CASE REPORT

MOLECULAR DIAGNOSIS OF CAPRINE ORF VIRUS (ORFV) FROM PENANG, MALAYSIA

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ABSTRACT. This case report describes the molecular diagnosis of contagious ecthyma caused by orf virus (ORFV) detected from two Jamnapari crossed goats from Penang. The epithelium samples from the infected goats were sent to the Veterinary Research Institute of Malaysia in Ipoh, for disease diagnosis. Molecular diagnosis via polymerase chain reaction with high-GC pan chordopoxviruses primer and Sanger sequencing confirmed that the ORFV to be the causative agent. This report also served as a baseline data for the Malaysian ORFV infection.

Keywords: orf virus, contagious ecthyma, contagious pustular dermatitis, pox, goat

INTRODUCTION

Contagious ecthyma is caused by the orf virus (ORFV) that belongs to the genus parapoxvirus, subfamily Chordopoxvirinae of the Poxviridae family (Zhao *et al*., 2010). Alternatively, the disease also has been referred as orf, contagious pustular dermatitis, scabby mouth, sore mouth or malignant aphtha (Spyrou and Valiakos, 2015). ORFV has a global distribution and effects a wide range of domestic and wild animals including sheep and goat. In Malaysia, cases of ORFV infection have been reported since 1935 with three different local isolates identified from goats (Zamri-Saad *et al.,* 1992; Bala *et al.,* 2018).

ORFV infection causes high morbidity but low mortality rate (Zhao *et al.,* 2010). Often, young animals are more susceptible to ORFV infection than the older animals, and can lead to high mortality due to secondary infection. The hallmark of ORFV infection includes infectious pustules around the nostrils, lip, mouth, tongue, ear, between toe and occasionally at the udder and teats (Zhao *et al*., 2010; Abdullah *et al*., 2015; Bala *et al*., 2018).

ORFV is a zoonotic pathogen with reported human cases (Bergqvist *et al.,* 2017). Those working with animals (veterinarians, farmers, butchers) have higher risk of exposure to ORFV infection following direct contact with infected animal of indirectly from fomites or contaminated materials in conjunction with skin trauma (Bayindir *et al*., 2011; Nougairede *et al*., 2013; Bala *et al.,* 2018). In humans, the lesions are self-limiting with painful pustules often seen on the hands, fingers and occasionally other body parts

(Bayindir *et al.,* 2011; Bergqvist *et al.,* 2017). Although the lesions are not life-threatening, the infections can limit a patient's daily activities, bringing substantial economic impact. In addition to its zoonotic potential, ORFV can cause significant detrimental impact to livestock industries and trade.

CASE HISTORY

Two epithelium tissues (case ID: VRI-006507-2018) were presented to the Veterinary Research Institute, Ipoh for disease diagnosis by the Bukit Tengah Regional Veterinary Laboratory, Penang. The samples was taken from two male Jamnapari crossed goats and is suspected for contagious ecthyma. The animals were from a private goat farm located in Kampong Tasek Cempedak, Simpang Ampat, Penang. The general aim of this study was to identify the causative agent. Tissue samples were minced using sterile scissors and forceps, then homogenised with sterile pestle in a mortal with sterile sand and phosphate buffer saline supplied with faetal calf serum and antibiotics (penicillin, streptomycin and kanamycin). The homogenate was then centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected and stored at -20 °C for DNA extraction.

Molecular diagnosis

DNA was extracted using NucleoSpin® Virus kit (Macherey-Nagel GmbH & Co.KG, Germany) according to the manufacturer's recommendation and further stored at -20 °C. Detection of the ORFV was through polymerase chain reaction (PCR) using high-GC pan chordopoxviruses universal primers based on Li *et al.* (2010). The high-GC pan

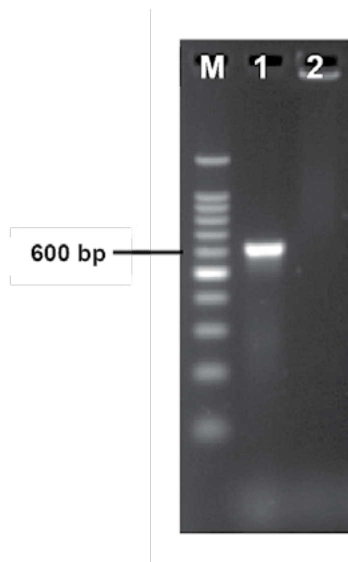

Figure 1. Agarose gel (1.5%) electrophoresis of ORFV amplified with high-GC pan chordopoxviruses universal primers by PCR. Lane M: 100 bp DNA ladder (Promega, USA). Lane 1: VRI-006507-2018, Lane 2: no template control.

