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First report of contagious ecthyma (orf) outbreak in goats of Andaman and Nicobar Islands

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ABSTRACT

Goat constitutes almost 42.1 % of the total livestock population of the A & N islands. Generally the livestock are free from many dreaded diseases which are prevalent in mainland, India. However, in the present study the outbreak of contagious ecthyma (Orf) in goats of Andaman and Nicobar Islands was investigated and confirmed by PCR assay. The outbreak of orf was reported from different villages of the South Andaman. A total of 171 clinical cases of contagious ecthyma were reported during the different outbreak reported during the year 2017. The scab samples from the affected goats were collected and processed for extraction of viral DNA. Nested PCR assay was done by using the forward and reverse primers of parapox virus. The results revealed the confirmation of the outbreak of Contagious ecthyma (orf) virus in the goats of Andaman & Nicobar Islands for the first time.

Key words: Andaman, Contagious ecthyma (orf), Goat, Nested PCR.

INTRODUCTION

Andaman and Nicobar group of islands is strategically and biologically very important part of India located in Bay of Bengal. It is almost 1300 km away from mainland ports towards east-southern side in the Bay of Bengal and encompasses about 572 islands of which 36 islands are inhabited. These islands constitute one of the richest repositories of biodiversity in the whole of South and South East Asia. The region is also one among the 22 agro-biodiversity hot spots in India. The livestock population is approximately 1.80 lakhs which comprises of cattle, goat, pig and buffalo. The island is surrounded by approximately 1900 km of coastal length which is approximately 1/3rd of the country total coastal line. As per livestock census of 2012, the cattle, buffalo, goat, pig and poultry population in the Island is 45, 608, 7850, 64, 602, 35, 401 and 10, 80, 228 (nos) respectively. The major bottlenecks for low productivity are indiscriminate inbreeding and free mixing of the animals, dilution of genetic superiority, long inter-calving period, inadequate availability of feed and fodder and high parasitic load.

The livestock and poultry of Andaman and Nicobar islands in general are free from most of the dreaded diseases which are prevalent in mainland India *viz.* black quarter, rinderpest, haemorrhagic septicaemia, rabies, contagious bovine pleuro-pneumoniaetc (Sunder *et al.*,2005; Sunder, 2014). Except for three incidences of foot and mouth disease (FMD) outbreak in 1985, 1989 and 2005 and three incidence of swine fever outbreak in 1967, 1987 and 2000, no other outbreak of infectious or contagious disease has been reported so far in livestock of this territory (Sunder *et al.* 2005; Sunder *et al.*,2018).Goat constitutes about 42.1% of the total livestock population in the Andaman & Nicobar Islands and is an integral part of the livestock system (Sunder *et al.*,2018).The disease pattern in the goats indicated the sero prevalence of *Brucella melitensis, Mycoplasma capri* and *Leptospirosis* (Sunder *et al.*,2005; Sunder 2014).The other diseases like pox virus, contagious ecthyma (orf), enterotoxaemia and gastrointestinal parasitism are also observed (Sunder *et al.*,2005). In the last few years, seasonal and sporadic incidence of contagious ecthyma (orf) in goats has been observed in the Islands. However, no reference is available for confirmatory diagnosis of the disease in goats. This paper describes the first confirmatory diagnosis of the orf outbreak in Andaman & Nicobar Islands.

Orf (*contagious pustular dermatitis or contagious ecthyma*) is one of the most widespread, benign or contagious, communicable, zoonotic, economically important viral diseases caused by parapox virus of the subfamily Chordopoxvirinae, family Poxviridae (Pal *et al.*, 2013).The disease is transmitted through direct contact and through contaminated materials (Almagro *et al.*,1991).The genome of the parapox virus double stranded DNA of approximately 134-139 kbp with high GC content (approximately 64%). The gene (B2L) encodes for the envelope protein of the virus is highly immunogenic and pathogenic in nature. This gene has been targeted for detection of the orf virus (ORFV) from the clinical cases by PCR assay (Hosamani *et al.*, 2006). It usually causes diseases in sheep and goat however, some reports of zonotic

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transmission has also been reported (Tryland *et al.*, 2013). The disease is prevalent in worldwide and morbidity is very high usually ranges from 70-85 % causing huge economic losses (Peralta *et al.*, 2015). In India, this disease has been reported from many areas (Mondal *et al.*, 2006; Balakrishnan *et al.*, 2017), however, so far no outbreak or any confirmed cases of the orf has been reported from this Islands. In the present study detail study has been carried out to study the outbreak of orf during different season and confirmation by PCR assay.

