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Identification and Molecular characterization of Orf virus from Goats of Thanjavur District of Tamilnadu

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ABSTRACT: Contagious pustular dermatitis in goats as often referred as Orf or Scabby mouth being a viral etiology is described to be highly contagious and causing economic losses to the animal husbandry sector. Thanjavur District of Tamilnadu being an agrarian background and depend mostly on livestock and orf like contagious disease pose a serious threat at times affecting their livelihood which has been addressed by earlier and rapid diagnosis .Twenty one number of Orf suspected cases from three flocks of goats in Thanjavur District (Cauvery Delta Region, Tamilnadu) exhibited skin lesions such as erythema and pustules with crusty areas over the muzzle region and perianal areas. The scab material was collected from severely affected goats showing characteristic symptoms and subjected to viral DNA extraction and subsequently the conserved B2L gene was amplified by polymerase chain reaction. The PCR test carried out showed positivity of B2L gene in nineteen samples confirming the Orf virus. As a result, the PCR technique was found to be a swift tool for diagnosis which proved to help in advocating measures for further curtailment of Orf among goats in Cauvery Delta Region of Tamilnadu.

Keywords: Contagious ecthyma - Goat - Orf virus - B2L gene – PCR.

INTRODUCTION

Orf or Contagious ecthyma being a contagious viral disease and has been recorded throughout the world in goats and sheep (Mondal *et al*., 2006). The Orf virus (ORFV) affects mostly the epithelium of goat and it is a double-stranded DNA virus belonging to the genus Parapoxvirus (PPV) and family Poxviridae (Delhon *et al.,* 2004; Fleming *et al.,* 2015). The zoonotic nature of Orf has also been recorded among the animal stake holders and field practising veterinarians (Essbauer *et al.,* 2010; Nandi *et al.,* 2011). Nandi *et al.* (2011) reported that orf is a self-limiting disease and can be treated by symptomatic therapy along with topical dressing and antiseptics which is more beneficial for reducing the viral load in affected goats and administration of topical and systemic antibiotics can be used for treatment and management of the disease. Vaccination and strict biosecurity measures prevents occurrence of contagious ecthyma (Nettleton *et al.,* 1996). Outbreaks in India during the periods of late summer and winter was reported by Venkatesan *et al.* (2012). Lesions are confined to muzzle and lips of the affected kids and goats. In severe cases lesions were on skin of the eyes, feet, vulva, udder, and scrotum (De La Concha-Bermejillo *et al.,* 2003). Contagious ecthyma complicated with bronchopneumonia due to secondary bacterial complications are difficult to treat (Nandi *et al.,* 2011). The disease can be diagnosed based on the characteristic lesions and in more severe complicated cases confirmation by serological and nucleic acid-based techniques are essential (Venkatesan *et al.,* 2012). Contagious ecthyma was identified by clinical signs and confirmation was done by using polymerase chain reaction (PCR) (Peralta *et al*., 2018, Tedla *et al*., 2018). Polymerase chain reaction based on B2L gene-specific primers of contagious ecthyma was used for the confirmatory diagnosis of contagious ecthyma in sheep and goats by several researchers (Hosamani *et al*., 2006; Li *et al*., 2012; Ahanger *et al*., 2018; Venkatesan *et al*., 2018; Tedla *et al*., 2018). Contagious ecthyma virus outbreak among West African dwarf goats was also reported (Adedeji *et al*., 2017). Polymerase chain reaction differentiates orf virus from other pox viruses

such as sheep pox and goat pox (Kottaridi *et al.,* 2006; Hosamani *et al.,* 2009). The B2L gene of orf virus encodes the major envelop protein, which is highly immunogenic was used for the confirmation of the virus (Chan *et al.,* 2009; Friederichs *et al.,* 2014; Zhang *et al.,* 2014; Gelaye *et al.,* 2016). Hence for rapid confirmation of Orf in goats the work was carried out with three flocks of goats suspected for Orf viral infection in Thanjavur District of Tamilnadu.

MATERIALS AND METHODS

Sample collection. An outbreak of pox like disease was reported in three different goat flocks in Thanjavur region of Tamil Nadu. The non-descript goats maintained in open pasture system were examined for Orf viral infection and the clinical signs were recorded. One third of animals showed anorexia, dullness, cough, dyspnea and presence of crusty mucopurulent yellow nasal discharge with a rise in body temperature. Multiple discrete edematous nodular lesions with crust formation on the lips of affected animals were also observed. The skin scab samples were collected in 50 % glycerol saline and transported on ice to the laboratory for confirmative diagnosis. The samples were stored at -40^{o}C for further processing.

