A Piglet with Concurrent Polioencephalomyelitis due to Porcine Teschovirus and Postweaning Multisystemic Wasting Syndrome

Maki TAKAHASHI1)*, Yukio M. SEIMIYA1), Yoshihisa SEKI1) and Manabu YAMADA2)


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ABSTRACT. A piglet developed respiratory distress followed by difficulty in standing and unsteady gait. The lesions were characterized by polioencephalomyelitis with the predominant distribution in the brain stem, as well as lymphocyte depletion and histiocyte infiltration with cytoplasmic inclusion bodies in the lymphoid tissues throughout the body and interstitial pneumonia. Porcine teschovirus (PTV) antigens were found in the former lesions and porcine circovirus 2 (PCV2) in the latter two lesions. PTV genes were detected from the diencephalon. The results suggest that the piglet was concurrently affected with polioencephalomyelitis due to PTV and postweaning multisystemic wasting syndrome (PMWS) associated with PCV2. They also suggest that the immunosuppressive condition developing in PMWS may have facilitated the infection of the brain with PTV.

KEY WORDS: piglet, polioencephalomyelitis, porcine teschovirus.

A piglet with symptoms suggestive of limb paresis was examined pathologically and virologically. Respiratory disease caused by the highly virulent PTV-1 strains is characterized by polioencephalomyelitis with high morbidity and mortality [9]. Milder forms of the disease are caused by the less virulent PTV-1 and other serotypes of strains and frequently affect weaning piglets [9, 10, 19]. Although postweaning multisystemic wasting syndrome (PMWS) associated with porcine circovirus 2 (PCV2) occurs in the swine population throughout Japan [15, 17], there are no reports of the concurrence of PCV2-associated PMWS and PTV-induced polioencephalomyelitis. This study describes the pathological and virological findings in a piglet concurrently affected with both diseases.

Porcine teschovirus (PTV) strains within the family Picornaviridae are classified into 11 serotypes [9]. Teschen disease caused by the highly virulent PTV-1 strains is characterized by polioencephalomyelitis with high morbidity and mortality [9]. Milder forms of the disease are caused by the less virulent PTV-1 and other serotypes of strains and frequently affect weaning piglets [9, 10, 19].

Tissue blocks collected throughout the body were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of tissue were stained with hematoxylin and eosin (HE). Selected sections of the lung were stained with Grocott’s methenamine silver and those of the other organs with the streptavidin-biotin-peroxidase (SAB) method. The SAB method was applied using a SAB kit (Nichirei Corporation, Tokyo) and the following primary antibodies: mouse anti-PTV monoclonal antibody and rabbit anti-hemagglutinating encephalomyelitis virus (HEV) serum applied to the pons, medulla oblongata, cerebellum, spinal cord and its associated ganglia, anti-Pneumocystis carinii monoclonal antibody (ViroStat, U.S.A.) and rabbit anti-PRRSV serum to the lung and biotinylated porcine anti-PCV2 serum to the lung and parotid lymph nodes, at dilutions of 1:128, 1:2,000, 1:320, 1:8,000 and 1:160, respectively.

The principal histological lesions were observed in the CNS and were characterized by polioencephalomyelitis. A severe degree of changes was found in the pons and medulla oblongata, moderate changes in the spinal cord, spinal dorsal root ganglia and cerebellum and mild changes in the diencephalon, mesencephalon and trigeminal ganglia. The lesions were distributed throughout the pons and medulla oblongata without any location affected preferentially, and consisted of neuronal degeneration, neuronophagia, glial accumulation and perivascular cuffing. Affected neurons were swollen with chromatolysis or shrunken with an increased eosinophilic affinity. Their nuclei were eccentrically located and often accompanied by karyopyknosis, -rrhexis or -lysis. Neuronophagia was often found and microglial accumulation was disseminated in the lesions (Fig. 1). Perivascular cuffing was composed of two to three layers of cells in which lymphocytes were the predominant cell type. The involvement of the diencephalon and mesencephalon was limited to several nervous nuclei and adjoining white matter.

NOTE  Pathology

Postweaning Multisystemic Wasting Syndrome

*Correspondence to: TAKAHASHI, M., Iwate Prefecture Central Livestock Hygiene Service Center, 390–5 Sunagome, Takizawa-mura, Iwate 020-0173, Japan. e-mail: maki-t@pref.iwate.jp
Changes similar to those in the brain stem were present at all levels of the spinal cord and were more prominent in the lumbar part. The ventral horns were frequently affected with occasional involvement of the central intermediate substances and dorsal horns, as well as adjoining white matter. The cerebellar lesions were characterized by microglial accumulation predominantly distributed in the molecular layer, and accompanied by degeneration of several Purkinje’s cells and mild lymphocyte infiltration in the meninges adjacent to the lesions in the molecular layer. There was neuronal degeneration and satellite cell accumulation among neurons, as well as occasional perivascular cuffing in the affected ganglia. Rare changes in the cerebrum included microglial accumulation and mild perivascular cuffing in the cortex. No lesions were found in the peripheral nerves.

Varying degrees of lesions, consisting of lymphocyte depletion and histiocyte infiltration with numerous spherical, basophilic and cytoplasmic inclusion bodies, were seen in the lymphoid follicles and parafollicular zones in the lymphoid tissues throughout the body such as the parotidean (Fig. 2), mandibular, renal and mesenteric lymph nodes, spleen, thymus and Peyer’s patches. Multinucleated giant cells were occasionally concurrent in the lesions.

