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Pathogenesis of a genotype C strain of bovine parainfluenza virus type 3 infection in albino guinea pigs

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ABSTRACT

Bovine parainfluenza virus type 3 (BPIV3) is one of the most important of the known viral respiratory tract agents of both young and adult cattle and widespread among cattle around the world. Up to present, three genotypes A, B and C of BPIV3 have been described on the basis of genetic and phylogenetic analysis and only limited studies on the pathogenesis of the genotype A of BPIV3 infection in calves and laboratory animals have been performed. The report about experimental infections of the genotypes B and C of BPIV3 in laboratory animals and calves was scant. Therefore, an experimental infection of guinea pigs with the Chinese BPIV3 strain SD0835 of the genotype C was performed. Sixteen guinea pigs were intranasally inoculated with the suspension of SD0835, while eight control guinea pigs were also intranasally inoculated with the same volume of supernatant from uninfected MDBK cells. The virus-inoculated guinea pigs displayed a few observable clinical signs that were related to the respiratory tract disease and two of the sixteen experimentally infected guinea pigs died at 2 and 3 days post inoculation (PI), respectively, and apparent gross pneumonic lesions were observed at necropsy. The gross pneumonic lesions in guinea pigs inoculated with SD0835 consisted of dark red, slightly depressed, irregular areas of consolidation in the lung lobes from the second to 9th day of infection at necropsy, and almost complete consolidation and atelectasis of the lung lobes were seen at 7 days PI. Histopathological changes including alveoli septa thickening and focal cellulose pneumonia were also observed in the lungs of guinea pigs experimentally infected with SD0835. Viral replication was detectable by virus isolation and titration, real-time RT-PCR and immunohistochemistry (IHC) staining in the respiratory tissues of guinea pigs as early as 24 h after intranasal inoculation with SD0835. The results of virus isolation and titration showed that guinea pigs were permissive for SD0835 replication and exhibited a higher virus replication level in both lungs and tracheas. As well, the results of IHC staining implicated that the lungs and tracheas were the major tissues in which SD0835 replicated. Virus-specific serum neutralizing antibodies against BPIV3 were detected in virus-inoculated guinea pigs. The aforementioned results indicated that BPIV3 strain SD0835 of the genotype C was pathogenic to guinea pigs and could cause a few observable clinical signs, and gross and histologic lesions in virus-inoculated guinea pigs. Thus guinea pig is an ideal laboratory animal infection model for BPIV3 and would cast more light on the genotype C of BPIV3 infection process, in vivo tropism and pathogenesis or serve as a useful system for monitoring the pathogenesis of SD0835 and other BPIV3 isolates.

1. Introduction

Bovine parainfluenza virus type 3 (BPIV3) is a virus classified as a member within the genus *Respirovirus* of the family *Paramyxoviridae*. BPIV3 is one of the most important viral respiratory agents of both young and adult cattle and has been associated with the bovine respiratory disease complex (BRDC) development in feedlot cattle (Autio et al., 2007; Snowder et al., 2007). Recently, a very high seropositivity rate to BPIV3 was detected by ELISA in dairy cattle in Turkey and reached to 94.64%, which implicated that a very high level of BPIV3 infection occurred in dairy cattle (Kale et al., 2013). BRDC is commonly referred as "Shipping fever". BPIV3 was first isolated in 1959 from cattle showing signs of shipping

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fever (Reisinger et al., 1959; Hoerlein et al., 1959). The noted clinical signs are coughing, anorexia, pyrexia, nasal and ocular discharges, dyspnea and sometimes diarrhea. BRDC is economically important because it causes an increase in morbidity–mortality rates and precautions should be taken for its treatment and control of production losses (Irsik et al., 2006).

BPIV3 is widespread among cattle around the world. Recently four of the seven Australian isolates of BPIV3 were reported to be distinct from other three Australian isolates and the previously reported strains of BPIV3 and classified as genotype B (BPIV3b), and the other three Australian isolates and the previously reported strains of BPIV3 were classified as genotype A (BPIV3a) (Horwood et al., 2008). The detection, isolation or serological evidence of BPIV3 was not reported in China before 2008. However during 2008 there were many cattle identified with clinical signs considered to be consistent with BPIV3 infection. Four BPIV3 strains were isolated from bovine nasal swabs collected in Shandong Province. Phylogenetic analysis revealed that the four Chinese BPIV3 isolates were distinct from the reported genotypes A (BPIV3a) and B (BPIV3b) and appeared to be a potentially new genotype, which was tentatively classified as genotype C (BPIV3c) (Zhu et al., 2011). Shortly after that report, BPIV3c was isolated from cattle in Argentina and South Korea (Maidana et al., 2012; Oem et al., 2013), respectively, and BPIV3b was also isolated from water buffaloes in Argentina (Maidana et al., 2012). Up to present, BPIV3c was circulating in cattle in China and South Korea in Asia and in Argentina in South America, and BPIV3b was circulating in cattle in Australia and only in water buffaloes in Argentina, respectively.