chordopoxviruses universal primers were designed and proven to amplify the known high-GC content poxviruses (parapoxvirus, molluscum contagiosum virus, and crocodilepox virus strain Zimbabwe). The primer sequences are: ORF99 forward primer (5'CAT CCC CAA GGA GAC CAA CGA G3') and ORF99 reversed primer (5'TCC TCG TCG CCG TCG AAG TC3') which amplified amplicon with size about 630 bp. The PCR mixtures contained 1× GoTaq® Green Master Mix (Promega, USA), 5 µl of DNA isolated from the infected tissue and 0.2 µM of each primer that run in a final volume of 25 µL. The PCR thermal profile includes: 98 °C for 2 min as an initial activation step, followed by 30 cycles (98 °C, 10 s; 59 °C, 20 s; 72 °C, 30 s) and one cycle of 72 °C for 2 min, run on MyCycler™ thermal cycler (Biorad, USA). The PCR amplicon was gel electrophoresed on a 1.5 % agarose gel at 100 V for 45 min and visualised with Omega Lum™ G imaging system (Gel Company, USA).

A single band with approximately 630 bp was amplified (Figure 1), corresponding to the expected size of the primers set. The amplicon was sent to Apical Scientific Sdn. Bhd. for DNA sequencing using Sanger sequencing. The nucleotide sequences obtained were analysed with NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) to find regions of

Table 1: Nucleotide similarity between VRI-006507-2018 with others ORFV strains retrieved from GenBank

Strain	GenBank Accession	Species	Origin	Year	Nucleotide similarity (%)
SJ1	KP010356.1	goat	Fujian, China	2012	99.8
SY17	MG712417.1	sheep	Songyuan, China	2016	99.6
B029	KF837136.1	sheep-human*	Germany	1996	99.6
Marseille-2011	JQ596637.1	lamb-human*	France	2011	99.6
D1701	HM133903.1	sheep	Germany	no info	99.6
OV-IA82	AY386263.1	sheep	Iowa, USA	1982	99.6
NZ2	DQ184476.1	sheep	New Zealand	no info	99.6
NP	KP010355.1	goat	Fujian, China	2011	99.4
VA2010910054J	KF830861.1	sheep	Virginia, USA	2010	99.4
GO	KP010354.1	goat	Fujian, China	2012	99.2
YX	KP010353.1	goat	Fujian, China	2012	99.2
NA17	MG674916.2	goat	Fujian, China	2016	98.9
OV-HN3/12	KY053526.1	sheep	China	2012	98.9
NA1/11	KF234407.1	sheep	China	2011	98.9
OV-SA00	AY386264.1	goat	Texas, USA	no info	98.9

*Animal origin human isolates

similarity between our sequence to other archived sequences. At nucleotide level, VRI-006507-2018 is 99% identical to few ORFV strain isolated from goat, sheep and animal origin human isolates, confirming that VRI-006507-2018 is an ORFV (Table 1).

DISCUSSION

In this case report, an ORFV infection was molecularly diagnosed in goats from Penang. The result indicates that VRI-006507-2018 is closely related to ORFV strains isolated from goats and sheep which originated mainly from China (Table 1), in accordance to a previous study in Malaysia (Abdullah *et al.,* 2015). The availability of sequence data is crucial for the characterisation of the virus and the understanding of its epidemiology. Overall, our finding served as a baseline data and can contributes to future works in understanding the current Malaysian ORFV circulation and the disease burden in small ruminant.

Virus isolation through cell culture is regarded as the gold standard for detection of poxviruses (Chan *et al.*, 2007). An attempt to isolate the ORFV with cell culture was conducted in this study but unfortunately it was not successful. In general, parapoxvirus cultivation in cell culture is regarded difficult as success rate is low (Suzuki *et al.,* 1993). Nonetheless, molecular diagnosis through PCR for detection of the viral DNA and subsequent DNA sequencing for determining the identity of the encoded nucleotide sequence is particularly useful for rapid disease diagnosis. These methods have successfully diagnosed ORFV from field specimen samples (Chan *et al.,* 2007; Li et al., 2010; Zhao *et al.*, 2010; Abdullah *et al.*, 2015), proven to be rapid and accurate without the need for cell culture and positive isolate as seen in this case.

ORFV causes generalised or localised cutaneous lesions (Zhao *et al.*, 2010; Spyrou and Valiakos, 2015). Although ORFV infection is recognisable to most, however, it is often under-reported. In Malaysia, there are only four cases of ORFV reported so far (Zamri-Saad *et al.,* 1992; Bala *et al.,* 2018). As such, the limited records on the outbreak of ORFV infection coupled with its low severity and mortality, results in less attention being given to the impact of this infection. Apart from reporting the incidence of ORFV infection, this case report is of significance to human health as well due to its zoonosis factor. Given that VRI-006507-2018 is highly homologous with human isolates (Table 1), highlighting the need for further study into the prevalence of ORFV infection in both livestock and human.

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