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EPIDEMIOLOGICAL INVESTIGATION OF THE PESTE DES PETITIS RUMINANTS OUTBREAKS IN TUMKUR DISTRICT, KARNATAKA, INDIA

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Abstract- In this study, epidemiological investigation of the three outbreaks of peste des petits ruminants (PPR) in goats and sheep flocks with high morbidity and considerable mortality were recorded at Tumkur District, Karnataka, India during 2012-2013. Clinical samples were collected from the affected flocks/villages for laboratory investigation along with epidemiological parameters. The PPR virus (PPRV) antigen and nucleic acid was detected in the infected tissues/swab materials by sandwich enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction techniques (RT-PCR) and PPRV-specific antibodies were detected by competitive ELISA and indirect ELISA in serum samples. The PPR virus was isolated successfully in Vero cells after initial adaptation, and its identity was confirmed by RT-PCR assays. The significant epidemiological parameters observed were: young animals aged between 6 month to one year were severely affected than adult animals, which showed only few symptoms pertaining to PPR; changing pattern of disease in term of severity of gross lesions was observed in affected animals; source of infection was the introduction or purchase of animals from other source; etc. Given the importance of PPR and high productivity losses in small ruminants, outbreaks of the disease need to be carefully monitored, in spite of vaccination of the sheep and goats is undergoing under the National control programme on PPR.

Keywords- PPR, Epidemiology, Outbreaks, Sheep and goats, Karnataka, India

Introduction

Peste des petits ruminants (PPR) is an acute, highly contagious, notifiable and economically important transboundary viral disease of sheep and goats. The causative agent, PPR virus (PPRV) belongs to the *Morbillivirus* genus of *Paramyxoviridae* family. There is a single serotype of PPRV, but genetically grouped into four distinct lineages (I, II, III, and IV) based on partial sequence analysis of Fusion (F) gene [1,2]. Clinically, the disease is characterized by fever, ocular and nasal discharges, catarrhal inflammation of the ocular and nasal mucosa, necrotic lesions in oral cavity, enteritis, diarrhoea and bronchopneumonia followed by either death or recovery from the disease [3]. The mortality ranges from 50-90% and sometimes can be nil and morbidity can be 10 to 100%, even lower than 10% depending on circumstances [4]. Mortality and morbidity of disease are high when occurring in naive sheep and goat population. This transboundary nature of the disease is one of the main constraints in augmenting the productivity of small ruminants in enzootic regions like the parts of Africa, the Middle East, and the parts of Asia.

In India, PPR was first recorded in 1987 from Tamil Nadu [5] and it continues to be reported only from the Southern India until 1994. Later, a number of PPR outbreaks were reported from the northern states of India [6] with a solitary report in Indian buffalo in southern state [7]. Now, PPR is enzootic in India, and outbreaks occur in sheep and goats regularly throughout the country incurring huge economic losses [8]. It is a major constraint in small ruminant production incurring huge economic losses (estimated to be INR 1,800 million (US$ 39 million) annually in terms of morbidity, mortality, productivity losses with trade restriction [8,9].

For the proper control of PPR, there is need of base line epidemiological data on the disease prevalence in population, strong support of diagnostic methods and proper and timely vaccination of the susceptible population. National Institute of Veterinary Epidemiology and Disease Informatics (formerly PD_ADMAS) is a premier research institute under Indian Council of Agricultural Research (ICAR) carrying out research in the field of animal disease monitoring and surveillance, epidemiology and diagnostics. This study describes epidemiological investigation of the three confirmed PPR outbreaks occurred in Tumkur district in Karnataka state of India based on the virus isolation and detection of antigen, antibody, and nucleic acids of the PPRV in the clinical samples collected during the outbreak investigations.

Materials and Methods

Epidemiology of Outbreak and Clinical Picture

The outbreak of PPR occurred in an organized sheep farm in Gowraganahalli (Village I) in September 2012 and in villages Kemparayanahatti (Village II), Doddarampura (Village III) during March 2013, in Tumkur district of Karnataka. Outbreak occurred in local breed of goats and sheep aged between 2-3 years and few kids and lambs of age 4-6 months in Village II, III and Bannur breed of Sheep aged 3-4 years in village I. Farmer had sheep flock of 100 in

an organized farm in the Village I and animals were maintained for the fattening purpose. All the animals were purchased from Sindnur shandy in Raichur district of Karnataka. Five sheep died and rest of sheep was showing symptoms of PPR. The necropsy of the dead animals showed characteristic gross lesions of PPR [Fig-1].

