Pathological Observations on 2 Horses of Myeloencephalopathy Associated with Equine Herpesvirus 1 (EHV-1) Infection

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During the epizootic of EHV-1 infection involving 132 of about 2,000 racehorses at the Ritto Training Center of the Japan Racing Association in the western part of Japan, 1989, 7 horses developed paralysis after the onset of the febrile illness.

Of the 7 horses showing the development of neutralizing antibodies against EHV-1, 2 were necropsied for pathological studies. In horse No. 1, small malaciae were found in the capsula interna, pons and spinal cord. Sporadic vasculitis with thrombus, which caused malaciae, was observed in the small arteries and veins of the brain and spinal cord. Horse No. 2 had perivascular cuffing in the brain and demyelination in the spinal cord. Edema and demyelination was also noted in the rami labiales superiores of the trigeminus nerve and ischiadicus nerve. From these results, it was suggested that paralysis developed in horses was caused by myeloencephalopathy associated with EHV-1.

Key words: equine herpesvirus, meningoencephalomyelitis, myeloencephalopathy.

Introduction

Equine herpesvirus 1 (EHV-1) causes abortion in pregnant mares, respiratory diseases in young horses and paralysis to horses of all ages and breed groups1).

Although both male and female horses may be affected, pregnant mares seem to be especially at risk2,3). As compared with abortion and respiratory disease, paralysis appears to be relatively rare in the incidence rate4). The paralysis associated with EHV-1 infection is classified pathologically into myeloencephalopathy4) or meningoencephalomyelitis5) on the basis of histological findings, such as vasculitis in the small arteries and veins of the cerebrum and the spinal cord. Although myeloencephalopathy in horses associated with the EHV-1 infection has been observed in many countries abroad6-12), there have been no reports on the occurrence of this disease in Japan. Between January and February in 1989, 7 of 132 infected horses exhibited paralysis. This report describes the first occurrence of myeloencephalopathy among the horses with EHV-1 infection in Japan by showing pathological findings of 2 horses with paralysis during the epizootic of EHV-1 infection among racehorses.

Materials and Methods

History of the outbreak: The outbreak of EHV-1 infection occurred among about 2,000 racehorses in the Ritto Training Center of the Japan Racing Association in Shiga Prefecture, in Japan, during the period between just at the beginning of January and the middle of February in 1989. Of these horses, 132 showed common clinical signs such as fever and a
serous nasal discharge. Nasal swabs and serum samples for a virological and serological examination were collected from these horses and submitted to our station. Virological and serological examinations were performed as described previously\(^{13,14}\). Complement fixing (CF) antibody against EHV-1 increased significantly in 83 sera obtained from the 132 horses in the convalescent stage. In this epizootic, 10 strains of EHV were isolated from the nasal swabs and were identified as EHV-1 using the immunofluorescent antibody technique with the EHV-1 monoclonal antibody supplied by Dr. Allen\(^{15}\), University of Kentucky.

**Horses:** In this epizootic, 2 of 7 racehorses showed incoordination or paralysis in the hind limbs and were euthanized for unfavorable prognoses and examined pathologically.

Horse No. 1, a thoroughbred, male and 5 years old, showed a mild fever of 38.7°C on the 1st day of the onset of illness and incapable of standing, paralysis of penis and incontinence of urine on the 3rd day, and rose to his feet again on the 4th day. Incoordination in the hind limbs continued until the 14th day when the horse was euthanized. Horse No. 2 was a thoroughbred, female and 4 years old. A fever of 39.0°C were observed in the horse on the 1st day of the onset of illness and returned to normal the next day. Because of unilateral paralysis of the facial nerve, curving of the nose tip to the right side was observed and the horse fell into recumbent on the 7th day. Even after rising up on the 9th day, incoordination in the hind limbs and incontinence of urine remained until the 236th day when the horse was euthanized.

The CF antibody titer increased from 1:8 to 1:128 in horse No. 1 and from 1:4 to 1:256 in horse No. 2 immediately after ataxia. However, no virus was isolated from any of the organs or tissues such as the liver, spleen, kidneys, lungs, submandibular lymphnodes, inguinal lymphnodes, mesenteric lymphnodes, cerebrum, cerebellum, spinal cord and cereblosplian fluid of horse No. 1 and 2 which were necropsied on the 14th and 236th day, respectively.

