

Original Research Paper

Molecular Characterization of Contagious Pustular Dermatitis Virus in Goats of Sri Lanka

¹Susantha Piratheepan, ²Sumathy Puvanendran and ³Kalyani Perera

¹Department of Animal Science, Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka

²Animal Virus Laboratory, Veterinary Research Institute, Sri Lanka

³Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

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Corresponding Author:

Susantha Piratheepan
Department of Animal Science,
Faculty of Agriculture,
University of Jaffna, Jaffna, Sri
Lanka
Email: spiratheepan@univ.jfn.ac.lk

Abstract: Contagious Pustular Dermatitis (CPD), is a zoonotic disease of small ruminants causing severe deterioration of skin. Contagious Pustular Dermatitis Virus (CPDV) is an epitheliotropic virus belonging to the parapox virus. Accurate information on CPD in Sri Lanka is scarce. This study was conducted to investigate the prevalence and to characterize the CPD virus from goats in Sri Lanka and select a suitable CPDV strain as a vaccine candidate since no vaccines are available in Sri Lanka. Laboratory confirmation and phylogenetic analysis of CPDV were obtained through PCR and DNA sequencing respectively. Ninety-two scab samples from infected animals were collected in 50% Glycerol phosphate saline from the following districts of Sri Lanka: Vavuniya, Trincomalee, Anuradhapura, Jaffna, Mannar, Kilinochchi and Kandy. Viral DNA was extracted and PCR was performed with modifications by using the following primers ORF2F5'-CGAACTTCCACCTCAACCACTCC-3' and ORF2R5'-CCTTGACGACGATGTCGCCCTTCT-3'. The partial B2L gene was amplified. The B2L gene, which codes for an envelope protein, is highly conserved for CPDV. Laboratory analysis showed that eighty-six samples were positive for CPD viral DNA. In the phylogenetic analysis based on the partial B2L gene, the Sri Lankan strain was closer to the ORFV Assam/09 isolates (JN846834) from India. A12 strain (Anuradhapura) and ORFV Assam/09 isolates showed 99% similarity at the nucleotide levels. Sri Lankan isolates are closely related to each other. Therefore, one of the strains could be used as a vaccine candidate. Two isolates from India (Accession No JN 846834, KU128538) were closely related to isolates from the Anuradhapura, Mannar, Jaffna, and Kilinochchi districts. Two isolates from China (Accession No KU 199831 and KC 568397) were closely related to isolates from Vavuniya and Trincomalee.

Keywords: CPD, ORF Virus, Contagious, Zoonotic, Molecular Characterization, Nucleotide Sequencing

Introduction

Contagious Pustular Dermatitis typically causes lesions in the skin around the mouth. Hence in immune-compromised animals, the disease is very quickly developing and causing death (Couch, 1983). Contagious Pustular Dermatitis is caused by a virus called Contagious Pustular Dermatitis virus (CPD virus), as the disease is highly contagious, causing morbidity rates up to 100% and the fatality rate usually ranges between 5-15% (Haig and Mercer, 1998). In some untreated cases of contagious pustular dermatitis, the occurrence of secondary staphylococcal infection is quite frequent. Diagnosis is made based on clinical signs of CPD and the contagious nature of

the disease. The clinical signs of CPD include proliferative lesions at the hoof/horn junction and on the lips. Zoonotic contagious pustular dermatitis virus can spread to humans via direct contact with affected small ruminants or by utensils carrying contagious pustular dermatitis virus (Winter and Charnley, 1999). CPD virus is a member of the genus Parapoxvirus while the other members were, the pseudo cowpox virus, a bovine popular stomatitis virus that infects cattle, and the Para poxvirus of red deer in New Zealand. This viral protein interferes with the host's immune and inflammatory reactions, thereby delaying the recovery period and percentage (Zhang *et al.*, 2010). The envelope protein gene B2L and Virus Interferon Resistance (VIR) genes of the virus which are highly conserved used