DNA extraction and Polymerase chain reaction. The scab samples were homogenized in phosphate buffered saline to produce 20% tissue suspension. DNA extraction was carried out using QIAamp® DNA Mini kit from (QIAGEN, Germany) as per the manufacturer's instructions. B2Lgene specific forward and reverse primers were used to identify the orf virus (Hosamani *et al.* 2006) as shown in Table 1.

The PCR was carried out by using 200µl capacity thin wall PCR tubes with a final volume of 20µl. A reaction mixture was prepared as per the Table 2. The template concentration was adjusted to 100ng/µl. The PCR tubes containing the mixture was tapped gently and spun briefly. The PCR tubes with all the components were transferred to thermal cycler (Eppendorf). The PCR cyclic conditions are followed according to the recommendations of Gelaye *et al*. (2016) with slight modification as shown in Table 3.

Table 1: B2Lgene specific forward and reverse primers used for orf virus detection.

Sequence of specific primers used for amplification of B2L gene of CEV		
ORFVB2LF1	5-TCCCTGAAGCCCTATTATTTTTGTG-3	Expected amplicon size - 1137bp
ORFVB2LR1	5-GCTTGCGGGCGTTCGGACCTTC-3	

Table 2: Composition of reaction mixture for PCR amplification of CEV genes.

Sr. No.	Components	Quantity (µl)
1.	Taq master mix red – (2x) (Amplicon, USA)	10.0
2.	Forward primer (10pmol/µl)	1.0
3.	Reverse primer (10pmol/µl)	1.0
4.	Template DNA	5.0
5.	Nuclease free water	3.0
Total		20.0

Table 3: Steps and conditions for B2L gene amplification.

Sr. No.	Steps	Temperature	Time
1.	Initial denaturation	95^{o}C	5 min.
2.	Denaturation	95^{o}C	50 sec.
3.	Annealing	56^{o}C	60 sec.
4.	Extension	72^{o}C	90 sec.
5.	Step 2-4 for 35 cycles		
6.	Final extension	72^{o}C	7 min.
7.	Holding temperature	4^{o}C	Hold

The amplified products were analyzed by electrophoresis on a 1.2% agarose gel containing 0.5 ng/ml ethidium bromide in Tris-acetate-EDTA (TAE) buffer (Lawal *et al.* 2017). The amplicons were viewed using a GelDoc imaging system (BioRad, CA, USA).

RESULTS AND DISCUSSION

Contagious pustular dermatitis is likely to be an increasingly important health issue among goats because vaccination is currently unavailable and the disease may reduce the marketable weight of the live animal. The disease usually affects young animals and is easy to diagnose when the lesions are confined to lips, muzzle and teats. However, the clinical diagnosis may become complex when the disease is more severe and lesions are present in the atypical locations and sometimes confusing with goat pox. The clinical signs seen in the goats included multifocal to coalescing ulcerated lesions in the epidermis of the muzzle and lips (Fig. 1). No visible lesions were observed in other locations.

Scab samples were found positive for contagious ecthyma virus by the polymerase chain reaction was also reported by Gelaye *et al*. (2016). The disease causes morbidity up to 100% and the mortality between 5%-15% (Housawi *et al.,* 1991; Constable *et al.,* 2017), however in the present incidence it was found that the morbidity in two flocks is 30 percent and in other flock 16 per cent and no mortality in all

the three farms, which was lower than the previous reports. Low morbidity and mortality might be due to early detection and culling of the affected animals with adaptation of proper biosecurity measures. The present outbreak was recorded in 7 months to two years aged goats during the late summer as described by other researchers (Bouznach *et al.,* 2013; Maan *et al.,* 2014).

Diagnosis based on pathologic examinations and clinical signs are inaccurate and virus isolation is thought to be standard procedure, but it is a time-consuming one (Chan *et al.,* 2007). Nowadays, the PCR technique has become widely used to amplify the desired genomic fragments from specimens and become an important technique to diagnose and to differentiate orf virus infection in field specimens (Inoshima *et al.,* 2000). To confirm the causative agent B2L gene was amplified in this study. The expected PCR fragments, approximately 1137 bp in length, were obtained from DNA which had been extracted from the lesions (Fig. 2) and this result is in accordance with Zhang *et al.* (2010).

Fig. 1. Goat - Orf lesions.