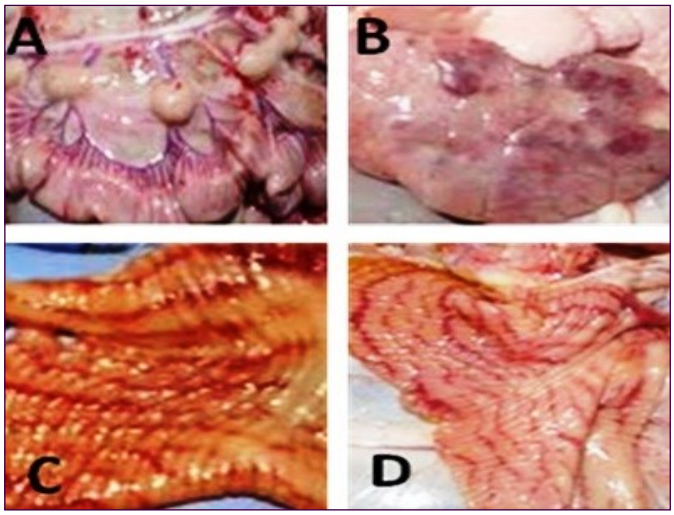

Fig. 1- Post Mortem lesions of PPR affected sheep showing A. Enlarged, edematous and congested intestinal mesenteric lymph nodes, B. congestion and consolidation of lobes of lungs C. Colon showing discontinuous streaks of congestion and hemorrhages (Zebra marking) on the mucosal folds. D. Blood vessels congestion and hemorrhages around the ileo-caecal junction.

In village II the flock comprised of 50 sheep and 20 goats per house and the animals in the village were not vaccinated for PPR. Approximately 300 animals were affected out of 1000 animals in the village as per reports by the farmers. Clinical signs observed include congestion of mucus membrane, watery diarrhea, blood mixed nasal discharge, pyrexia, ulcers in oral cavity, cough with bronchitis, dullness, anorectic and prostration etc. In Village III, 1000 animals were affected out of 2500 animals according to report of the farmers. Clinical signs such as congestion of mucus membrane, nasal discharge, crust formation in nostrils [Fig-2], ulcers on tongue, pyrexia, cough and diarrhea were observed.

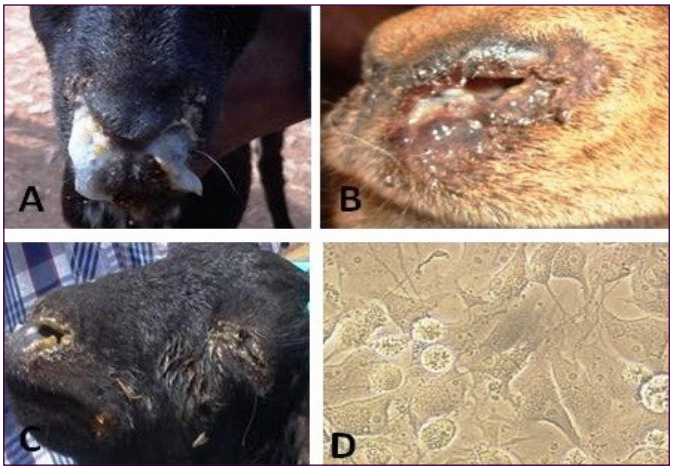

Fig. 2- PPR affected animals showing the Clinical signs A. Profuse nasal discharge (Village II) B. Profuse mucopurulent catarrhal exudates, erosion and ulceration of the nasal mucosa (Village I) C. Catarrhal exudates crusts over and occludes the nostrils and eye (Village III). Fig.1D. PPRV isolates infected Vero cells showing characteristic cytopathic changes (CPE) such as rounding and syncytia with degenerative changes on day 4 post-infection (×400 magnifications).

Clinical Materials

Upon request from the local state veterinarians, investigation team of NIVEDI deputed for the disease diagnosis and control measure. Clinical samples were collected during three outbreaks investigations by the team. Clinical samples (blood, serum, swabs and post mortem samples) were collected from both ailing and dead goats and sheep which included affected, non-affected and dead animals with different age groups during the investigation and were subjected for laboratory investigations [Table-1].

