ECRONICON OPEN ACCESS

EC VETERINARY SCIENCE
Research Article

Detection of Antibodies against *Peste Des Petits* Ruminants Virus in Nepal

Khanal DR[1]*, Shrestha S[2], Khanal S[1], Paudyal N[1] and Naletoski I[3]

[1]*National Animal Health Research Centre (NAHRC), NARC, Khumaltar, Lalitpur, Nepal*

[2]*Himalayan College of Agricultural Sciences and Technology (HICAST), Kathmandu*

[3]*International Atomic Energy Agency, Vienna, Austria*

***Corresponding Author:** Khanal DR, National Animal Health Research Centre (NAHRC), NARC, Khumaltar, Lalitpur, Nepal.

Received: March 23, 2021; **Published:** June 24, 2021

Abstract

Peste des Petits Ruminants (PPR) has been considered as one of the most devastating transboundary diseases of wild and domestic small ruminants. A study was undertaken during 2014 - 2015 to detect the presence of antibodies against PPR virus (PPRV) among sheep and goats from 16 clusters representing three altitudinal gradients ranging from 150 to 2300 metres above sea level (masl) from southern low lands (terai: < 500 masl) to northern middle hills (500-1800 masl) and mountains (1800 - 2300 masl). A total of 552 sera samples from 524 goats, 16 chyangra (high mountain goat) and 12 sheep collected between February 2014 and December 2015 were screened using competitive enzyme-linked immunosorbent assay (c-ELISA) kits. Of the 552 sera tested, overall seropositive samples were 190/552 (34.42%) with the highest prevalence in goats of low land terai (62/138) (44.93%) followed by that of middle hills 74/229 (32.31%) and mountains 54/185 (29.19%). Species-wise prevalence was 29.38% (154/524) in goats and 37.5% (6/16) in chyangra. None of the sera from sheep was positive for PPRV antibodies. Though the vaccination history is unknown, this study has demonstrated the presence of PPRV antibodies in serum samples of goats across all altitudes. The gradient prevalence of PPRV antibodies in goats of terai, middle hills and mountains is indicative of proportional vaccination coverage in those areas. However, detection of antibodies against PPRV in chyangra dwelling in high mountains of Nepal in the absence of preventative PPR vaccination programme is suggestive of ongoing circulation of PPR virus.

Keywords: *PPR; Chyangra; c-ELISA; Vaccination*

Introduction

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of the wild and domestic small ruminants with mortality reaching up to 90% within a few days of infection. After its first reported outbreak in 1942 from Côte d'Ivoire, PPR has been spreading at an alarming rate in the last two decades, reaching regions previously not infected and putting hundreds of millions of small ruminants at risk. The annual loss incurred due to PPR is estimated to be in between 1.4 to 2.1 billion US$. These losses have been attributed to poverty, malnutrition, social and economic instability, and conflict [1].

According to the Food and Agriculture Organization (FAO), the loss incurred annually due to PPR is estimated to be more than $2 billion, mainly affecting the small herders and poor rural households that depend on the animals for milk, meat, wool and leather both for their use and trade [2]. Approximately 80% of the world's sheep and goat population is threatened by PPR [3]. Because of its clinical

incidence and the restrictions on animal movements, PPR is a disease of major economic importance [4]. After successful eradication of rinderpest in 2011, the World Organization for Animal Health (OIE) and the FAO have aimed for the global eradication of rinderpest's less famous cousin, PPR [5] by 2030 [6]. The overall undiscounted cost of eradication was estimated at 3.1 billion and the benefit-cost ratio for the most likely scenario was estimated at 33.8 [7].

Scientific communities across the world are convinced that PPR is eradicable [3]. As part of the solidarity campaign for the global eradication of PPR, this exploratory study was aimed at detecting the seroprevalence of PPRV antibody in the small ruminants with no clear history of vaccination, especially in goat population across different ecological zones of Nepal.

