Veterinary Microbiology 142 (2010) 408–415

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Identification and phylogenetic analysis of an Orf virus isolated from an outbreak in sheep in the Jilin province of China

Kui Zhao [a,1], Deguang Song [a,1], Wenqi He [a,*], Huijun Lu [b], Bingbing Zhang [a], Chao Li [c], Keyan Chen [a], Feng Gao [a,**]

[a] College of Animal Science and Veterinary Medicine, Jilin University, 5333 Xi'an Road, Changchun 130062, China
[b] Key Laboratory of Zoonosis, Ministry of Education, Institute of Zoonosis and Animal Research Center, Jilin University, Changchun 130062, China
[c] College of Animal Science, Jilin Agricultural University, Changchun 130118, China

ARTICLE INFO

Article history:
Received 18 June 2009
Received in revised form 26 September 2009
Accepted 2 October 2009

Keywords:
Orf virus
Small-tailed Han sheep
ORFV 011(B2L) gene
ORFV 059(F1L) gene
Phylogenetic analysis

ABSTRACT

This study investigated an acute outbreak of contagious ecthyma dermatitis that occurred in November 2008 in a herd of 180 small-tailed Han sheep in the Jilin province of China. The pathological findings of this case revealed severe vascular proliferation, viral cytopathic changes in keratinocytes by vacuolar degeneration, ballooning degeneration, and eosinophilic cytoplasmic inclusions, suggesting that the disease could have been caused by an Orf virus (ORFV) infection. Immunohistochemistry, indirect immunofluorescence (IFA), and transmission electron microscopy supported the diagnosis of ORFV infection. Finally, the pathogen of the disease was identified using polymerase chain reaction (PCR) and sequences of major envelope protein genes (ORFV 011(B2L) and ORFV 059(F1L)). The full-length ORFV 011(B2L) and ORFV 059(F1L) genes were cloned and sequenced. The nucleotide and amino acid sequences of ORFV 011(B2L) and ORFV 059(F1L) genes in these outbreaks were analyzed, and their phylogenetic trees were constructed. The phylogenetic studies of ORFV 011(B2L) genes and ORFV 059(F1L) genes showed that the ORFV-Jilin province isolate clustered in different branches and was closer to the ORFV-Mukteswar 67/04 isolate and the ORFV-OV/C2 isolate, respectively. To date, there is no report on the molecular characterization of any Orf virus with other isolates around the world in mainland China. Further, the above results may provide some insight into the genotype of the etiological agent responsible for the contagious ecthyma dermatitis outbreak in the Jilin province, and could also provide a comparative view of the central coding region genomics of parapoxvirus (PPV).

1. Introduction

Parapoxviruses (PPVs) represent one of the eight genera within the chordopoxvirus (ChPV) subfamily of the *Poxviridae*, which include the Orf virus (ORFV), the bovine papular stomatitis virus (BPSV), pseudocowpoxvirus (PCPV), PPV of red deer in New Zealand (PVNZ) and PPV of the grey seal (Nettleton et al., 1995; Robinson and Mercer, 1995; Mercer and Haig, 1999; Becher et al., 2002). PPVs are epitheliotrophic viruses affecting a wide range of species including sheep, goats, camels, red deer, reindeer, squirrels, seals, grey seals, Weddell seals, musk ox, and PPV isolates can cause non-systemic, eruptive skin disease (Yeruham et al., 1994). The viruses are sometimes transmissible to humans through direct contact (Robinson and Balassu, 1981), and diseases caused by PPV have been reported in many countries including North and South

* Corresponding author. Tel.: +86 431 87835376; fax: +86 431 87836150.
** Corresponding author.
E-mail addresses: hewq@jlu.edu.cn (W. He), gf_jldxnxb@yahoo.com.cn (F. Gao).
[1] These authors contribute equally to the manuscript.

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doi:10.1016/j.vetmic.2009.10.006

America, New Zealand, Finland, Germany, Norway, Japan, Italy, the United Kingdom, Western Australia, South Asia and, recently, Antarctica. The Orf virus is the prototype species of the *Parapoxvirus* genus, and it primarily causes contagious ecthyma in goats, sheep, and other ruminants worldwide (Glover, 1928; Haig and Mercer, 1998; Büttner and Rziha, 2002).