to measure genetic variation among parapoxvirus. CPDV is a highly contagious transboundary pathogen. Poxviridae family are oval in shape and enveloped viruses containing a Double Strand (ds) DNA genome. The genetic diversity of parapox virus was also determined by comparing the full lengths of CPDV 011, 059 (ORFV059Fw1: 5'-CTCGGTCAAGGACTGGATA-3'; ORFV059Rv1: 5'-GATGGCTGGATGGTGCA-3), 109, 110 and 132 genes with reference strains. These genes are sequenced from CPD virus isolates in different parts of the world. Through PCR it has been identified that the length of the B2L gene is approximately 1460 bp and it encodes 379 amino acids (Zhang *et al.*, 2010). It is a highly conserved gene and codes the highly virulence envelope protein of the CPD virus. Almost all the phylogenetic analysis data published worldwide are based on highly conserved genes. In Sri Lanka, only auto-vaccine is produced. During the outbreak of the disease, if the farmer wishes to protect the animals, they give scabs that contain more virus particles to the laboratory to get the auto-vaccine produced, to protect against the further spreading of the infection and control the disease in a herd. Furthermore, in Sri Lanka, there is no vaccination program to prevent the occurrence of CPD.

Materials and Methods

Molecular Characterization of the CPD Virus Strains

Under sterile conditions, 1 g of scab sample was weighed and washed with phosphate-buffered saline. Then Phosphate Buffered Saline (PBS, pH = 7.2) was added to make a 40% suspension and it was subjected to 3 freeze-thaw cycles between 37-20°C. After the last freeze-thaw cycle, the suspension was clarified at 3000 rpm for 20 min at 4°C using a centrifuge (Hanil Science Industrial, Germany).

DNA Extraction Polymerase Chain Reaction

One ml of supernatant after centrifuge was transferred to a microcentrifuge tube and stored at -20°C in a freezer (Biobase, Hong Kong). Two hundred microliter of 40% scab suspension was transferred to a sterile microcentrifuge tube and the DNA was extracted using a commercial DNA extraction kit (purelink genomic DNA extraction kit, Invitrogen, USA). A polymerase chain reaction was performed on the extracted DNA based on the method described by Hosamani *et al.* (2006) with slight modifications and the multiplication reactions were carried out in a thermal cycler (applied biosystem, U.S.A). The thermocycler was set as initial denaturation at 94°C for 5 min, amplification conditions for one cycle of the PCR reaction were 50 sec at 94°C (denaturation), 50 sec at 55°C (annealing), and 59 sec at 72°C (DNA synthesis). Each cycle was repeated 35 times and the final cycle was completed by a final extension for 10 min at 72°C. All PCR assays were included with proper controls. Table 1 represents the number of scab samples collected from various districts of Sri Lanka.

Table 1: The number of scabs collected from CPD-affected goats of different districts of Sri Lanka

Districts	Number of scabs collected
Anuradhapura (NC)	10
Ampara (E)	4
Puttalam (NW)	6
Trincomalee (E)	8
Jaffna (N)	22
Batticaloa (E)	6
Kandy (C)	2
Kilinochchi (N)	8
Vavuniya (N)	22
Mannar (N)	4
Total	92

DNA Sequencing and Phylogenetic Analysis

The PCR products of partial B2L genes (ORFV011) were sequenced (automated nucleotide sequencing). The B2L gene was sequenced and a phylogenetic tree was made to see the genetic relationship among isolates (Hosamani *et al.*, 2006). The sequenced B2L genes were compared with those available in the GenBank database using the MAFFT online software (<http://mafft.cbrc.jp/alignment/server/>) program (Katoh *et al.*, 2002). The nucleotides were analyzed by the Clustal W method (Mahmoud *et al.*, 2010; Mayr *et al.*, 1981; Tamura *et al.*, 2007) for the identical level of sequences.