Table 1- Details of the samples and their results screened for peste des petits ruminants virus antigen and antibodies

Name of Village with Geo coordinate	Species	Clinical specimens	No. of Samples Screened	No. of samples Positive in different assays / tests				
				s-ELISA	c-ELISA	I-ELISA	RT- PCR	Isolation
Gowraganahalli, Koratagere Taluk, N-16° 37.063' E-075°12.682'	Sheep	Blood	3	3	-	-	3	-
	Sheep	Tissue (Lymph node, Intestine, Spleen)	3	3	-	-	3	+/- (Sheep)
	Sheep	Serum	23	-	4	2	-	-
		Total	29	6	4	2	3	-
Kemparayanahatti, Chikkanayakanahalli Taluk, N 13° 27.831' E 076° 40.695'	2 kids, 2 sheep and 2 Goat	Oral Swab	6	3	-	-	4	+ (Kid)
	2 Goat and 2 Sheep	Rectal swab	4	1	-	-	1	+ (Goat)
	3 Goat 2 Sheep	Nasal swab	5	1	-	-	3	-
	4 Goat 4 Sheep	Serum	8	1	7	7	-	-
	Sheep and Goat	Blood	2	0	-	-	0	-
		Total	19	6	7	7	8	
Doddarampura, Chikkanayakanahalli Taluk, N 12° 47.000' E 077° 14.554'	2 kids, 2 Goat , 2 Sheep	Oral swab	6	3	-	-	4	+ (Kid)
	2 Goat and 2 Sheep	Rectal swab	4	0	-	-	0	-
	2 Goats, 2 kid and 3 Sheep	Nasal swab	7	4	-	-	4	+ (Goat)
	4 Sheep & 3 Goats	Serum	7	0	6	7	-	-
	Sheep and Goat	Blood	2	0	-	-	-	-
		Total	21	7	6	7	8	-

s-ELISA-sandwich ELISA, c-ELISA-Competitive ELISA, I-ELISA-Indirect ELISA, RT-PCR- reverse transcription-polymerase chain reaction, + positive for isolation, - Nil

ELISA and RT-PCR Assays

The serum samples were tested for the presence of PPRV-specific antibodies by using competitive enzyme-linked immunosorbent assay (c-ELISA) kit and Indirect ELISA as described earlier [10,11]. PPR c-ELISA kit was used for detection of PPRV antibodies in terms of percentage inhibition (PI) as per method described earlier. Samples with PI of ≥ 40% were considered positive for the presence of PPRV antibodies. All the clinical samples were initially screened by sandwich ELISA (s-ELISA) [12] and reverse transcription-polymerase chain reaction (RT-PCR) assays for detection of PPRV antigen or nucleic acid.

The total RNA was extracted from clinical materials by using RNA easy kit (RNeasy®Minikit Qiagen Inc, Valencia, CA, USA), and the RT-PCR was performed using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit for first stand synthesis and subsequently second strand PCR was performed using virus-specific reported primers sets [13-15] and PCR Master mix reagents (Ampliqon, A/s, Denmark).

Virus Isolation

For virus isolation, swabs collected in sterile PBS (0.5ml), freeze thawed three times and which were highly positive by s-ELISA [Table-1] were selected. Samples from same village and species were pooled and treated with Gentamicin in Dulbecco's Minimum Essential Medium (DMEM, Sigma,USA) and filtered using 0.45µ filter. One to 1.5ml filtrate was inoculated onto Vero cell monolayer (80% confluent) by adsorption method. Cells were incubated at 37° C for one hour with intermittent shaking to allow adsorption of virus. The virus inoculums was then decanted and the infected cells were washed with DMEM (serum free) and added with maintenance medium (2% foetal calf serum with DMEM) for further incubation at 37° C for 6-8 days. Media was changed on alternate days with 2% DMEM and flasks were regularly observed for the appearance of cytopathic effect (CPE). Passaging of the virus continued until visible CPE observed in the infected cells. To investigate the specificity of the isolated virus, RT-PCR was performed as described earlier. Total RNA extracted from cell culture-adapted isolates was subjected to complementary DNA synthesis and subsequent PCR amplification of F and N gene sequences as described earlier using specific primers.

