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INFECTIOUS DISEASE

Pathological Characterization of an Outbreak of Porcine Reproductive and Respiratory Syndrome in Northern Vietnam

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Summary

In 2007, a highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) emerged in Vietnam and spread to nearly all regions of the country by 2010. Ten representative pigs of different age groups, infected naturally with HP-PRRSV in northern Vietnam in 2010, were used to characterize the pathological features of the infection. Infection was confirmed using reverse transcriptase polymerase chain reaction and viral isolation. The clinical signs and gross findings in these pigs included high fever (>40.2°C), red skin, blue ears, anorexia, respiratory distress, diarrhoea, haemorrhagic pleurisy and lymphadenopathy. Reproductive failure was the main clinical feature in sows. PRRSV infection-associated microscopical lung and lymph node lesions were observed frequently, regardless of age of the animals. Lung lesions were characterized by interstitial pneumonia and were occasionally associated with haemorrhage and fluid exudation following alveolar collapse. Lymph nodes exhibited characteristic haemorrhage and apoptosis, lymphocytic depletion and disorganization secondary to fibrosis and capillary formation. Haematoxylin and eosin staining or caspase-3 immunohistochemistry revealed apoptosis induction in various tissues and organs, particularly the lymph nodes and lungs. Primarily haemorrhagic microscopical lesions were observed commonly in other organs including the spleen, liver, heart and kidney. Immunohistochemical examination revealed HP-PRRS antigen in the lung, lymph node, liver and kidney macrophages, and lung and kidney epithelial cells. Pigs infected naturally with HP-PRRS in the field have multisystemic disease characterized by marked apoptotic cell death.

Keywords: highly-pathogenic porcine reproductive and respiratory syndrome virus; pathological characterization; pig; Vietnam

Introduction

Porcine reproductive and respiratory syndrome (PRRS), also called 'blue ear' disease, is typically characterized by a high fever and respiratory distress in young pigs and is accompanied by high rates of mortality and reproductive failure in pregnant sows (Collins *et al.*, 1992; Wensvoort *et al.*, 1992; Batista *et al.*, 2002). This syndrome is considered one of the most important infectious diseases in pigs and has caused serious economic losses to the pork industry worldwide (Pejsak *et al.*, 1997; Neumann *et al.*, 2005). PRRS was first recognized in the USA in 1987 (Keffaber, 1989) and rapidly disseminated worldwide. In 2006, a highly pathogenic form of the PRRS virus (HP-PRRSV) that caused outbreaks and resulted in high mortality in both young and adult animals (including sows) was first reported in China (Tian *et al.*, 2007; Feng *et al.*, 2008) and spread rapidly into Vietnam and other neighbouring countries (Metwally *et al.*, 2010;

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Theary *et al.*, 2010; Ni *et al.*, 2012; Tornimbene *et al.*, 2015). In 2007, HP-PRRS was first confirmed in Hai Duong, a northern province in Vietnam; since then, the outbreak has spread to many other provinces and has presented many challenges in attempts to mitigate production losses (Tung *et al.*, 2011). By 2010, HP-PRRSV continued to affect nearly all provinces in Vietnam (Phu, 2011).

To date, several studies have characterized HP-PRRSV under experimental conditions (Zhou *et al.*, 2008; Hu *et al.*, 2013; Do *et al.*, 2015). However, pathological studies of this virus are lacking, and information regarding the characteristics of HP-PRRSV in cases of natural infection has rarely been reported. This study was conducted to characterize the pathological features and examine the distribution of viral antigen in tissues from pigs of different ages that were infected naturally with HP-PRRS during the northern Vietnamese outbreak of 2010.

Materials and Methods

Animals

The study used 10 representative pigs from three farms in Ha Noi, Bac Giang and Thai Binh provinces. The animals were grouped according to their ages: a weaning group (1–2 months), a grower group (2–4 months) and a sow group (1–3 years) (Table 1). Relevant information about these pigs, including the vaccination history, breed and age, was collected from the farm owners. Each pig was marked and ear-numbered and kept separately in isolation facilities.

Clinical and Gross Pathology

Clinical signs and rectal temperatures of each animal were monitored. Pigs were killed and subjected to necropsy examination after they became moribund or 10 days after first showing clinical signs. Serum samples were collected prior to necropsy examination.

Histopathological Examination

Samples of lung, tonsil, lymph nodes, kidney, spleen, liver and heart were taken from each animal at necropsy examination and fixed in 10% neutral buffered formalin. Tissues were processed routinely and embedded in paraffin wax. Sections (3–4 μm) were stained with haematoxylin and eosin (HE).

Immunohistochemistry

Immunohistochemistry (IHC) with mouse monoclonal antibody specific for PRRSV SR-30/SDOW-17 (Rural Technologies, Brookings, South Dakota, USA) was performed to examine the distribution of PRRSV in the tissues. Tissue samples were incubated with the antibody for 60 min at 37°C, followed by treatment with a polymer reagent (Envision, Dako, Tokyo, Japan) as described by Lan *et al.* (2006). Other immunohistochemical markers, including Iba1 (Dako) and CD163 (Biorad, Hercules, California, USA) for macrophage detection and AE1/AE3 cytokeratin (Dako) for detection of epithelial hyperplasia, were used to identify other characteristic lesions or changes in infected tissues. Caspase-3 (Cell Signalling, Tokyo, Japan) IHC was used to evaluate the typical hallmarks of apoptotic cells in selected lymphoid organs (i.e. lymph node, tonsil and spleen) and in the lung, liver, kidney and heart (Porter and Jänicke, 1999). The reaction products were 'visualized' with 0.05% 3-3′-diaminobenzidine and 0.03% H_2O_2 in Tris-HCl buffer; the sections were counterstained with Mayer's haematoxylin for 1 min and rinsed, dehydrated, cleared and mounted with coverslips.

Polymerase Chain Reaction and Virus Isolation

Total RNA was extracted from serum and lung tissue samples using TRIzol LS (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocol. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed according to the instructions provided by the kit manufacturer (One Step RNA PCR Kit, Takara Bio Inc., Otsu, Shiga, Japan), using the following open reading frame (ORF)-7 primer sets: forward, 5′-CAGCCAGTCAATCAGCTGTG-3′ and reverse, 5′-TCGCCCTAATTGAATAGGTG-3′ (Cheng-qian, 2009). The amplification products were analyzed using 1% agarose gel electrophoresis and ethidium bromide staining.

For virus isolation, RT-PCR-positive serum and/or lung homogenate samples were inoculated into Marc145 cells, which were subsequently maintained in Dulbecco's Minimal Eagle's Medium (DMEM; Wako, Japan) supplemented with antibiotics and 10% fetal calf serum. After allowing viral adsorption to the cells for 1 h at room temperature, fresh medium was added and the cells were cultured at 37°C in an enriched CO_2 atmosphere to allow observation of the cytopathogenic effect (CPE).

Immunocytochemistry of Tissue Cultures

Immunocytochemistry was conducted to confirm the presence of virus in tissue cultures. Cultured Marc145 cells in 48-well microtitre plates were inoculated with homogenized tissue samples, followed by

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