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Molecular confirmation of Peste des petits ruminants (PPR) disease outbreak in cross bred Jamunapari goats in Tamil Nadu

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Abstract
Peste des petitis ruminants (PPR) is an acute febrile, extremely contagious and infectious disease of goats along with high morbidity as well as fatality rate. The present study was carried out to investigate and confirm natural outbreak of Peste des Petits Ruminants (PPR) in cross bred Jamunapari goats in Namakkal district of Tamil Nadu, India. The characteristic clinical signs of PPR were observed in affected animals including high fever (more than 40 °C), catarrhal nasal discharge, crusts around the nostrils, lachrymal discharge, erosive-ulcerative oral lesions covered with gray to yellow pseudo membrane on the gums, lips, tongue and palates and also diarrhea. Nasal swabs were taken from clinically affected goats and stored at -80^{0}C until processing. The collected nasal swabs were pooled and RNA was extracted and converted into cDNA. M gene specific Reverse Transcription PCR (RT-PCR) was carried out and found positive for PPR virus infection. The PPRV was isolated in Vero cells and showed the characteristic CPE viz. rounding, syncytia and detachment of cell sheet. It was observed that cross bred Jamunapari breed was alo susceptible to PPR infection. Hence, the breed susceptibility may be considered while implementing control and eradication programme for PPR.

Keywords: Jamunapari goat breed, Peste des petits ruminants (PPR) virus, M gene, reverse transcription PCR, Vero cells

Introduction
Peste des petits ruminants (PPR), also known as Kata, Goat plague, is an acute highly contagious viral disease that causes serious economic losses due to high morbidity and mortality rates (Dhar *et al*., 2002) [7]. PPR is economically important viral disease which affects mainly sheep and goats, sometimes wild small ruminants and camel (Balamurugan, *et al*., 2012) [4]. PPR is highly contagious viral disease caused by *Morbilli* virus belongs to the family Paramyxoviridae. The typical form of PPR is associated with anorexia, pyrexia, ulceration, necrosis of mucous membranes, sores in mouth, mucopurulent nasal and ocular discharges, pneumonia, inflammation of the gastrointestinal tract (GIT) and diarrhea (Abubakar *et al*., 2011; OIE, 2014) [2, 11]. In India first PPR outbreak was recorded in Tamil Nadu in the year1987 (Shaila *et al*., 1989) [19]. At present PPR has been enzootic and reported regularly from different parts of the country among small ruminants and incurring significant economic losses in terms of morbidity, mortality, and loss of productivity (Singh *et al*., 2004) [21]. Hence the present study confirms the PPR outbreak in organized cross bred Jamunapari goats by M gene specific Reverse Transcription PCR (RT-PCR) and virus isolation in Vero cells.

Materials and Methods
The present PPR disease outbreak was investigated and recorded in an organized cross bred Jamunapari goat farm in Namakkal district of Tamil Nadu during the month of August 2020. Totally 65 goats which were less than two and half years of old. They were purchased from local sandy without the history of vaccination against PPR and also reported that three does were aborted and five goats were died two days before disease investigation. All the goats were reared in intensive system i.e., elevated slatted floor system. Disease investigation was carried out to find out the cause of disease. Nasal swabs, ocular swabs, peripheral blood smear, faecal samples and blood samples in aborted goats were collected from clinically affected goats.

Microscopic Examination
Faecal samples and peripheral blood smears were examined for the presence of parasitic ova and protozoan parasites as described by Soulsby (1982) [22].

Bacterial Culture Examination
Nasal swab were inoculated into nutrient agar, blood agar, Mac conkey agar, anaerobic agar and Sabouraud dextrose agar plates as per Quinn *et al.* (1994) [14].

Rose Bengal Plate Test (RBPT)
To differentiate the cause of abortion, blood samples were collected from aborted does and serum was separated to carry out Rose Bengal plate test as per OIE, (2018).