Results

The morbidity, mortality, and case fatality rates of disease in villages I, II, and III were 66.6, 8, & 5%; 30, 40 & 100% and 20, 8 & 5% respectively. The results of clinical materials [Table-1] revealed that most samples from each of the outbreak were positive for PPRV antigen or nucleic acid when tested by s-ELISA and RT-PCR assays. The analysis of serum samples with PPR c-ELISA kit and indirect ELISA demonstrated the presence of PPRV- specific antibodies. Further, the PPRV was successfully isolated from clinical specimen in Vero cells at passage level 6 after 4 days of infection [Fig-2], and these isolates were designated as PPRV Ind. Tumkur 2013 (goat origin).

Discussion

Karnataka state is known to be hyper endemic area to PPR. Tumkur district is bordered by Chikkaballapura, Chithradurga, Hassan, Mandya, Ramnagar, Bangalore rural and Andhra Pradesh. Movement of animals is a common factor between these places during trade. Although the origin of disease could not be traced, movement of animals could be the reason in Villages II and III. As no vaccination was carried out, 30% of young ones can be expected per year and they will be at risk of acquiring infection as reported earlier [8]. Generally, the flocks were mainly of sheep population with few goats. The present outbreak (March 2013) of the disease was reported first in goats and later transmitted to sheep as they were kept together. The clinical signs of the disease as well as high morbidity and mortality were mainly recorded in sheep because of more flock size. The disease was tentatively diagnosed by clinical signs but confirmation was based on various laboratory tests/assays. In spite of more sheep population, virus isolation succeeded was from goats, which indicates more severity of disease in goats. As survey started a month later, during this time, majority of the PPR-affected animals had either recovered or were in the recovery phase i.e., in which, sheep and goats that recover from PPR develop an active lifelong immunity. Actually the outbreak investigation started after a month in Village II, III and after 15 days in Village I. Based on clinical signs and laboratory test it was confirmed as PPR. The animals in village I were procured from Raichur shandy (Local Market) where thousands of animals comes from different districts of Karnataka and neighboring Andhra Pradesh and Maharashtra states. During trade, in contact might be triggering factor to cause disease in them as a introduction of infected animals under transport stress. Even though PPR outbreaks occur throughout the year, the incidence is mostly observed between October and March, with a peak during February [16] in Karnataka. Hence the appropriate time to vaccinate flocks is, in the months of July to September as reported earlier [16]. Sheep and goats should be vaccinated from four months of age. Intensive vaccination campaign programmes should be followed with aim of complete village flocks instead of individual or household flocks as reported earlier [17].

On analysis of the livestock disease database available at NIVEDI, Tumkur district has reported PPR regularly since 2003 except during 2009 [Fig-3] A. Maximum number of outbreaks was recorded during 2005 later on it declined due to regular vaccination programme carried out in the district. The disease has been recorded least during 2011 due to implementation of national control programme. On enquiry with the local veterinary authorities, it was learnt that decrease in the incidence of the disease leads to reluctance by farmers towards vaccination. Thus, less or no vaccination during 2011 and 2012 could have lead to building up of susceptible population and thereby occurrence of the disease. The exact source of the infection could not be ascertained. However, based on the history, initially goats were affected in both villages (II and III), later transmitted to sheep in that villages. Procuring of animals in the flock in Village I without proper vaccination details, quarantine measures and clinical history might have contributed to the spread of the infection. The outbreak villages were depicted geo coordinately in Google earth Map using Quantum GIS (QGIS) 1.8.0 software [Fig-3] B, where the shortest distance between infected villages were shown. However, the complete epidemiological details of these outbreaks were not known, but it could be assumed that goats and sheep had died of the disease since morbid materials were positive for PPR on laboratory examinations, and death was probably due to PPR. The affected goats or sheep were not vaccinated against PPR earlier. Therefore, the detection of PPRV antibody and antigen could have been only resulted from field infection with PPRV since the virus is endemic in the Southern part of India [18,19].