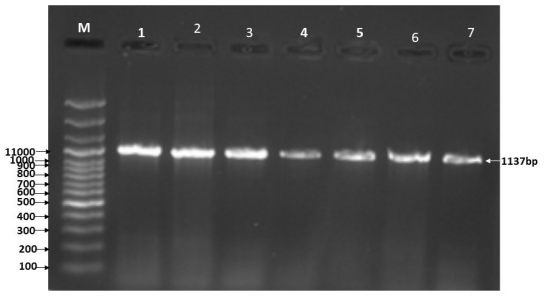

Fig. 2. 1137bp amplified product (Goat-Orf).

CONCLUSIONS

From the present investigation it is concluded that the skin lesions in goat were caused by orf viruses (ORFV) and confirmed by molecular method such as Polymerase chain reaction and the challenges posed by sudden occurrence of orf in goats in Thanjavur region was controlled successfully by application of early interventions by separation and treatment of ailing animals and advocation of biosecurity measures in the farm.

FUTURE SCOPE

In the epidemiological point of view, additional sequence analysis and functional assays of various immunomodulatory protein genes of the orf virus needs to be conducted for the purpose of developing suitable control measures in future.

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Conflict of Interest. None.

REFERENCES

Adedeji, A. J., Maurice, N. A., Wungak, Y. S., Adole, J. A., Chima, N.C., Woma, T. Y., Chukwuedo, A. A. and Shamaki, D. (2017). Diagnosis of orf in West African dwarf goats in Uyo, Akwa Ibom state, Nigeria. *Afr. J. Infect. Dis., 11*(2), 90-94.

Ahanger, S.A., Parveen, R., Nazki, S., Dar, Z., Dar, T., Dar, K.H., Dar, A., Rai, N. and Dar, P. (2018). Detection and phylogenetic analysis of orf virus in Kashmir Himalayas. *Virus disease, 29*(3), 405-410.

Bouznach, A., S. Hahn, Y. Stram, S. Menasherov, N. Edery, N. Shicaht, G. Kenigswald and Perl, S. (2013). Case Report: Contagious Ecthyma - Deviations in the Anatomically Appearance of Lesions in an Outbreak in Lambs in Israel. *Israel Journal of Veterinary Medicine, 68* (4), 246 – 251.

Chan, K. W., Lin, J. W., Lee S. H., Liao, C. J., Tsai, M. C., Hsu, W. L., Wong, M.L. and Shih, H. C. (2007). Identification and phylogenetic analysis of orf virus from goats in Taiwan**.** *Virus Genes 35,* 705-712.

Chan, K. W., Yang, C. H., Lin, J. W., Wang, H. C., Lin, F. Y., Kuo, S. T., Wong, M. L. and Hsu, W. L. (2009). Phylogenetic analysis of parapoxviruses and the C-terminal heterogeneity of viral ATPase proteins. *Gene*, *432*(1-2), 44–53.

Constable, P. D., Hinchcliff, K. W., Done, S. H. and Grunberg, W. (2017). A textbook of the diseases of cattle, horses, sheep, pigs and goats, 11th edn, Saunders Elsevier, Edinburgh London.

De La Concha-Bermejillo, A., J. Guo Z., Zhang and Waldron D. (2003). Severe persistent orf in young goats. *J. vet. Diagn. Invest., 15*, 423-431.

Delhon, G., Tulman, E. R., Afonso, C. L., Lu, Z., De la Concha-Bermejillo, A., Lehmkuhl, H. D., Piccone, M. E., Kutish, G. F. and Rock, D. L. (2004). Genomes of the parapox viruses orf virus and bovine popular stomatitis virus. *Journal of Virology, 78*(1), 168–177.

Essbauer, S., Pfeffer, M. and Meyer, H. (2010). Review Zoonotic poxviruses. *Veterinary Microbiology, 140*(1), 229–236.

Fleming, S. B., Wise, L. M. and Mercer, A. A. (2015). Molecular genetic analysis of orf virus: A poxvirus that has adapted to skin. *Viruses*, *7*(3), 1505-1539.

Friederichs, S., Krebs, S., Blum, H., Wolf, E., Lang, H., Von Buttlar, H. and Büttner, M. (2014). Comparative and retrospective molecular analysis of parapoxvirus (PPV) isolates. *Virus Research*, *181*, 11–21.

Gelaye, E., Achenbach, J. E., Jenberie, S., Ayelet, G., Belay, A., Yami, M., Loitsch, A., Grabherr, R., Diallo, A. and Lamien, C. E. (2016). Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008–2013. *Virol. J., 13*, 34.

