude of 320 m above mean sea level. The data for the experiment involves 231 Avikalin lambs and 398 Malpura lambs born during 2014 and 2015. The selection criteria for the animals under study was as below. Only lambs born with pedigree information, true breed characteristics and within a cohort were taken for study. Animals of both the sexes were included in the study with correction for fixed effect 'sex' in the responding variable. All the animals under the study belong to same age group, *i.e.* 'weaner' with mean age at vaccination 106.3 days (SD = 21.5) for 2014 born and 107.2 days (SD = 17.3) for 2015 born lambs. As part of the regular prophylactic measure, dams of all the lambs were vaccinated in the month of December 2013. All the animals were kept under semi-intensive management system. Concentrate mixture was offered *ad libitum* to suckling lambs from 15 days of age till weaning (90 days). After 3 weeks of age till weaning, lambs were sent for grazing for 3 h each in morning and evening, but not along with their dams. During the post-weaning period in addition to 8–10 h grazing and dry fodder supplementation, 300 g of concentrate mixture was provided in the evening hours. The grazing area consisted of forestland with natural fodder trees like Khejri (*Prosopis cineraria*), Ardu (*Ailanthus spp.*), and Neem (*Azadirecta indica*). Bushes and surface vegetation including the improved pastures of *Cenchrus ciliarisis*. Due to scarce grazing resources from March to June, the sheep were supplemented with hay of *Cenchrus*, Cowpea, and *Dolichos*; pala leaves (*Zizyphus*) and fodder tree loppings.

2.2. Vaccination and sampling

As part of the scheduled vaccination program, the animals were vaccinated (1 ml subcutaneous) with freeze dried live attenuated PPR virus ('*Sungri* 96' strain) vaccine containing a PPR virus titre $\geq 10^{2.5}$ $TCID_{50}$ (Raksha-PPR, Indian Immunologicals, India). Whole blood was collected aseptically by jugular vein puncture from the lambs on '0' day of vaccine delivery (0DPrV), 14 days post vaccination (DPV), 21DPV, 28DPV and 45DPV for sera separation. Sera was collected and stored at −20 °C until testing.

2.3. Competitive ELISA for detection of antibody against PPR vaccine

Sera were tested by competitive Enzyme Linked Immuno-Sorbent Assay (C-ELISA) at ICAR-CSWRI Animal Genetics laboratory using the commercially available PPR C-ELISA kit, developed by IVRI Mukteswar India, as per the protocol (Singh et al., 2004a,b). Samples were tested in duplicate and plates were read in ELISA reader to obtain optical density (OD) values at 492 nm wavelength. Percent Inhibition (PI) values for C-ELISA were calculated as: 100 − {(OD of test sample/OD of monoclonal antibody control) × 100}. Samples having >40% PI by C-ELISA are usually considered as positive by PPR vaccine (Singh et al., 2004b), however in current study we kept PI cut off at 50% for evaluating response stringently.

2.4. Environmental determinant analysis

The percent protected individuals after administration of PPR virus vaccine were categorized as per DPV and the graph was plotted using Excel (MS Office, 2010). The 0DPrV and 28DPV antibody titer obtained by C-ELISA were converted to PI and then used as the phenotype for analyzing the variability in the immune response. The least squares analysis of variance model involved breed, cohort (year of birth), season of birth (Season 1: January-February; Season 2: July-September), sex of the animal, age of the animal at the time of vaccination as effects. Analysis was carried out using SPSS (2005). The statistical model used was as follows:

$$Y_{ijklm} = \mu + B_i + C_j + S_k + X_l + b(A_{ijklm} - = A) + \varepsilon_{ijklm}$$

Where, $\boldsymbol{Y_{ijklm}}$ = Observation for vaccine response (PI) on m^{th} animal born to breed *i*, cohort *j*, season *k*, of sex *l*; $\boldsymbol{\mu}$ = the intercept; $\boldsymbol{B_i}$ = breed effect; $\boldsymbol{C_j}$ = cohort effect; $\boldsymbol{S_k}$ = season effect; $\boldsymbol{X_l}$ = the effect of sex of lamb; $\boldsymbol{b(A_{ijklm} - =A)}$ = the covariable effect of age at vaccination; and $\boldsymbol{\varepsilon_{ijklm}}$ = residual error term.

The linear regression of age at vaccination on 0DPrV and 28DPV PI values was studied using the linear regression equation (SPSS, 2005). Here age at vaccination was used as independent variable and PI for 0DPrV and 28DPV was responding variable. The equation was plotted to see, how age affects the titre.

2.5. Estimation of heritability for PPR vaccine response

Responding variables used for genetic analysis were the antibody titre generated as a result of PPR vaccine elicited immune response at 28DPV that were converted to PI values. Genetic analysis was done using 398 records at 28DPV for Malpura lambs born to 265 dams sired by 67 sires and 187 records for 28DPV for Avikalin lambs born to 150 dams sired by 21 sires. Genetic parameter estimates for the traits under study such as additive direct variance, maternal permanent environmental effect and residual effect and their ratios to the total phenotypic variance resulting in to heritability (h^2) and proportionate permanent environmental effect (c^2) were calculated by Animal model using a single-trait linear model. Two linear models used were as follows:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_a a + \varepsilon \quad (1)$$

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_a a + \mathbf{Z}_{pe} pe + \varepsilon \quad (2)$$