Results and Discussion

Laboratory analysis showed eighty-six samples out of ninety-two were positive for CPD viral DNA. A PCR assay was developed (modified to the existing procedure) and performed to obtain the partial B2L gene (the envelope protein-coding gene). Since the B2L gene sequences have been widely used for molecular epidemiological studies of Para poxviruses the primers were designed to get 507 bp products. The amplified PCR products from the samples obtained were 507 bp for the partial B2L gene. This result indicates the presence of CPD virus in the scabs, which were collected from affected goats as shown in the Plate 1 clear band, were obtained and a DNA ladder in the 12th position was used to find out the length of the products. As this is the first study in Sri Lanka it helps to differentiate different dermatitis viruses from CPD. Plate 1 shows the scabs due to the CPD virus and the animal was severely suffering from CPD. Plate 2 shows the goats affected by CPD and scabs are formed mainly around mouth, oral commissures and muzzle.

Phylogenetic Analysis

Maximum Likelihood

Thirty-five B2L sequences of CPDV from other countries were used to build the tree. The blue circles denote Sri Lankan isolates. The CPDV isolates were collected from goats in Anuradhapura, Trincomalee, Jaffna, Vavuniya, Kandy, Kilinochchi, Ampara, Puttalam, Batticaloa and Mannar districts for molecular studies. The isolated Sri Lankan strains demonstrated a high degree of identity among themselves.

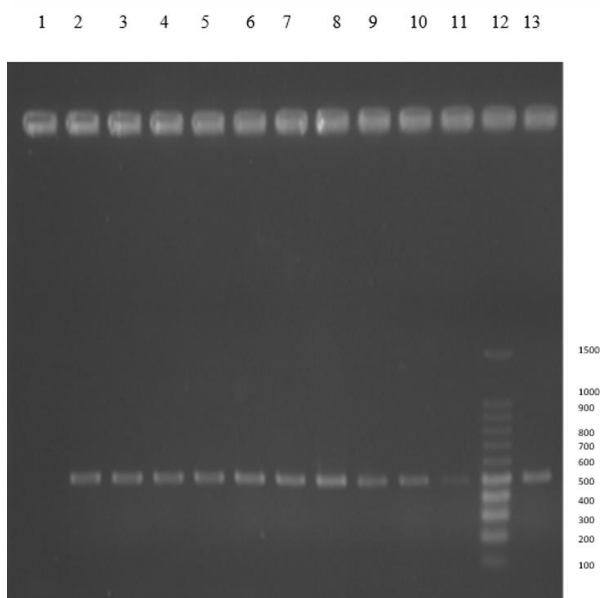


Plate 1: CPD positive samples obtained clear and strong bands with the Gel Image system

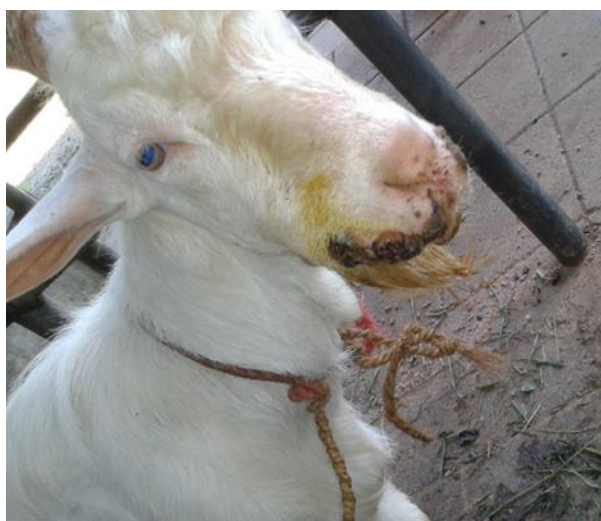


Plate 2: Scabs due to contagious pustular dermatitis

The phylogenetic trees from both maximum likelihood (Fig. 1.) and neighbor-joining methods were the same. In the phylogenetic trees, there are two main clusters. In cluster I there are 35 foreign isolates and ten isolates from Sri Lanka. In cluster II there are three foreign isolates. In cluster I there are six sub-clusters and the local isolates fall in separate sub-clusters. All these isolates fall into three sub-clusters. Four isolates from Anuradhapura, Mannar, Jaffna, and Kilinochchi are closely related and fall into a sub-cluster. Isolates from Vavuniya and Trincomalee fall into a sub-cluster but isolates from Kandy

and some isolates from Trincomalee are closely related and falls into a sub-cluster. These Sri Lankan isolates were closely related among themselves.