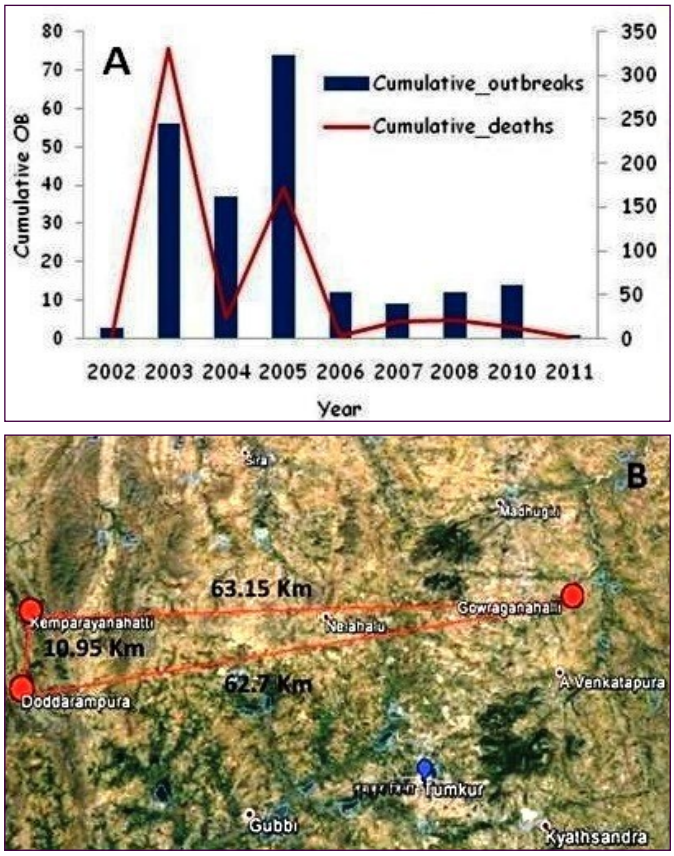

Fig. 3- Year wise outbreak profile of Tumkur district, Karnataka, India **(A)**. Outbreak villages were depicted geo coordinately in Google earth Map using QGIS 1.8.0 software **(B)**.

The serum samples collected from apparently infected and in contact animals showed varying percentage inhibition from 6 to 47, 16 to 88, and 37 to 89 in village I, II and III, respectively by c-ELISA. This showed that the animals were under various stages of infection, as the outbreak was noticed in flocks before the incident was investigated. PPR virus was detected by s-ELISA and highly positive samples were subjected for virus isolation in Vero cell lines. The passaged viruses during adaptation showed characteristic CPE of PPRV after 4 days post infection (dpi) in Vero cells at passage level 6. Earlier, Balamurugan et al. [20] isolated the PPRV from the sheep and goats in an outbreak at Jhansi and Revati in Uttar Pradesh and Bhopal in Madhya Pradesh, at earlier passage levels after 8 to 10 dpi in Vero cells. In this study, the isolates of PPRV was characterized by RT-PCR, which gave specific amplicons size products with respect to the gene specific sequences. This indicates the isolated virus was the PPRV. Further characterization of the virus by gene sequences and phylogenetic analysis is required for the classification of the lineages and also to know the circulating virus in the Tumkur district and cause of outbreak. However, the phylogenetic analyses of different isolates including vaccine strains showed only PPRV lineage IV is in circulation in India since the disease was first reported [1,2,21].

In general, the significance epidemiological and socio economical parameters observed were: young animals between 6 month to 1 year old age were severely affected; adult animals showed only few symptoms of the PPR; changing pattern in term of severity of gross lesions observed in animals; source of infection in most of the case was the introduction or purchase of animals from other source; mortality and morbidity was observed moderately; most of the loss due to death and loss in weight of animals; feed and fodder loss due to off feed, wastage, spoilage etc; more labour hours were spent for the maintenance of the isolated animals as well as treatment; delay in recovery of investment, loss due to cost of treatment and vaccination, etc.,

In conclusion, the epidemiology of the outbreak, clinical signs, virus isolation, detection of PPRV antigen/antibody by RT-PCR assays and ELISA, and the identity of the virus have confirmed that the outbreaks were caused by PPRV. Given the importance of PPR and high productivity losses in small ruminants, outbreaks of the disease need to be carefully monitored in spite of vaccination of the sheep and goats is undergoing in the National control programme (NCPPPR). Farmers and other livestock handlers should be educated on prevention and control measures such as vaccination of animals at proper time, good hygienic practices, restriction on movement of infected animal, quarantine measures and biosecurity levels within and between flocks/village. This needs to be addressed to limit the spread and severity of PPR outbreaks and thus can reduce the economic impact of PPR in India.

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Conflicts of Interest: None Declared.

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