Materials and Methods

Site selection

A total of 16 sites from three distinct geographic regions (terai, middle hills and mountains) from south to north altitudinal gradients across the east-west length of the country, as shown in figure 1, were selected. Terai region with elevation less than 500 metres above sea level (masl) comprised of five sites (Chitwan, Bara, Banke, Bardia and Kanchanpur); middle hills with altitudes ranging from 500 - 1800 metres comprised of six sites (Surkhet, Lamjung, Tanahun, Lalitpur, Sindhuli and Dhankuta) and five sites (Jumla, Mustang, Rasuwa, Sindhupalchowk and Dhankuta) were selected from the mountain region with altitude ranging from 1800 - 2300 metres. Two government farms, Regional Agricultural Research Station (RARS) Banke and Goat Research Station (GRS), Bandipur were also included in the study.

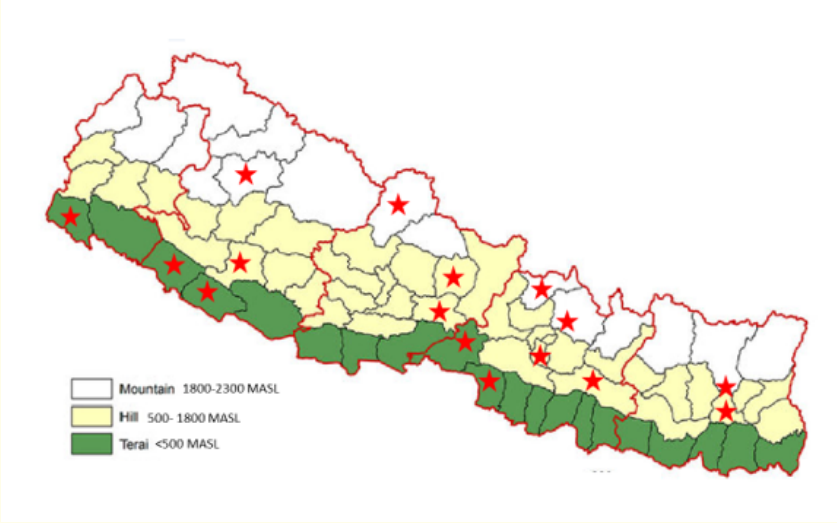

Figure 1: *Geographic regions of Nepal showing sample collection sites denoted by* ★ .

Sample collection

Field stockpersons, then Animal Health Research Division (AHRD) staffs and intern were mobilized for serum collection from small ruminants. The intern and AHRD staffs collected sera from the different government and private farms (n = 184). In addition, sera from the serum bank of AHRD (n = 368) were also utilized for this exploratory study to analyze the seroprevalence of PPRV antibodies. A total

of 552 sera collected from 524 goats, 12 sheep and 16 chyangra (Table 1) were stored in the deep freezer (-80°C) until the time of testing at AHRD laboratory.

Assay procedure

A commercial ELISA kit (IDVet, France) designed to detect antibodies directed against the nucleoprotein of the PPR virus in serum or plasma was used. This uses technology developed by FAO Reference Laboratory (CIRAD-EMVT, Montpellier, France). The wells are coated with purified recombinant PPR nucleoprotein (NP). The samples to be tested and the controls are added to the micro-wells. Anti-NP antibodies, if present form an antibody-antigen complex which masks the NP epitopes. An anti-NP-peroxidase (HRP) conjugate is added to the micro-wells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex. After washing the substrate solution (TMB) is added to eliminate the excess conjugate. The resulting colouration depends on the number of specific antibodies present in the sample to be tested. In the absence of antibodies, a blue solution appears which becomes yellow after the addition of the stop solution. In the presence of antibodies, no colouration appears. The microplate is read at 450 nm. Based on the earlier report [8] the relative sensitivity (94.5%) and specificity (99.4%) was used for the data analysis.

Results

Out of 552 samples tested, PPRV antibodies were detected in 190 samples, the details are elaborated in the table 1.

