



E-ISSN: 2320-7078  
P-ISSN: 2349-6800  
JEZS 2017; 5(2): 1366-1368  
© 2017 JEZS  
Received: 17-01-2017  
Accepted: 18-02-2017

**SS Begum**

Department of Veterinary  
Epidemiology and Preventive  
Medicine, College of Veterinary  
Science, AAU, Khanapara,  
Guwahati-781022, Assam, India

**G Mahato**

Department of Veterinary  
Epidemiology and Preventive  
Medicine, College of Veterinary  
Science, AAU, Khanapara,  
Guwahati-781022, Assam, India

**D Muthuchelvan**

PPR Laboratory, Indian  
Veterinary Research Institute,  
Mukteswar, Nainital Dist.,  
Uttarakhand-263138, India

**D Chaudhary**

Indian Veterinary Research  
Institute, Mukteswar, Nainital  
Dist., Uttarakhand-263138,  
India

**M Hussain**

ICAR-National Research Centre  
on Yak, West Kameng District,  
Dirang-790101, Arunachal  
Pradesh, India

**N Nashiruddullah**

Division of Veterinary  
Pathology, Faculty of  
Veterinary Sciences & Animal  
Husbandry, Sher-e-Kashmir  
University of Agricultural  
Sciences & Technology-Jammu,  
RS Pura-181102, Jammu &  
Kashmir, India

**KK Rajak**

Indian Veterinary Research  
Institute, Mukteswar, Nainital  
Dist., Uttarakhand-263138,  
India

**Correspondence****SS Begum**

Department of Veterinary  
Epidemiology and Preventive  
Medicine, College of Veterinary  
Science, AAU, Khanapara,  
Guwahati-781022, Assam, India

## Molecular identification of *Peste-des-petits-ruminant virus* from goats of Assam state of North-East India

**SS Begum, G Mahato, D Muthuchelvan, D Chaudhary, M Hussain, N Nashiruddullah and KK Rajak**

**Abstract**

Peste des petits ruminants (PPR), which literally means “Plague of small ruminants” is an economically important disease of sheep and goats that is highly contagious with morbidity and mortality rates being as high as 100 per cent and 90 per cent, respectively. The present study was undertaken as a part of PhD research work to identify *Peste-des-petits-ruminant virus* from suspected PPR outbreak sample in goats of Assam state of North-East India. Five clinical samples were collected from a PPR suspected outbreak from goats of Darrang district of Assam, and the samples were tested by PPR Sandwich ELISA kit developed by Division of Virology, IVRI, Mukteswar, Uttarakhand, India. Out of the five samples, one sample was positive for PPRV, and the viral RNA was isolated from the sample using Trizol method. Later the RNA from the sample was subjected to direct cDNA by RT-PCR, and this cDNA was used for expression of N (368 bp) and F (372 bp) gene of PPR virus using custom designed primers. Expression of the N and F gene confirmed the presence of PPRV in the clinical materials and was rented for quick action to be taken for control of the disease.

**Keywords:** Goat, PPR, RT-PCR, sandwich ELISA

**1. Introduction**

*Peste-des-petits-ruminant* (PPR) is one of the devastating diseases of goats and sheep. The disease is often associated with high morbidity and mortality, and is more severe in goats than in sheep [1, 2]. The clinical symptoms include high fever coupled with oculonasal discharges, pneumonia, stomatitis, and gastroenteritis leading to severe diarrhoea followed by death or recovery. Considering the economic importance of the disease, FAO and OIE has initiated the global campaign to eradicate it by 2030 (<http://www.fao.org/ppr/en/>). In India, the disease was first reported in 1989 in Tamil Nadu state [3] and is now being reported from other parts of the country too [4, 5]. Currently, the disease is endemic and the outbreaks occur throughout the country and in all seasons. To combat the disease, an indigenously developed live attenuated vaccine and monoclonal antibody based diagnostics are available; and with this, the Government of India has launched a national control program in 2010 with an aim to vaccinate all the susceptible sheep and goats [4]. Though vaccination is being practised for this disease, different authors have reported a variety of response of the animals to the vaccine with regards to age, breed, season etc. [5, 6].

