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Pathogenicity of blood orf virus isolates in the development of dairy goat contagious pustular dermatitis

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ABSTRACT

Contagious pustular dermatitis is an exanthematous zoonotic disease caused by the orf virus. Pandemic outbreaks of this disease cause great economic losses, while the pathogenesis of this disease still remains obscure. In this study, blood samples were collected from 628 asymptomatic goats across China for PCR-based virus detection. We detected the orf virus in the blood of asymptomatic goats. Moreover, the orf virus obtained from the blood of infected goats was infectious and induced typical symptoms of contagious pustular dermatitis after inoculation of uninfected dairy goats. In summary, our data provide evidence that asymptomatic animals may be carriers of orf virus. Our findings should contribute to elucidating the details underlying the pathogenesis of contagious pustular dermatitis.

1. Introduction

Pustular dermatitis is an acute, contagious epithelial zoonotic infectious disease (Guo et al., 2003) that has been reported in various animals, such as goats, sheep, several other small ruminants and humans (Hasheminasab et al., 2016; Zhang et al., 2014). Contagious pustular dermatitis is characterized by vesicles, papules and crusty and rapidly growing scabs on the lips and nose of infected animals (Abrahao et al., 2009; Zhao et al., 2010). Symptoms usually persist for three to four weeks (Lojkic et al., 2010) and the disease exhibits a high morbidity rate and low mortality rate (Guo et al., 2003). Infections of sheep and goats are usually self-limiting (Guo et al., 2003). However, if viral infection is accompanied by a bacterial co-infection, such as *staphylococci*, *streptococci* or *corynebacteria*, the mortality rate can approach 90% (Fleming et al., 2017; Gelaye et al., 2016). In China, this disease has been reported in several provinces, including Gansu, Qinghai and so on (Yu et al., 2017; Zhang et al., 2016; Zhao et al., 2010).

The orf virus, a member of the genus *Parapoxvirus* of the family *Poxviridae*, causes contagious pustular dermatitis. The genome of the orf virus is a linear double-stranded DNA molecule with a G + C content as high as 64% (Wang et al., 2016) and a genome length of 138 kb (Chi et al., 2015). *B2L* and *VIR* are conserved genes (Friederichs et al., 2014; Yogisharadhya et al., 2017). Therefore, *B2L* and *VIR* of orf virus are used as markers for orf virus detection.

In previous studies, diagnosis of orf virus infection usually was based on clinical symptoms, serological results and DNA-based detection of the virus in pathological tissues (Ou et al., 2016; Yang et al., 2016; Yang et al., 2015). However, orf virus detection in asymptomatic animals has been rarely investigated. In the present study, we found that orf virus isolated from blood could serve as a novel predictor of contagious pustular dermatitis in dairy goats. In addition, we further established an effective method for the detection of orf virus in asymptomatic animals.

2. Materials and methods

2.1. Study subjects for epidemiological investigation of orf prevalence

A total of 628 asymptomatic dairy goats were included in this study. The goats originated from the following six areas of China: Qingdao, Kunming, Yulin, Yangling, Shilin and Hengshan within Shandong, Yunnan and Shaanxi provinces. All blood samples were collected from goats exhibiting no clinical proliferative dermatitis symptoms for at least 3 months. All samples were preserved by the addition of anticoagulant and analyzed by PCR.