MATERIALS AND METHODS

The outbreak occurred during the year 2017-2018 in Andaman local goats in various villages of South Andaman. Animals of all ages were affected and showed the typical lesions of contagious ecthyma (Fig 1). During the period, a total of 10 outbreaks were reported wherein 171 goats were affected and showed the typical clinical symptoms of contagious ecthyma. The disease mainly observed during the drier period of the year mainly in the month of August, September, October and February. Highest cases were observed in the month of September from the Middle Andaman zone. The clinical symptoms observed were mainly erythematous spots or swellings followed by formation of papules and then scab in and around the mouth, gums, inner thigh (Fig 1). Affected animals were dull and could not able to take feed. The morbidity of the disease was found to be more than 75%. However mortality was less than 5%. The attack rate in the population was found to be 27.8 %.

Scabs and swab materials were collected without any preservative as a source of virus from the clinically infected goats from the different villages of the South Andaman, India. The scabs were grounded in 10% suspension in phosphate buffered saline (PBS). The samples were processed aseptically and DNA extraction was done as per the method described in Quiagen pathogen kit standard according to the manufacturer's instruction.

Major envelope membrane glycoprotein (B2L) gene of the orf virus was targeted using PCR assay as per the standard protocol described by (Inoshima *et al.*, 2000). A set of three primer pairs in a semi nested PCR format was used. During the first step a set of pan-parapox primer (PPP-1) and pan-parapox primer (PPP-4) primers was used to generate the product. Later in semi nested PCR a set of inner primer PPP-3 was used with PPP-4 for amplification of 235 bp fragment of the B2L gene (Table 1). PCR was carried out with 2X PCR Taq mixture containing Taq DNA polymerase, $MgCl_2$ and dNTPs.DNA extracted from scab material was added to 25 µl reaction mixture containing 12.5 µl mix, 1 µl of each primers and 5 µl DNA. DNA was amplified in a thermal cycler (Eppendorf, Germany) by two step reaction: 95^{0}C for 9 min and five cycles of 94^{0}C for 1 min, 50^{0}C for 1 min and 72^{0}C for 1 min and 25 cycles of 94^{0}C for 1 min, 55^{0}C for 1 min and 72^{0}C for 1 min. Semi nested PCR was carried out using 5 µl of the first PCR product in the same conditions with PPP-3 and PPP-4 primers. After amplification, a 10 µl was electrophoresed through 1.5% agarose gel with ethidium bromide to visualize the amplicon B2Lof 235 bp.

Amplified products were purified using a commercial gel extraction kit (GCC biotech India Pvt. Ltd, India) and sequence information was generated by dideoxy fingerprinting. Sequence information generated was submitted for Gen Bank accession numbers (MH730658-MH730659). Blast searches were used to retrieve the homologous sequences from the GenBank database. Sequence alignment was done by using the Clustal W method (Thompson *et al.* 1994). Phylogenetic analysis was done by Neighbour-joining method with 1000 bootstrap replications using MEGA software version X (Kumar *et al.*, 2018).

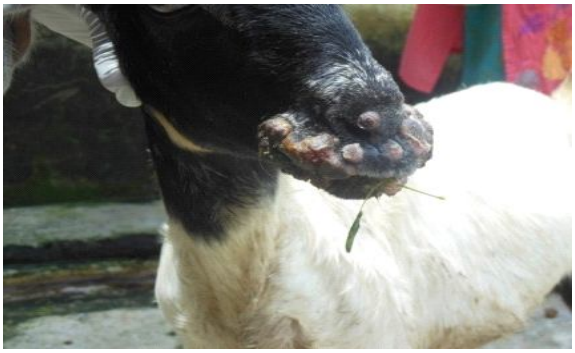

Fig 1: Clinical signs in affected goats. Scaby and ulcerated cauliflower like lesions around lips.

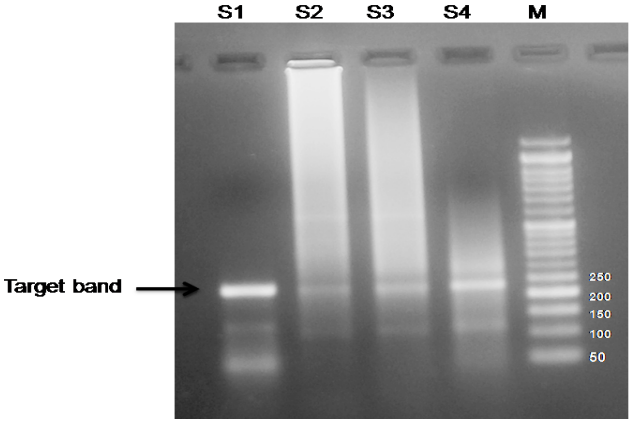

Fig 2: Showing 235 bp partial fragment of major envelope membrane glycoprotein (B2L) of orf virus (Lane S1 to S4, M=50 bp ladder).