Interstitial pneumonia with fungal infection was observed in the pulmonary lobes. The alveolar septa were moderately thickened due to histiocyte infiltration and occasional hyperplasia of hypertrophic type II pneumocytes, accompanied by foamy or acidophilic “honeycomb” materials in the alveoli (Fig. 3-A). The materials were stained as round or crescent-shaped and empty cysts, and were mostly situated in clusters with Grocott’s methenamine silver staining.

PTV antigens were present in the neuronal cytoplasm in the CNS (Fig. 1, inset), PCV2 antigens in the histiocytic cytoplasm and inclusion bodies in the lymph nodes (Fig. 2, inset) and pulmonary alveolar septa (Fig. 3-B) and *P. carinii* antigens in the pulmonary alveoli (Fig. 3-C). Neither HEV nor PRRSV antigens were found in the CNS or the lungs.

Portions of the diencephalon, lung and tonsils were obtained at necropsy for virological examinations. For PTV isolation, the tissues of the diencephalon were homogenized in Earle’s medium and inoculated onto porcine kidney cell line (CPK) cultures that were subsequently observed for seven days. Three further passages were made.

Viral RNA was extracted from the diencephalon for PTV and from the pulmonary tissues for PRRSV with a commercial kit (TRIzol-LS reagent, Invitrogen, U.S.A.) and ampli-
concurrent infections with PCV2 and PRRSV (ORF7 region) [12], as described previously. The amplified products were purified with a commercial kit (Montage-PCR, Millipore, U.S.A.) and sequenced directly with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, U.S.A.) and the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, U.S.A.). The sequences of the closest relatives of the products were retrieved from DNA databases based on the BlastN program network service available at the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp).

To detect CSFV antigens, tonsil tissues were frozen in liquid nitrogen, sectioned at 5 µm using a cryomicrotome, fixed in cold acetone and stained with anti-CSFV antibodies (Kyoto-Biken, Kyoto).

The RT-PCR products of 321 base pairs were amplified from the diencephalon and sequenced. The homologies between this sequence and those of other PTV strains including F65, Talfan, PS36, UKG173/74, DS183/93 and Vir1626/89 were 96.7 to 95.2%, with a variation of nine to 13 nucleotides. Based on the results, the origin of the viral RNA was identified as PTV. Neither cytotoxic or infectious genes nor amplified products were obtained from the diencephalon or the lung. No CSFV antigens were detected from the tonsils, neither.

The CNS lesions observed in the present piglet with respiratory and nervous symptoms were characterized by polioencephalomyelitis, with the predominant distribution in the brain stem. PTV antigens and its genes were detected from the lesions. Neither HEV nor CSFV antigens were found in the CNS lesions or the tonsils. The results indicate that the piglet was affected with polioencephalomyelitis due to PTV.

It is thought that the predominant lesions of PCV2-associated PMWS consist of lymphocyte depletion and histiocytic infiltration with characteristic inclusion bodies in many lymphoid tissues, as well as interstitial pneumonia [1, 3, 15, 17], and that the presence of infectious or noninfectious cofactors in addition to PCV2 is required for the development of PMWS with clinical symptoms [1, 15]. Polymyocystis carinii is commonly considered an opportunistic fungal pathogen that may cause fatal interstitial pneumonia in immunocompromised animals and humans [2, 8].

The lesions mentioned above were observed in the lymphoid tissues and pulmonary lobes of the present piglet, and the latter lesions were accompanied by fungal infection. PCV2 antigens were found in the lesions of both organs and P. carinii antigens in the pulmonary lesions. Neither PRRSV antigens nor its genes were detected from the pulmonary lesions. The present results suggest that the respiratory distress in the piglet was associated with PMWS caused by concurrent infections with PCV2 and P. carinii. And the fact of P. carinii infection also suggest that the piglet was immunologically suppressed.

Although the initial replication of PTV in piglets occurs in the tonsils and intestinal tract with the subsequent viremic stage leading to infection of the CNS, most infections under field circumstances have a subclinical course without CNS involvement [7, 9]. It is reported that humoral antibodies are important as a defense mechanism against PTV infection in piglets, and that a lack of these antibodies results in the development of symptoms suggestive of encephalomyelitis [4, 9]. It is also suggested that pigs severely affected with PMWS suffer immunosuppression resulting from the altered populations of cells participating in the immune system response [3, 16, 17]. The previous results seem to suggest that the immunosuppressive condition associated with PMWS may have helped PTV to infect the CNS in the present piglet.

The differential diagnoses for PTV-induced CNS lesions in pigs include those caused by other neurotropic viruses such as HEV, PRV and CSFV. Pathogenic examinations were required to differentiate PTV from HEV infections, since both CNS lesions were similar in quality and distribution [5, 7, 14]. On the other hand, PTV-induced lesions may be differentiated from those due to PRV or CSFV. The former lesions were distributed predominantly in the gray matter of the brain stem and spinal cord [5, 7, 19], while the latter two lesions were found in both the grey and white matter throughout the CNS [13, 18]. Observation of intranuclear inclusion bodies in the neurons and glial cells [11, 13] or of encephalomyelitis with a principal lesion made up of perivascular cuffing [6, 18] would further facilitate the diagnoses of PRV or CSFV infection. Further studies will be needed to clarify the pathogenesis by which PTV infects the CNS.

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