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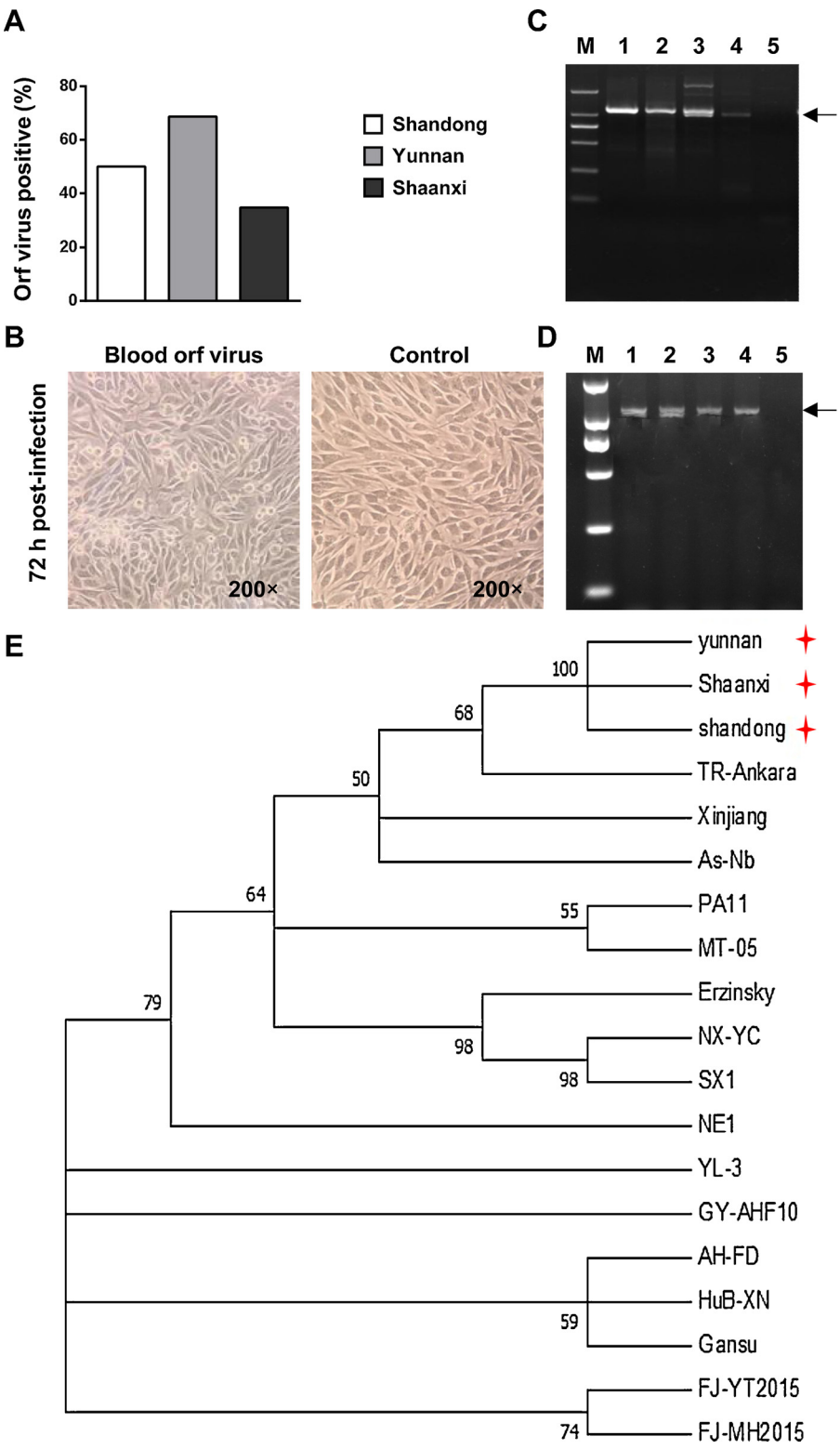

Fig. 1. Verification of orf virus isolated from the blood of asymptomatic dairy goats. (A) The prevalence of blood orf virus varied among provinces. Prevalence rates of viral infection in Shandong, Yunnan and Shaanxi were 50.0%, 68.7% and 34.7%, respectively, with a total prevalence of 47.8%. (B) Isolated blood orf virus was inoculated into cultures of bovine testicular epithelial cells. Cytopathic characteristics of cells after infection are visible from the rounding up of cells, loss of cell adherence to a flask, and other morphological changes. CPE was observed and photographed at 72 hpi at a magnification of 200×. (C) PCR amplification of *B2L* from the viral genomic DNA extracted from bovine testis epithelium cells infected by orf virus originated from Yunnan, Shandong and Shaanxi. The marker used was the DL2000 DNA marker. The amplicon length was 1137 bp, as indicated by an arrow. (D) PCR amplification of *B2L* from viral genomic DNA extracted from scabs that were derived from the control group and orf virus group as described at materials and methods. The amplification length was 1137 bp as indicated by an arrow. M: DL2000 DNA marker. Lane 1: DNA extracted from scabs of NO. 1; Lane 2: DNA extracted from scabs of NO. 2; Lane 3: DNA extracted from scabs of NO. 3; Lane 4: DNA extracted from scabs of NO. 4 ; Lane 5: DNA extracted from scabs of NO. 5 (the control group). (E) Phylogenetic analysis based on the nucleotide sequence of the complete *B2L* gene. The phylogenetic tree was constructed using the neighbor-joining algorithm using MEGA7 with bootstrap analysis performed based upon 1000 trials. All sequences were collected from NCBI. The red star indicates Shaanxi, Yunnan and Shandong isolates in this study (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

2.2. Virus isolation

Virus detection was performed by analyzing the blood of all goats and tissue samples (buccal mucus, whey, urine and fecal samples) of symptomatically-infected goats. For tissue samples, purtenances were washed three times with PBS and homogenized in 50% w/v PBS using a tissue mortar. Both orf-positive blood samples and tissue homogenates from the orf-infected goats were freeze-thawed three times and clarified by centrifugation at 8000 rpm for 15 min. After adding an equal volume of PBS and passing diluted supernatants through 0.22 μM filters, all blood sample filtrates and random various tissue sample filtrates from symptomatic animals were inoculated into cultures of bovine testicular epithelial cells. The bovine testicular epithelial cell is a primary cell line that is prepared and preserved in our lab as described before (Higaki et al., 2013).

Cell cultures were monitored daily for cytopathic effect (CPE) and