Contagious ecthyma, also known as Orf, contagious pustular dermatitis, infectious labial dermatitis, scabby mouth, or sore mouth, is a disease with a worldwide distribution (De la Concha-Bermejillo, 1995; Haig and Mercer, 1998). The disease is characterized by proliferative and self-limiting lesions around the muzzle and lips (scabby mouth) of infected animals, and sometimes also affects the gums and tongue, especially in young lambs. Feet, teats and eyelids can be affected occasionally. Rarely, the lesions may extend into the esophagus, stomach, intestines, or respiratory tract. The disease usually lasts for 3–4 weeks and resolves in 1–2 months. The disease has a very high morbidity; although mortality is rare and usually does not exceed 10%, mortality rates of up to 10% and 93% have been reported in lambs and kids (Mazur and Machado, 1990, 1998; Gumbrell and McGregor, 1997). The disease is frequently severe enough to create substantial welfare problems in flocks (Robinson and Balassu, 1981). This in turn has an economic impact on sheep farmers due to decreases in production.

Orf virus infections are found ubiquitously wherever sheep and goats are farmed in China, but infections are only occasionally reported, probably because of its low severity and low economic consequences. In this research, we studied an outbreak of Orf virus infection in sheep and verified the identity of the virus by PCR. The full-length ORFV 011(B2L) and ORFV 059(F1L) genes of the Orf virus isolated from sheep in the Jilin province were cloned and sequenced. This is the first phylogenetic analysis of the Orf virus isolated in mainland China in comparison with other isolates from around the world.

2. Materials and methods

2.1. Disease outbreaks

The natural outbreak of the Orf virus among sheep occurred in a farm with 180 small-tailed Han sheep for meat production, including 120 breeders and 60 lambs at the age of 1–6 months old, in the Jilin province of China during November 2008. Of the 60 lambs in the flock, 25 developed proliferative lesions around the mouth, including all the 12 lambs purchased from a nearby farm before 20 days. In young kids, lesions appeared on the junctions of the lips, the muzzle, as well as on tongue. Lesions were nodular, varying in size between approximately 3 and 8 mm. There was slight increase in the body temperature in infected lambs. Two of the affected 6-week-old lambs died due to inanition owing to suckling difficulty caused by the severe mouth lesions found in this age group. The morbidity and the mortality of the disease were 25 in 180 (13.9%) and 2 in 180 (1.1%), respectively. In the 60 lambs, the morbidity and the mortality of the disease were 41.7% and 3.3%, respectively. Among 25 infected animals, 23 recovered 12–18 days after clinical signs first appeared.

2.2. Necropsy and morphological examination

Biopsies of the skin and lip of affected sheep were sent for pathologic evaluation. Biopsy samples collected from the submitted carcasses were fixed in 10% buffered formalin for histological examination or were un-fixed for electron microscopic evaluation, for virus isolation, and for PCR analysis. Necropsies were performed on the brain, heart, liver, spleen, lungs, kidney and skin. Organ samples of the carcasses were fixed as above. The tissue samples were trimmed, dehydrated, embedded in paraffin, stained with hematoxylin and eosin (H&E) and examined with light microscopy.

2.3. Immunohistochemical detection

Briefly, 4-μm thick sections were obtained, placed on silanized slides, deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked by 3% (v/v) H_2O_2, and the antigen was retrieved by microwave in citrate buffer (10 mM, pH 6.0) at 98 °C for 15 min. The primary antibody used was rabbit anti-ORFV polyclonal antibody (kindly provided by the Jilin Institute for Veterinary Research, diluted at 1:100). The biotinylated secondary antibody was used at a dilution of 1:5000. An avidin–biotin-peroxidase complex was used to reveal antigen–antibody reaction. Staining was achieved using diamino benzidine (DAB) as a peroxidase substrate (Maixin Biotechnology Company, Fujian, China). Subsequently, slides were counterstained with hematoxylin. The primary antibody was replaced with normal rabbit serum as a negative control.

2.4. Indirect immunofluorescence assay (IFA)

The scab specimens collected from skin lesions were triturated in 0.01 M PBS. The homogenized samples were then centrifuged at 3000 r/min for 20 min at 4 °C. The clarified supernatants were passed through 0.45 μm filters and used to inoculate a confluent monolayer of Madin–Darby bovine kidney (MDBK) cells grown on 6-well cell culture plates (Costor, corning incorporated, USA) in MEM supplemented with 10% fetal bovine serum (FBS) and penicillin (100 U/ml); streptomycin (100 μg/ml); and nystatin (20 μg/ml). Incubated cells were kept in a CO_2 incubator supplying 95% air and 5% CO_2. Normal cell controls were maintained in a similar manner. The cells were observed daily with an inverted microscope for any cytopathic effects (CPE). The cells showing CPE were subjected to an indirect fluorescence antibody (IFA) test employing rabbit anti-ORFV polyclonal antibody (kindly provided by the Jilin Institute for Veterinary Research, diluted at 1:100). The secondary antibody used was FITC-conjugated goat anti-rabbit IgG (1:500 dilution in Evans Blue). After immunostaining, the normal control and inoculated cells were observed under a fluorescence microscope.