The etiological agent, *Peste-des-petits-ruminants virus* (PPRV) belongs to the genus *Morbillivirus*, subfamily *Paramyxovirinae*, family *Paramyxoviridae* and order *Mononegavirales* [7], and is similar to other important pathogens of the *Morbillivirus* genus like rinderpest, measles and canine distemper. The virus occurs as a single serotype and based on molecular epidemiological studies, it is classified into four distinct lineages using partial N, F gene or H gene (I-IV) [8, 9]. Earlier, the lineages I-III had been reported in Africa, and lineage IV was mainly restricted to the Middle East and Asia. At present, the lineage IV is fast spreading in many countries of Africa [2], and being a single serotype, the vaccine virus of one lineage should protect others also.

Assam state is the gateway of North-Eastern India. It is located in the Eastern Himalayas and shares her international border with Bhutan and Bangladesh. The state harbours 5.18 and 61.69 million sheep and goats respectively (<http://dahd.nic.in/dahd/WriteReadData/Livestock.pdf>), and about 63% of the state's working force is engaged in agriculture and allied activities.

Backyard farming is one of the main socio-economic activities of the residents of this state, and since goat meat is preferred in the state, goat rearing is practiced widely<sup>[10]</sup>. PPR is considered as an emerging/re-emerging disease in Assam, and to design effective control measures of any disease, it is imperative to understand the epidemiological pattern in the region where it prevails. Although, the epidemiology of PPR has been fairly studied, reports on the prevalence and incidence are very limited for the North-Eastern India<sup>[9, 10]</sup>. Here we confirmed the presence of PPR disease in the Assam state of North-Eastern India using ELISA and RT-PCR.



(A) (B)

**Fig. 1:** PPR suspected goats (A) showing ocular secretion and (B) ulceration and bleeding of the gum at Punia village of Darrang District in June 2013.

## 2.2 Sandwich ELISA

The sandwich ELISA was performed in MAb based PPR sandwich ELISA (s-ELISA) kits that was developed by ICAR-Indian Veterinary Research Institute, Regional Station, Mukteswar, Nainital District, Uttarakhand, India. In brief, all the steps were carried out in 100  $\mu$ L reaction volume and incubated at 37  $^{\circ}$ C. After each incubation step, the plates were washed three times with 100  $\mu$ L/well PBS containing 0.05% Tween-20 (PBST). The microtiter plate was coated with capture antibody @ 1:4000 dilutions in phosphate buffered saline (0.01 M PBS, pH 7.4  $\pm$  0.2) and then the wells were blocked with 50  $\mu$ L blocking buffer (0.01 M PBS containing 0.1% Tween20 and 0.5% goat serum negative for PPRV antibodies). The PPR suspected samples were added @ 50  $\mu$ L in duplicate wells. The reference positive antigen (Vero cell derived purified PPRV antigen) and the PPRV reference negative antigen (healthy uninfected Vero cell antigen) were added in quadruplicate wells. The detection antibody (4G6 MAb raised against N-protein of PPRV) diluted 1:20 in blocking buffer was added (100  $\mu$ L/well) to all the wells. The anti-goat horseradish peroxidase conjugate (Sigma-Aldrich, USA) was used @ 1:5000 in blocking buffer and OPD substrate solution (OPD 1 mg/ mL containing 4  $\mu$ L 3% H<sub>2</sub>O<sub>2</sub>) was added and incubated at 37  $^{\circ}$ C for 15 min followed by addition of 50  $\mu$ L of 1 M H<sub>2</sub>SO<sub>4</sub> to stop the reaction. The optical density was measured at 492 nm in an ELISA reader (Tecan, Switzerland).

## 2.3 Virus isolation

For virus isolation, confluent B95a cells were infected with 0.2 ml of filtered (0.45  $\mu$ ) swab fluids and blindly passaged for four times. The typical cytopathic effect (CPE) of PPRV was noted in the infected cells. The cultured cells were freeze-thawed three times to harvest the virus.

## 2. Materials and methods

### 2.1 Ethical approval

In June 2013, a PPR like disease was reported in goats from the Punia village of Darrang District to North East Regional Disease Diagnostic Laboratory (NERDDL), Khanapara, Assam. A team of experts visited the area and interviewed the farmers about clinical signs and symptoms, morbidity and mortality. A total 39 out of 65 goats succumbed to the disease, and from five animals (Fig. 1), clinical materials were collected which were initially screened with s-ELISA<sup>[11]</sup> and shipped under cold chain to Indian Veterinary Research Institute (IVRI), Mukteswar Campus for further characterization.