Geographic region	Site	Number	Positive	Percent Positive
Terai (< 500 masl)	Bara	27	15	55.56
	Bardia	28	14	50.00
	Banke (RARS)*	19	19	100.00
	Kanchanpur	37	3	8.11
	Chitwan*	27	11	40.74
	Sub total	138	62	44.93
Middle Hills (> 500 - 1800 masl)	Surkhet	51	9	17.65
	Lamjung	23	3	13.04
	Tanahun (GRS)*	39	32	82.05
	Lalitpur*	6 Sheep	0	0.00
	Sindhuli*	92	27	29.35
	Dhankuta	18	3	16.67
	Sub total	229	74	32.31
Mountains (> 1800 - 2300 masl)	Jumla	21 goats + 6 Sheep	2 goats	7.41
	Mustang	16 (Chyangra)	6	37.50
	Rasuwa	47	3	6.38
	Sindhupalchowk*	7	2	28.57
	Dhankuta (Murtidhunga)	88	41	46.59
	Sub total	185	54	29.19
	Grand Total	552	190	34.42

Table 1: *Geographic region, sites and numbers of sera collected and tested for PPRV antibodies from small ruminants.*
**: Sera collected by AHRD staffs and the intern (n = 184). Other sera (n = 368) were from AHRD serum bank.*

The apparent prevalence was calculated to be 34.4% (95% CI: 30.4 - 38.3) while the true prevalence was 35.9% (95% CI: 31.6 - 40.0). The highest seropositive samples were from goats of the low land terai, where the apparent prevalence was calculated to be 44.9% (95% CI: 36.6 - 53.2) while the true prevalence was 47.2% (95% CI: 38.3 - 56.1). In the middle hills, the apparent prevalence was calculated to 32.3% (95% CI: 26.2 - 38.3) while the true prevalence was 33.6% (95% CI: 27.1 - 40.1). The lowest prevalence was found in mountains, where the apparent prevalence was calculated to be 29.1% (95% CI: 22.6 - 35.7) while the true prevalence was 30.3% (95% CI: 23.2 - 37.3).

None of the 12 sera from sheep were positive for PPRV antibodies. In case of chyangra (high mountain goat), the apparent prevalence was calculated to be 37.5% (95% CI: 13.7 - 61.2) while the true prevalence was 39.2% (95% CI: 13.7 - 64.7).

Discussion

Most of the sera obtained from 14 clusters other than two government farms (Banke and Tanahun) lacked information on vaccination status. In Mustang (mountain region) wherein vaccination is not routinely practiced compared to terai and middle hills, the presence of PPRV antibodies in chyangra (high mountain goat) is suggestive of on-going circulation of PPR virus. Those chyangra rearing nomadic farmers were not aware of any history of PPR vaccination. Although this work was not designed to deliver any epidemiological parameters of PPR in Nepal, this exploratory investigation has indicated that the virus is circulating in the country. Although the number of sera tested from sheep were low in number compared to that of goats, none of them were positive for PPRV antibodies and indicating that lower susceptibility of sheep compared to goats. Due to proximity and open borders of Nepal to India and China, PPR spread to and from both sides is possible. In the government farms (RARS, Banke and GRS, Bandipur) where regular vaccination is routinely practiced, the seroconversion was around 88%. This fact infers that despite absolute vaccination, there could be less seroconversion. As the level of observed seroconversion is higher than the recommended herd immunity threshold (80%) this could confer protective herd immunity [1,9] to reduce the transmission of infection in the population sufficient to eliminate virus.

Although the current work was limited only to ELISA testing, further work on molecular characterization of PPRV is yet to be done for better understanding of the lineage prevalent in Nepal. In other parts of the world, the molecular epidemiology, based on the sequence comparison of a small region of either the N or the F gene, has revealed the existence of four distinct lineages (I-IV) of the virus [10]. Until June 2018, PPR had never been detected in Europe but on 24th June 2018 the Bulgarian authorities reported cases of PPR in sheep in the village bordering with the Thrace region of Turkey. It was the first occurrence of PPR in Bulgaria and in the European Union (EU). The source of PPR infection in Bulgaria was not clear, it could have been the illegal movement of animals, contaminated materials or humans [10]. Due to open, unregulated cross border trade of live animals between India and Nepal and to a lesser extent to Tibetan region of China sharing common grazing lands, there are high chances of contracting and spread of disease in both the southern terai and northern high mountain regions of Nepal.

In agreement with our finding, one Ethiopian study has documented the presence of PPRV antibody in the animals not vaccinated against PPR or rinderpest [11]. The seroprevalence of PPRV antibody detected were: camels 10%, cattle 16%, goats 22% and sheep 23%, thus confirming natural transmission of PPR virus under field conditions. However, a recent study at Ivory Coast found that cattle are dead-end hosts for PPRV and do not play an epidemiological role in the maintenance and spread of PPRV [12].