Haemto Biochemical Analysis
The routine haematological examination of whole blood samples viz. haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and differential leukocyte count and biochemical analysis of serum such as Glucose, Protein, Albumin, BUN, Creatinine, AST, ALT, ALP, GGT, Total Cholesterol, Calcium, Phosphorus, Total Bilirubin and Direct Bilirubin from PPR-infected goats were carried out using Semi Auto Analyzer in Centralized Clinical Laboratory, Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)
RNA was extracted using RNA isoplus and RNA quality and concentration was checked using Nanodrop. cDNA was synthesized using first strand cDNA synthesis kit (Thermo Fisher Scientific) as per manufacturer's instruction. PCR was carried out for M gene as per Rai *et al.*, (2010). The final volume of 20 µl of reaction mixture consisting of 3 µl of cDNA, 1 µl of 10 pmol of each forward (F-5'-CTT GAT ACT CCC CAG AGA TTC-3') and Reverse primer (R-5'-TTC TCC CAT GAG CCG ACT ATG-3') and Red dye Master mix 10 µl (Ampliqon) and nuclease free water 5 µl. The reaction mixture was placed in a Labnet thermal cycler with following conditions: 95 ºC for 3 minutes; 29 cycles of 94 ºC for 30 seconds, 60 ºC for 30 seconds, 72 ºC for 30 seconds, final extension at 72 ºC for 10 minutes. 10 µl of PCR product was electrophorosed on 1.5% agarose gel containing 2.5 µg/ ml of ethidium bromide and visualized in Bio Rad Gel documentation system.

Virus Isolation In Vero Cell Culture
RT-PCR positive pooled nasal swabs samples were centrifuged for 8000 rpm 10 min and filtered through 0.22 µ syringe filter. The filtrate was inoculated in to Vero cells monolayer in coverslip and cytopathic effects were observed. Uninoculated Vero cell monolayer was kept as control.

Results and Discussion
The present study was carried out to investigate the PPR disease outbreak in a cross bred Jamuapari goat farm. Out of 65 goats 41(63%) developed clinical disease and five animals (7.7%) were died and four goats were aborted irrespective of their gestation, but most of them were in midterm of pregnancy. Rahman *et al.* (2018) [15] reported that the local Black Bengal goats (33%) appear less susceptible to PPR than Jamunapari (46%) goats. The occurrence of PPR was significantly higher in the Jamunapari breed than Black Bengals (Rony *et el*., 2017) [16]. Out of 41 affected 35 goats were purchased from local sandy without history of vaccination against PPR. Introduction of newly purchased goats into existing flock is considered to an epidemiologically significant event in the spread of PPR. Similar mode disease transmission has been reported previously by Asmar *et al*, (1980); EMPRES, (1999) [8]. Affected goats showed high fever (41 °C) catarrhal nasal discharge, crusts around the nostrils, lacrimal discharge, erosive-ulcerative oral lesions on the gums, lips, tongue and palates, abortion and enteritis (Fig1, 2 & 3). Both the sexes goats were affected. Similar observations were recorded in previous reports (Rony *et el*., 2017; Meher *et al*. 2017) [16, 10]. Furthermore, the goats were reared in intensive system of rearing which resulted in close contact between animals and enhanced the spread of disease. The close contact and seasonal conditions may be the reason for high morbidity and mortality. For PPR to spread, close contact between infected and susceptible animals (Ozkul *et al*., 2002) [12] and inhalation of aerosols produced by sneezing and coughing of infected animals is needed for disease outbreak (Saliki, 1998) [18]. Balamurugan *et al.* (2012) [4] also reported the seasonal influence on PPR outbreaks in India i.e. PPR outbreaks are most frequent during wet or cold dry months (April to February).

In this study to differentiate enteritis due to coccidiosis and helminthic infection faecal samples were examined and no parasititic ova and no blood protozon in peripheral blood smear could be detected by microscopic examination. To rule out the bacterial infection in clinically affected goats, bacterial culture was carried out in nasal swabs and no bacteria of etiological importance could be isolated as previously reported by Roy *et al.* (2010) [17]. All five aborted goats were found negative against Brucellosis by RBPT. Aububakar *et al*. (2008) [1] was reported high rate of abortions (28-45%) with all the stages of pregnancy during and after PPR outbreak and their serum samples were found positive for PPR antibodies by competitive ELISA (c ELISA).