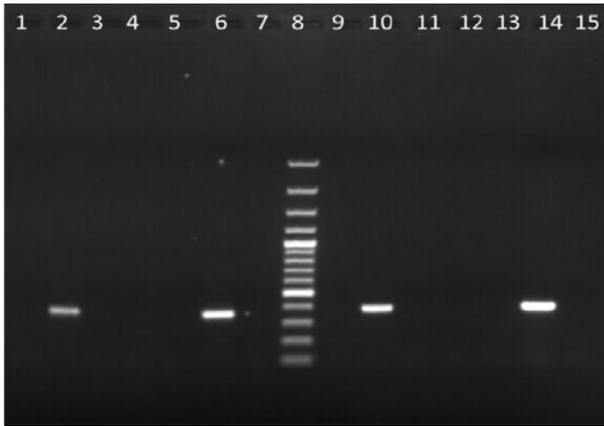
### 2.4 RT-PCR

For molecular characterization, the total RNA was extracted from clinical materials or culture supernatants using RNeasy kit (Qiagen, USA), and the RT-PCR was performed using virus-specific primers sets targeting N or F genes as used in earlier studies<sup>[8, 9, 12]</sup>. In brief, the total RNA was extracted from clinical material using RNA easy kit (RNeasy® Minikit QiagenInc, Valencia, CA, USA), and RT-PCR was performed using Qiagen Revert Aid First Strand cDNA Synthesis Kit for first strand synthesis and subsequently PCR was performed using gene specific primer sets<sup>[13]</sup>. The amplicons were run in 1.5% agarose gel post amplification.

## 3. Results and discussion

Earlier, the lineage IV viruses were reported only from Asia. But now, it is being reported in many parts of Africa, and thus emphasizing the need for continuous surveillance<sup>[2]</sup>. Intense surveillance and molecular epidemiology has to be performed for rapid control of this disease. The presence of PPR in the state of Assam has been reported earlier<sup>[10]</sup>, and here, we have investigated a suspected outbreak of PPR in goats at Punia village of Darrang district of Assam. The morbidity, mortality and case fatality rate of the outbreak was 100%, 60% and 60% respectively, and from the ailing animals, three rectal and two nasal swabs were collected and tested in s-ELISA. All the five samples were found to be positive by s-ELISA. The filter sterilized fluids from the nasal swabs were used for virus isolation in B95a cells and the CPE typical of PPRV were noted after five blind passages. The culture supernatant as well as the original clinical materials was subjected to diagnostic RT-PCR with N gene and F gene specific primers<sup>[9]</sup>. The PCR gave specific amplicon size products (N gene, 368 bp and F gene, 372 bp), and specific RT-PCR further confirmed the identity of the virus as PPRV

(Fig.2). Molecular characterization of peste-des-petits ruminants virus (PPRV) has also been reported from an isolate of an outbreak in the Indo-Bangladesh border of Tripura state of North-East India [14]. The causative agent of this outbreak was confirmed by sandwich ELISA, virus isolation and N gene based RT-PCR and sequencing as being the involvement of lineage IV PPR virus. Although, in the present study the causative agent could be confirmed as PPR, yet the origin of the virus could not be traced. Introduction of new animals purchased from the endemic region without proper quarantine could have caused the outbreak for which restriction on movement of animals and farmer awareness campaign is needed. Also further characterization of the PCR amplicons by gene sequencing and phylogenetic analysis is required for identification of the lineage type and to know the molecular details of the virus circulating in the region.



**Fig 2:** Agarose gel electrophoresis of RT-PCR products, Lane 1-5: PPR suspected samples; Lane 6: Positive control (N gene specific); Lane 7: No template control; Lane 8: 100bp plus DNA ladder; Lane 9-13: PPR suspected samples; Lane 14: Positive control (F gene specific); Lane 15: No template control.

#### 4. Conclusion

The clinical signs, virus isolation, detection of PPRV by s-ELISA and RT-PCR assays had confirmed that the outbreak was caused by PPRV. Due to rapid spread and high productivity loss in small ruminants, PPR outbreaks need to be controlled rapidly. At the time of implementation of the control measure programs, farmers and other livestock handlers are needed to be educated on prevention and control measures of PPR such as vaccination, restriction on animal movements etc. so that the small ruminant's productivity in the region can boost.

#### 5. Acknowledgement

The authors are thankful to the Director IVRI, for providing permission to carry out part of the research at IVRI-Mukteswar, Uttarakhand, India. The authors are also thankful to the Dean and Director Research (Vety.) AAU, Khanapara Campus, Guwahati-22, Assam, and the Director and Dr. Paresh Sharma (Virologist), North East Regional Disease Diagnostic Laboratory, Khanapara, Guwahati-781022, Assam for necessary help and the facilities provided during the study.