Table 1: Primers used in semi nPCRamplificationof CE viral amplicon.

Virus	Gene	Sequence (5'-3')	Predicted Size
Orf	PPP-1	5'gtc gtccac gat gag cag ct-3'	235bp
	PPP-3	5'-gcg agt cc gaga agaata cg-3'	
	PPP-4	5'-tac gtgggaagcgcctcg ct-3'	

RESULTS AND DISCUSSION

Contagious ecthyma (orf) is a highly contagious disease of sheep and goat. In the present outbreak, all age groups of goats were found to be affected. The age of the infected goats ranged from 3 months to 4 years. No difference was recorded in terms of severity of infection with respect to sex, however, the infection was more pronounced in the kids. Clinical signs were pyrexia (105^0F-106^0F), erythematous spots, formation of papules and scab in and around the mouth, tongue, gums, inner thigh. The disease was reported mainly during the drier period of the season. Usually the affected goats recovered from the infection after antibiotic treatment in 2-3 weeks. The lesions were observed mainly in the mouth, tongue, abdomen, teat and gums. Morbidity was found to be very high (75%) however, the mortality was found to be low (5%). The mortality in the kids is reported to be high due to secondary bacterial infection of the wound (Gelberg, 2007). The virus can affect different parts of the body and in the present study also the lesions were observed in the mouth, gums, teats, inner thigh and abdomen region. The outbreak was mainly found in the different villages of South Andaman district of Andaman & Nicobar Islands. A total of 171numbers of clinical cases were observed.

Contagious ecthyma is a seasonal and widespread viral disease of small ruminants of age groups in Andaman Islands as an endemic disease in various regions of India. In the present outbreak the disease was mainly reported during the drier months i.e. during the months of August to January. Similar outbreaks of orf had been reported from different parts of India *viz.*, Uttarakhand (Venkatesan *et al.*, 2011), Assam (Bora *et al.*, 2015), Uttar Pradesh (Kumar *et al.*, 2014), Rajasthan (Mann *et al.*, 2014).The scab samples were collected from different areas of the outbreak. A total of 9 scab samples were collected and processed. The presence of orf virus DNA was analysed by PCR. The analysis of the semi nested PCR revealed the confirmation of the Orf virus in the scab samples collected from nine goats (Fig 2). The product size was approximately 235 bp in all the positive samples. There were multiple bands produced during the first PCR, however, in the second PCR specific product of approximately 235bp was amplified. Mondal and his associates also reported similar findings when they also used the same set of primers for amplification of 235 bp amplicons (Kumar *et al.*, 2018). All the samples were found positive by PCR assay. The present confirmative diagnosis of orf is the first of its kind of molecular detection and confirmation of outbreak of CE from Andamans. As per the literature, molecular identification of orf from Andaman state has not been reported. This disease has got significant economic impact on poor farmers (Perry *et al.*, 2002) as it directly affects the feed intake of goats. The outbreak of CE is being recorded in goats in the month of August to September to January. The seasonal outbreak of orf had been reported in the month of September 2012 in the Muzzaffarnagari sheep flock at CIRG, Makhdoom, India (Kumar *et al.*, 2014). The