Two isolates from India (Accession No JN 846834, KU128538) were closely related to isolates from the Anuradhapura, Mannar, Jaffna, and Kilinochchi districts. These districts are in the Northern and North Central provinces of Sri Lanka. Distance-wise India is closer to the above-mentioned districts and there was close trade associated with Sri Lanka which could be the reason for closely related isolates. Two isolates from China (Accession No KU 199831 and KC 568397) were closely related to isolates from Vavuniya and Trincomalee. Although it is unable to find the reason for this finding, it could be due to the importation of animal products or animals. India and China are closer to each other, and Chinese isolates could be closely related to Indian isolates. According to Fleming and Mercer (2007) the Para Poxviruses (PPVs) are antigenically and genetically closely related among themselves and the present study also supported the above finding. Even though these isolates are from one country or even the same districts within the country they are not 100% identical, they fall into different sub-clusters and the time they evolved from primitive strains is also different. Some strains from the Trincomalee and Vavuniya districts evolved earlier.

According to the phylogenetic analysis based on the partial B2L gene, the Sri Lankan strain was closely related to the ORFV Assam/09 isolates (JN846834) from India. CPD A12 strain and ORFV Assam/09 isolates showed 99% similarity at the nucleotide levels.

CPDV infection of goats and sheep is generally neglected worldwide due to the severity and ignorance of the farmers. Since CPDV infections cause severe economic loss to the farmer, it is urgent and important to adopt rapid and precise pathogen detection methods for screening samples from animals presenting pox-like lesions. The screening for asymptomatic carriers as well as wildlife is also needed to identify potential reservoirs.

Zoonotic CPD

Since the CPD virus was reported to have zoonotic potential, from the present study no human infections were reported therefore it is not possible to completely rule out the presence of zoonotic CPDV infections in Sri Lanka, as human infections were self-limiting and occur without the presence of severe disease. According to Bayindir *et al.* (2011); Lederman *et al.* (2007); Leavell *et al.* (1968); Uzel *et al.* (2005), CPD was associated with occupational infection in humans with lesions characterized by large, painful nodules on hands and, less frequently on the face.



Fig. 1: Phylogenetic tree

Conclusion

This study describes for the first time, the molecular characterization of ORFV isolates collected from goats in Sri Lanka. This study revealed that CPD is prevalent in over 50% of the goat farms in the 10 districts included in the study. Although it causes a severe economic impact on production, the disease is neglected by farmers and Animal health officers. Though Contagious papular dermatitis is endemic in Sri Lanka, yet there is no vaccination program to control the disease. Laboratory confirmation and phylogenetic analysis of CPDV revealed that Sri Lankan isolates were closely related. This is of utmost importance finding to select a suitable strain to be used as a vaccine candidate. Further studies such as adopting a strain in cell culture, characterizing cell culture adopted virus, and selecting a suitable method to inactivate the virus should be done for a successful

vaccine production which will ultimately help to control and eradicate this disease from Sri Lanka.

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Author's Contributions

Susanthaa Piratheepan: Conducted and led the comprehensive research process, encompassing conceptualization, experimentation, data collection, analysis and interpretation.

Sumathy Puvanendran: Provided overall supervision, guidance and advisement throughout the research project.

Kalyani Perera: Contributed to the project as an advisor, offering valuable insights and oversight across various phases of the research.

Ethics

Authors declare no any conflict of interest. Permission was obtained from Department of animal production and health and each farmers' used in research.

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