As the international community has been developing strategies to eradicate PPR, significant efforts need be invested to understand the epidemiology of the disease at national and international levels, especially in developing countries. Therefore, this exploratory investigation indicates the need for the development of a much more comprehensive epidemiological study of the disease in the country aimed to: i) understand the distribution pattern of the disease, ii) patterns of dissemination and iii) possibilities to break the chain of the spread using scientifically justified and recognized control measures. To achieve these goals, Nepal will need substantial organizational and technical

capacities within its national veterinary disease control system, as well as significant contribution of the international organizations, such as FAO, IAEA and OIE. After global rinderpest eradication in 2011, PPR has been identified as the next candidate disease for eradication by adopting similar strategies and approaches [7]. Rapid detection, prompt culling of infected herds, movement restriction and disinfection of the farm premises are important steps in limiting the spread of PPR in the newer areas as part of the effective containment measures.

Current modality of PPR control is by isolation and disinfection of the contaminated environment, and administration of a live-attenuated vaccine, which provides a strong immunity. However, maintenance of cold chain for vaccine efficacy has still been a major constraint in tropical and subtropical countries. Mass vaccination of sheep and goats in endemic countries using the thermostable vaccine might be a pragmatic approach to control PPR in the first phase of disease eradication. Like in FMD vaccine, the development of a marker vaccine for DIVA may help in serosurveillance to differentiate the infected animals from vaccinated animals to control PPR disease [13].

Conclusion

Seroprevalence study has indicated that PPRV is circulating in Nepal as evident from the detection of PPRV antibodies in chyangra (high mountain goat) where PPR vaccination is not routinely practiced. Due to the endemicity of PPRV, it is important to perform molecular characterization and sequencing of the virus for better understanding of the epidemiology, lineage for the development of effective vaccine strains that can confer long-lasting immunity as part of the global eradication campaign.

Acknowledgement

International Atomic Energy Agency (IAEA) deserves special thanks for providing the diagnostic kits and ELISA reader through TCP NEP5002.

Bibliography

1. Njeumi F., *et al*. "Global strategy for the control and eradication of PPR". In FAO and OIE. FAO (2015).
2. FAO and OIE. "Peste des petits ruminants Global Eradication Programme". FAO and OIE.
3. Baron MD., *et al*. "Peste des Petits Ruminants Virus". In Advances in Virus Research (2016).
4. Lefèvre PC and Diallo A. "Peste des petits ruminants". In Revue scientifique et technique (International Office of Epizootics) (1990).
5. Baron MD., *et al*. "Peste des petits ruminants: A suitable candidate for eradication?" *Veterinary Record* (2011).
6. Parida S., *et al*. "Pester des petits ruminants". *Veterinary Microbiology* 181.1-2 (2015): 90-106.
7. Mariner JC., *et al*. "The Opportunity To Eradicate Peste des Petits Ruminants". *The Journal of Immunology* 196.9 (2016): 3499-3506.
8. Libeau G., *et al*. "Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein". *Research in Veterinary Science* 58.1 (1995): 50-55.
9. Yirga A., *et al*. "Post-vaccination herd immunity against peste des petits ruminants and inter-vaccination population turnover in small ruminant flocks in northwest Ethiopia". *Preventive Veterinary Medicine* 174 (2019): 104850.
10. Niedbalski W. "Occurrence of peste des petits ruminants and its increasing threat to Europe". *In Medycyna Weterynaryjna* 75.8 (2019): 459-464.

11. Abraham G., *et al*. "Antibody seroprevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia". *Preventive Veterinary Medicine* (2005).

12. Couacy-Hymann E., *et al*. "Experimental infection of cattle with wild type peste-des-petits-ruminants virus – Their role in its maintenance and spread". *Research in Veterinary Science* 124 (2019): 118-122.

13. Sen A., *et al*. "Vaccines against peste des petits ruminants virus". *In Expert Review of Vaccines* (2010).

Volume 6 Issue 7 July 2021