#### 6. Competing interests

The authors declare that they have no competing interests.

#### 7. References

1. Abu-Elzein EME, Hassanien MM, Al-Afaleq AI, Abd-Elhadi MA, Housawi FMI. Isolation of peste des petits

ruminants from goats in Saudi Arabia. *Veterinary Record*. 1990; 127(12):309-310.

2. Parida S, Muniraju M, Mahapatra M, Muthuchelvan D, Buczkowski H, Banyard AC. Peste Des Petits Ruminants. *Veterinary Microbiology*. 2015; 181(1-2):90-106. doi:10.1016/j.vetmic.2015.08.009.
3. Shaila MS, Purushothaman V, Bhavasar D, Venugopal K, Venkatesan RA. Peste Des Petits Ruminants of Sheep in India. *Veterinary Record*. 1989; 125(24):602.
4. Muthuchelvan D. Peste-Des-Petits-Ruminants: An Indian Perspective. *Advances in Animal and Veterinary Sciences*. 2015; 3(8):422-429. doi:10.14737/journal.aavs/2015/3.8.422.429.
5. Begum SS, Mahato G, Sharma P, Sharma K, Hussain M, Das BC *et al.* Seroprevalence of Peste Des Petits Ruminants in Goats in Assam, India. *Asian Journal of Animal and Veterinary Advances*. 2016; 11(3):210-212. doi:10.3923/ajava.2016.210.212.
6. Gowane GR, Akram N, Prince LLL, Prakash V, Paswan C, Sharma RC *et al.* Evaluation of variability in antibody response induced by vaccination against Peste des petits ruminants (PPR) in Malpura and Avikalinsheep. *Small Ruminant Research*. 2016; 144:104-108.
7. Afonso CL, Amarasinghe GK, Banyai K, Bao Y, Basler CF, Bavari S *et al.* Taxonomy of the Order Mononegavirales: Update. *Archives of Virology*. 2016; 161(8):2351-2360. doi:10.1007/s00705-016-2880-1.
8. Dhar P, Sreenivasa BP, Barrett T, Corteyn M, Singh RP, Bandyopadhyay SK. Recent Epidemiology of Peste Des Petits Ruminants Virus (PPRV). *Veterinary Microbiology*. 2002; 88(2):153-159.
9. Muthuchelvan D, De A, Debnath B, Choudhary D, Venkatesan G, Rajak KK *et al.* Molecular Characterization of Peste-Des-Petits Ruminants Virus (PPRV) Isolated from an Outbreak in the Indo-Bangladesh Border of Tripura State of North-East India. *Veterinary Microbiology*. 2014; 174(3-4):591-95. doi:10.1016/j.vetmic.2014.10.027.
10. Begum SS, Mahato G, Sharma P, Hussain M, Saleque A. Assessment of immune response to a lyophilized peste-des-petits ruminants virus vaccine in three different breeds of goats. *Veterinary World*. 2016; 9(6):568-571.
11. Singh RP, Sreenivasa BP, Dhar P, Bandyopadhyay SK. A Sandwich-ELISA for the Diagnosis of Peste Des Petits Ruminants (PPR) Infection in Small Ruminants Using Anti-Nucleocapsid Protein Monoclonal Antibody. *Archives of Virology*. 2004; 149(11):2155-2170. doi: 10.1007/s00705-004-0366-z.
12. Balamurugan V, Sen A, Venkatesan G, Yadav V, Bhanuprakash V, Singh RK. Isolation and Identification of Virulent Peste Des Petits Ruminants Viruses from PPR Outbreaks in India. *Tropical Animal Health and Production*. 2010; 42(6):1043-1046. doi:10.1007/s11250-010-9527-0.
13. Balamurugan V, Sen A, Saravanan P, Singh RP, Singh RK, Rasool TJ *et al.* One-Step Multiplex RT-PCR Assay for the Detection of Peste Des Petits Ruminants Virus in Clinical Samples. *Veterinary Research Communications*. 2006; 30(6):655-666. doi:10.1007/s11259-006-3331-3.
14. Muthuchelvan D, De A, Debnath B, Choudhary D, Venkatesan G, Rajak KK *et al.* Molecular characterization of peste-des-petits ruminants virus (PPRV) isolated from an outbreak in the Indo-Bangladesh border of Tripura state of North-East India. *Veterinary Microbiology*. 2014; 174(3-4):591-595.