Hosamani, M., Scagliarini, A., Bhanuprakash, V., McInnes, C. J. and Singh, R. K. (2009). Orf: An update on current research and future perspectives. *Expert Review of Anti-infective Therapy*, *7*(7), 879–893.

Hosamani, M., Bhanuprakash, V., Scagliarini, A. and Singh, R. K. (2006). Comparative sequence analysis of major envelope protein gene (B2L) of Indian Orf viruses isolated from sheep and goats. *Vet. Microbiol., 116*(4), 317-324.

Housawi, F. M., E. M. E., Abu Elzein, M. M., Amin Al and Afaleq, A. I. (1991). Contagious pustular dermatitis (orf) infection in sheep and goats in Saudi Arabia. *Veterinary Record 128,* 550–551.

Inoshima, Y., A. Morooka, H. and Sentsui, H. J. (2000). Detection and diagnosis of parapoxvirus by the polymerase chain reaction. *J. Virol. Methods, 84,* 201-208.

Kottaridi, C., Nomikou, K., Lelli, R., Markoulatos, P. and Mangana, O. (2006). Laboratory diagnosis of contagious ecthyma: Comparison of different PCR protocols with virus isolation in cell culture. *Journal of Virological Methods*, *134*(1-2), 119–124.

Lawal, N., Hair-Bejo, M., Arshad, S. S., Omar, A. R. and Ideris, A. (2017). Adaptation and molecular characterization of two Malaysian very virulent infectious bursal disease virus isolates adapted in BGM-70 cell Line. *Adv. Virol.,* (1–19).

Li, W., Ning, Z., Hao, W., Song, D., Gao, F., Zhao, K., Liao, X., Li, M., Rock, D. L. and Luo, S. (2012). Isolation and phylogenetic analysis of orf virus from the sheep herd outbreak in northeast China. *BMC Vet. Res., 8*(1), 229.

Maan, S., Kumar, A., Batra, K., Singh, M., Nanda, T., Ghosh, A. and Maan N. S. (2014). Isolation and molecular characterization of contagious pustular dermatitis virus from Rajasthan, India. *Virus Dis., 25*(3), 376–380.

Mondal, B., Bera, A. K., Hosamani, M., Tembhurne, P. A., Bandyopadhyay, S. K. (2006). Detection of Orf virus from an outbreak in goats and its genetic relation with other parapoxviruses. *Vet. Res. Commun*., *30*, 531–539.

Nandi, S., Ujjwal, K. D. and Chowdhury, S. (2011). Current status of contagious ecthyma or Orf disease in goat and sheep—A global perspective. *Small Ruminant Research, 96* (1-2), 73–82.

Nettleton, P. F., J. Brebner, I., Pow, J. A., Gilray, G. D., Bell and Reid, H. D. (1996). Tissue culture-propagated orf virus vaccine protects lambs from orf virus challenge. *Veterinary Record, 138,* 184-186.

Peralta, A., Robles, C. A., Micheluod, J. F., Rossanigo, C. E., Martinez, A., Carosio, A. and König, G. A. (2018) Phylogenetic analysis of orf viruses from five contagious ecthyma outbreaks in Argentinian goats. *Front. Vet. Sci.*, *5* (6), 134.

Tedla, M., Berhan, N., Molla, W., Temesgen, W. and Alemu, S. (2018). Molecular identification and investigations of contagious ecthyma (orf virus) in small ruminants, North West Ethiopia. *BMC Vet. Res., 14*(1), 13.

Venkatesan, G., De, A., Arya, S., Kumar, A., Muthuchelvan, D., Debnath, B. C., Dutta, T. K., Hemadri, D. and Pandey, A. B. (2018). Molecular evidence and phylogenetic analysis of orf virus isolates from outbreaks in Tripura state of North-East India. *Virus disease, 29*(2), 216-220.

Venkatesan, G., Bhanuprakash, V., Balamurugan, V., Bora, D. P., Prabhu, M., Yogisharadhya, R. and Pandey A. B. (2012). Rapid detection and quantification of Orf virus from infected scab materials of sheep and goats. *Acta virologica, 56*, 81-83.

Zhang, K., Liu, Y., Kong, H., Shang, Y. and Liu, X. (2014). Comparison and phylogenetic analysis based on the B2L gene of orf virus from goats and sheep in China during 2009-2011. *Archives of Virology*, *159*(6), 1475–1479.

Zhang, K., Lu, Z., Shang, Y., Zheng, H., Jin, Y., Jijun, H. and Liu, X. (2010). Diagnosis and phylogenetic analysis of Orf virus from goats in China: a case report. *Virol., J., 7*, 78.

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