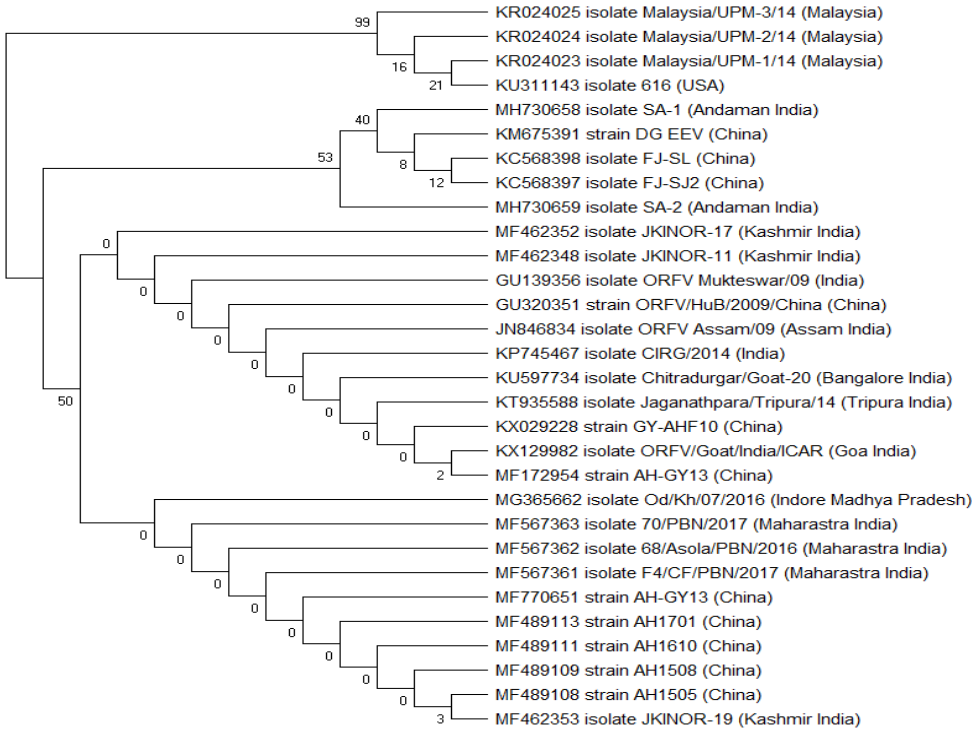

Fig 3: Phylogenetic analysis based on nucleotide sequence of MH730659 and MH730658. The phylogenetic relationship was constructed by the Neighbor-Joining algorithm using Mega 10.0 software.

disease has also been recorded during the early summer (April-June) from Rajasthan (Mann *et al.,* 2014) (north western region of India). These reports on the temporal trend of outbreak of orf indicate that the disease can occur anytime during the year. Typical lesions of orf as described in our report have also been recorded (Radostits *et al.,* 2006).

The clinical symptoms mostly appear on the mouth, nostrils, lips, nasal mucosa and in rare cases in teats also. The lesions in the udder and teats are mostly seen in the does which are in milking. The diagnosis of the disease is usually done on the basis of appearance of the clinical lesions and laboratory confirmation. However due to the advent of molecular biology tools mainly the PCR with amplification of the virulence gene, the confirmatory diagnosis has become more authentic. In the present study, the outbreak of the orf has been confirmed by the amplification of the B2L gene by nested PCR assay which targets the final amplification product of 235 bp (Mondal *et al.,* 2006).

Presently there are eight complete genome sequence of ORFV available in the Gene bank. The ORFV is the most studied virus is the parapox virus because of its availability and distribution throughout the globe. There are also reports which suggest that the same set of primers have been used for amplification of other fragment of 594 bp from the clinical samples for the diagnosis of parapox virus infection in reindeer (Tryland *et al.,* 2013).However, the use of nested But the semi-nested primers in this study have confirmed the presence of orf virus in the scab samples. The present finding is in accordance with the earlier results of the nPCR assay using B2L gene primers (Hosamani et *al.,* 2006; Ferede *et al.,*2014) indicating the emergence of this trans-boundary disease in these islands which is being isolated from the rest of India. Diagnosis of orf viral antigen in scab samples could be efficiently done by semi-nested PCR as it detects low copy number of viral DNA and which has been found to be more efficient diagnostic method for orf virus in goats.

The present investigation is the first report of the confirmatory diagnosis of Orf outbreak in goats from Andaman & Nicobar Islands. However, detail study is required to establish the epidemiology and virus lineage. Reposts suggested that the pathogenesis of the ORFV is due to the presence of the vascular endothelial growth factor gene which induces the endothelial cells to promote vascular permeability (Harvey *et al.,* 2015). However worldwide studies have been carried out to study the pathogenesis of the ORFV and several genes have been identified for the pathogenesis (Peralta *et al.,* 2015). Whole genome sequence analysis of the ORFV has been done and furthermore, the whole genome of the virus and phylogenic analysis has been carried out from various isolates (Kumar *et al.,* 2014, Martins *et al.,* 2014).The virulence genes *viz*. orf 020, orf112, orf117, orf 127 and orf132 are mostly located in the terminal region of the genome (Upton *et al.,* 2003). In the present study we report the first confirmed clinical outbreak of orf in goats by molecular identification of the virulence gene of the ORFV.

Sequence information of partial B2L gene of two samples was generated and was submitted for Gen Bank accession numbers (MH730658-MH730659). A phylogenetic analysis of the Andaman isolates and other isolates from neighbouring countries has been shown in Fig 3. From the phylogenetic analysis it has been found that Andaman isolates formed cluster with three Chinese isolates (DGEEV, FJ-SL and FJ-SJ2).

CONCLUSION

It is concluded that the contagious ectyma outbreak was confirmed in the A & N islands for the first time by PCR assay and detail study is required to study the epidemiological investigation and genetic lineage of the virus. Due to its less mortality rate, it has been not given much importance by the livestock owners or goat rearers.

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