The routine haematological examination (Table.1) of the PPR-infected goats only in two goats showed mild reduction in RBC, Hb and PCV values indicative of transient anaemia due to stress and recent abortion goats and might be widespread haemorrhages throughout the body. This decrease in the values of Hb and erythrocyte count was also reported earlier reports (Das *et al*. 2015; Begum, *et al*. 2018) [6, 5]. In serum biochemical study (Table 2), total protein and albumin level were significantly lower in PPR affected goats. Similar findings were also recorded by others workers (Das *et al*., 2015; Begum *et al*., 2018; Aziz *et al*., 2019) [6, 5]. This lower value might due to damage to the glomeruli that causes increase in permeability that leads to passing of high level of protein from blood to urine. There was reduction in blood glucose level and high level of ALT was noticed. This may be due to liver damage as a result of PPR infection (Begum *et al*., 2018; Aziz *et al*., 2019) [5]. There was no significant difference in other serum biochemistry values of the PPR infected goats

For confirmation of PPR disease outbreak, RT-PCR for M gene was carried out and in which 191 bp product specific for PPR virus was obtained and confirmed that the present outbreak was due to PPR virus (Fig.4). The findings are in accordance with the findings of Rai *et al.* (2010). In this investigation study, PPRV was successfully isolated in Vero cells and cytopathic effects were observed after three blind passages. The CPE observed were rounding of cells,

detachment of cell sheet, syncytia formation (Fig. 5a & 5b). The PPR virus was successfully isolated in Vero cells and produced characteristic CPE for PPR virus viz. rounding and syncytia which are in accordance with the findings of Kumar *et al*. (2013) [9]. PPR is one of the economically important diseases of small ruminants. The economic loss in India due to PPR disease of goats is estimated as 843.53 million US$ (Singh *et al*. 2014) [20]. Hence, earlier diagnosis is very much essential to control the PPR outbreak. Due to higher multiple birth percentage and higher milk yield Jamunapari breeds are also highly susceptible to PPR virus infection. Hence, strict implementation biosecurity measures, quarantine of newly purchased animals and regular vaccination programme should be practiced to control the disease.

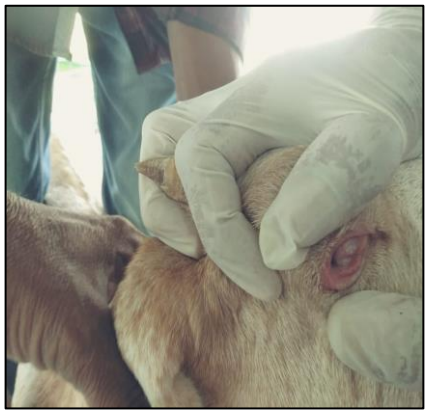

Fig 1: Congested ocular mucous membrane of PPRV infected cross bred Jamunapari breed of goat

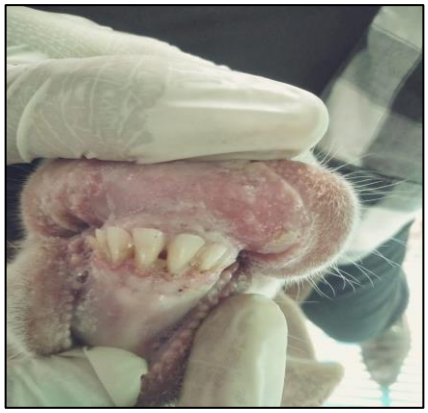

Fig 2: Ulcerative oral lesions on the gums and lips of PPRV infected cross bred Jamunapari breed of goat