So far, only limited studies on the pathogenesis of the genotype A of BPIV3 infection in calves and laboratory animals were conducted and reported (Van der Maaten, 1969a,b; Tsai and Thomson, 1975; Simmons et al., 2002). The pathogenesis of the genotypes B and C of BPIV3 infection in calves and laboratory animals was scant. Recently a report had described an experimental infection model using the genotype C of a Chinese BPIV3 strain SD0835 in Balb/c mice and had presented data indicating that viral infection could induce alterations in lungs and tracheas, and the detailed descriptions of histologic lesions had been presented (Dong et al., 2012). However no clinical signs and gross lesions could be observed in infected Balb/c mice during the whole experiment period. Therefore other experimental animal infection model being more susceptible to SD0835 might provide more facilities for the future research. The present paper reports the pathogenesis of SD0835 of BPIV3c infection in guinea pigs.

2. Materials and methods

2.1. Virus and cells

The Chinese strain SD0835 of BPIV3c (Zhu et al., 2011) was used in this study. MDBK cells were cultivated in minimum essential medium (MEM, GIBCO) supplemented with 10% fetal bovine serum (BIOCHROM AG, Germany) and kept at 37 °C. For the production of SD0835 used for guinea pig infection, MDBK cell monolayers were inoculated with SD0835. The infected cells were harvested and titrated, then aliquoted and stored at −70 °C until use. The virus stock had a titer of 1 ml $10^{7.25}$ $TCID_{50}$ by titration in MDBK cell monolayer cultures as described previously (Dong et al., 2012).

2.2. Animals and experimental infection procedure

A total of 24 specific pathogen free, female albino guinea pigs (weighing less than 400 g at the start of the experiment) were purchased from a commercial breeder, fed standard diet, and housed under controlled condition in individually ventilated cages and were randomly divided into two groups. Sera collected from all guinea pigs before the experiment were measured for virus-specific serum neutralizing antibodies with BPIV3 strain SD0835, and no virus neutralization antibody was detected. 16 guinea pigs (numbered as p1–p16) were anesthetized by intraperitoneal injection with 10% chloral hydrate, and 200 μl (3.6×10^6 $TCID_{50}$) of a suspension of SD0835 was instilled intranasally; 8 control guinea pigs were also instilled intranasally with the same volume of supernatant from uninfected MDBK cells. To determine the replication of BPIV3 in guinea pigs, two animals in infected group and one animal in control group were euthanized on day 1, 3, 5, 7, 9, 11 and 13 post inoculation (PI). Immediately following euthanasia, sera and samples of heart, kidney, liver, lung, spleen and trachea were harvested from each animal. Every sample of infected guinea pigs euthanized at each time point was divided into three parts as follows: one for virus isolation; one for histopathological examination and immunohistochemistry staining; one for real-time RT-PCR. All experimental animal procedures were approved by the Office of Laboratory Animal Management of Heilongjiang Province, China.

2.3. Clinical observations and pathological examinations

Clinical monitoring of guinea pigs was performed daily throughout the study. Specifically, observations were made regarding the guinea pig's mental alertness, body condition, activity level, and clinical signs of respiratory disease. Rectal temperatures were also recorded daily. At necropsy, a thorough post-mortem examination was performed, and samples of heart, kidney, liver, lung, spleen and trachea were collected and weighed. Upon removal, lungs were examined and extent of macroscopic lung lesions was estimated.

2.4. Histopathological examination and immunohistochemistry

The collected tissues were processed by routine histopathological procedures: specimens for histological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopy observations. Equivalent tissue sections from mock-inoculated guinea pigs were also processed and examined as controls.

Tissue sections were also examined by immunohistochemistry (IHC). Primary antibody of murine monoclonal antibodies against nucleocapsid protein of BPIV3 (developed in our laboratory) at a dilution of 1 in 1000 was applied overnight at 4 °C. The sections were treated using the protocols as previously described for detection of viral antigens of BPIV3 in experimentally infected Balb/c mice using IHC (Dong et al., 2012). The control sections were also examined.

2.5. Virus isolation and titration, and detection of viral RNA by a quantitative RT-PCR

Partial organs of heart, kidney, liver, lung, spleen and trachea were removed aseptically and prepared as fresh tissue homogenates with pestles (Axygen Inc., USA) at necropsy of guinea pigs. The homogenates were frozen and thawed twice and clarified by low-speed centrifugation and the supernatant fluids were harvested and stored in small volumes at −70 °C until use. Virus isolation and titration were performed in MDBK cell monolayer cultures as described previously (Dong et al., 2012).

The quantitative RT-PCR (qRT-PCR) is also described as real-time RT-PCR and its procedure is based on a TaqMan assay and two primers and a fluorogenic probe for BPIV3 were designed using Beacon Designer 7 from the matrix gene sequence of BPIV3 strain SD0835 (GenBank accession number HQ530153). The sequence of two pairs of primers, designed M-F (forward) and M-R (reverse) was located in Matrix region of BPIV3. The primers M-F (5′-GGA TGT