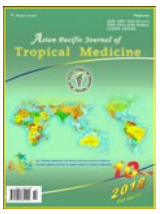

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Infection of Borna disease virus in healthy animals in northern China

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ABSTRACT Borna disease virus (BDV) is a non-segmented, single negative stranded RNA virus. It is highly neurotropic, but the infection is not cytopathic to the infected cells. BDV is widely infected in vertebrates, causing abnormal behavior and multiple pathological changes. Horses and sheep are the main natural hosts of BDV. Borna disease (BD) is characterized by progressive mononuclear encephalomyelitis. The BD clinical manifestations include ataxia, depression, rotation movement, mandatory standing, hitting objects with body and paralysis, and they usually last for 1-3 weeks. BDV infection causes a significant health problem in animals and economic loss for the society. However, BDV natural infection frequency in the raised animals in northern China, is poorly understood, but is required for understanding BDV infection epidemiology. BDV infection can be diagnosed through detecting viral proteins, antibodies and nucleic acid. The fluorescence based quantitative PCR is commonly used for BDV diagnosis because of the superior sensitivity. The assays for detecting plasma BDV specific antibodies include immunofluorescence, Western Blot and ELISA. Western Blot offers comparatively high specificity, which can detect different antibodies on the same blot/strip, and is often used as the confirmatory assay. This study investigated the infection prevalence of BDV in the meadow that raised horses and sheep in northern China. Blood samples were collected from a herd of healthy horses and sheep that were meadow raised in Hailin City, Heilongjiang Province, China. The uncoagulated blood samples were used to isolate peripheral lymphocytes and plasma. We selected Mongolian horses which are stocky, with relatively short but strong legs and a large head. The selected Han sheep is relatively small, has short tail and weighs 35-45 kg on average. BDV p40 mRNA was detected by Taqman based quantitative RT-PCR in lymphocytes of horses and sheep. BDV His-phosphoprotein (P) and His-nucleoprotein (N) fusion proteins were isolated with Histidine coated beads and the purity and immunological activity of the isolated viral proteins were assessed and verified by SDS-PAGE and Western Blot. Plasma BDV antibodies in horses and sheep were detected using the purified fusion proteins as antigens. Quantitative PCR assay showed that 50.00% (16/32) of the tested horse samples were positive for BDV p40 mRNA, and 20.83% (5/24) positive for BDV p40 mRNA in the sheep samples. The purified BDV His-P fusion protein in SDS-PAGE electrophoresis migrated at expected 24 kD size. A known specific BDV P antibody verified the immunogenicity of the purified BDV P in Western Blot, and BDV persistently infected human oligodendrocytes as positive control. The purified BDV His-N fusion protein appeared at the expected 40 kD position and the immunogenicity was also verified. The positive rates of BDV P antibody and N antibody were 11.11% (4/36) and 5.56% (2/36) in the tested horses, respectively. The positive rates of BDV P antibody and N antibody were 13.16% (5/38) and 7.89% (3/38) in the tested sheep, respectively. In conclusion, we utilized the expressed BDV His-P and His-N fusion proteins as antigen and established a modified Western Blot assay for detecting plasma BDV antibodies. Our data showed the direct evidence that natural BDV infection occurred in the meadow raised healthy horses and sheep in northern China.

Keywords: Borna disease virus; Horse; Sheep; Northern China

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