Fig 3: Enteritis in PPRV infected cross bred Jamunapari breed of goat

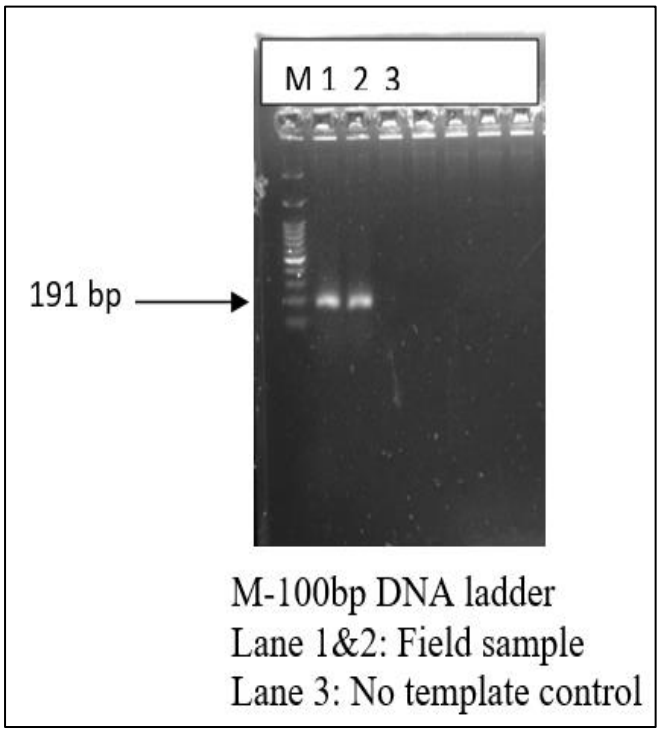

Fig 4: Agarose gel electrophoresis of M gene of PPRV

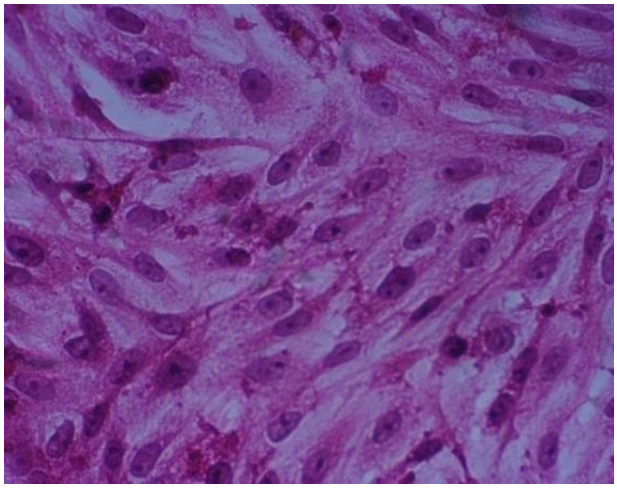

Fig 5a: Uninfected Vero cell monolayer (H&E) (200X)

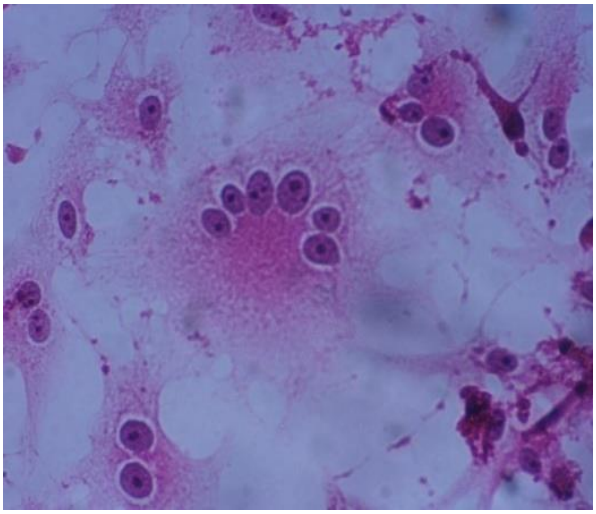

Fig 5b: PPRV infected Vero cells showing rounding of cells, detachment of cell sheet, syncytia formation (H&E) (200X)

Table 1: Haematological values of PPR infected cross bred Jamunapari goats

Sl. No.	Parameters	Animal No. 1	Animal No. 2	Animal No. 3	Animal No. 4
1	RBC ($x10^6$/cmm)	12.7	13.8	10.5	10.6
2	Hb (g/dl)	11.5	15.2	8.7	9.8
3	PCV (%)	38	42	30	33
4	WBC ($x10^3$/cmm)	13.21	7.95	18.81	10.44
5	Neutrophils (%)	30	28	14	10
6	Lymphocytes (%)	59	56	75	80
7	Eosinophils (%)	8	10	4	2
8	Monocytes (%)	3	6	7	8

Table 2: Serum Biochemical values of PPR infected cross bred Jamunapari goats

Sl. No.	Parameters	Animal No. 1	Animal No. 2	Animal No. 3	Animal No. 4
1	Glucose (mg%)	30	26	21	24
2	Protein (g%)	5.90	6.06	5.64	5.10
3	Albumin (g%)	1.93	2.60	2.11	1.61
4	BUN (mg%)	16	17	15	20
5	Creatinine (mg%)	1.03	0.30	0.74	0.85
6	AST (U/L)	126.4	102.6	178.8	164.5
7	ALT (U/L)	49.7	49.1	26.9	34.2
8	ALP (U/L)	57	34	68	65
9	GGT (U/L)	7.2	4.4	4.3	4.5
10	Total Cholesterol (mg%)	77	65	72	71
11	Triglycerides (mg%)	25	26	22	20
12	Calcium (mg%)	8.6	9.1	12.4	10.6
13	Phosphorus (mg%)	4.73	3.97	5.74	6.13
14	Total Bilirubin (mg%)	0.54	0.78	1.07	0.25
15	Direct Bilirubin(mg%)	0.44	0.58	0.93	0.22

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