**Pathological examination:** Two horses were euthanized with an injection of a mixture of a suxamethonium chloride solution (Succine: produced by the Yamanouchi Phamaceutical Co., Ltd., Tokyo) and thiopental sodium solution (Ravonal: produced by the Tanabe Phamaceutical Co., Ltd., Osaka). Necropsies were carried out for 12 hrs after euthanasia for horse No. 1 and immediately for horse No. 2. Histopathological samples were collected from the major organs and tissues with special attention to the central nervous system (CNS) and the facial nerve and the sciatic nerve of the peripheral nervous system. They were fixed in a 10% neutral buffered formalin solution, dehydrated through an ethanol series, embedded in paraffin and cut into sections. The sections were stained with hematoxylin and eosin (HE). Some representative sections were also stained with alcian blue, Luxol fast blue (LFB), silver, phosphotungustic acid hematoxylin (PT AH) and periodic acid-Schiff (PAS).

**Examination of tissues by immunoperoxidase method:** In paraffin embedded sections of the CNS, virus antigens were examined by the avidin biotin peroxidase complex (ABC) method\(^{16}\) using the above men-
tioned anti-EHV-1 mouse monoclonal antibody\textsuperscript{15}). The anti-EHV-1 mouse monoclonal serum used for the primary antibody was diluted to a 1:2,560 concentration. The secondary biotinylated antibody and ABC reagent were obtained commercially (Vectastain, Vector Lab., Burlingame, U.S.A.).

**Results**

**Gross findings:** In horse No. 1, small focal malaciae as large as the head of a needle with a ring hemorrhage were found in the capsula interna of the left diencephalon and the pons of the metencephalon (Fig. 1). Loose connective tissue around the lower lumber cord and cauda equina was gelatinous in appearance. The cerebrospinal fluid was slightly turbid to cloudy. The follicles of the spleen were indistinct. The submandibular,inguinal, hepatic, splenic, tracheal and mesenteric lymph nodes were edematous. Congestion, petechiae and ecchymoses were observed in the liver, kidneys and lungs. Ecchymoses were also observed under the epicardium of the ventriculus cordis.

In horse No. 2, the skin of the pudendum femininum was chronically covered by wet crusts of dripping urine and exudate. At the time of skinning, hypodermal tissue of the whole body showed some dehydration. Most of the skeletal muscles especially those of hind limbs showed atrophy. The bladder contained an yellowish-brown, melon-size, muddy concretion (Fig. 2), and its mucosal membrane had ecchymoses. The lymphnodes such as the submandibular, inguinal, hepatic, splenic, tracheal and mesenteric lymphnodes were atrophic. The lymphoid follicles of the spleen as well as the lymph-nodes above mentioned were indistinct.

In both horses, no gross lesions were observed in any of the other organs and tissues.

**Histopathological findings:** In horse No. 1, the principal changes in the CNS were in the vasculature with necrotizing and inflammatory lesions in the walls of the blood vessels. Small muscular arteries, arterioles and venules were affected and they were widely distributed throughout the CNS and menings.

These vessels showed such lesions as endothelial swelling or detachment, edema of the intima, fibrinoid necrosis or degeneration of the media and infiltration of the small mononuclear cells in the adventitia (Fig. 3). Fibrinoid thrombi were often observed in the lumen of such affected vessels (Fig. 4). In the cerebrum, the affected vessels were numerous in the brain stem and pia mater cerebri. Small foci of malaciae were occasionally seen closely by the affected vessels (Fig. 5). Foci showed predilection for the white matter adjacent to the cortex cerebri and contained a number of lipid phagocytes and several spheloid bodies with hemorrhaging. Ring hemorrhages were occasionally present around the venules. In the spinal cord, pathological findings of the affected vessels were similar to those in the cerebrum. Distribution of the vascular lesions had a predilection for pia mater spinalis.

Ischemic alteration occurred in places of the white matter of the spinal cord, where the affected vessels were supplied. Foci of the white matter showed ballooned fiber sheaths and axonal swelling, sometimes with infiltration by the phago-
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cytes (Fig. 6). Although foci were scattered throughout the spinal cord, the funiculus lateralis and funiculus ventralis of the white matter were predominantly involved.

In horse No. 2, a slight to moderate perivascular cuffing was often observed in the pia mater encephali, the white matter adjacent to the cortex cerebri and the brain stem (Fig. 7). Most of the cells infiltrated were small mononuclear round cells and there were a few multinucleate giant cells (Fig. 8). Slight to moderate focal demyelination were observed sporadically throughout the spinal cord (Fig. 9). Demyelinated foci were also found in the funiculus lateralis and funiculus ventralis predominantly. In the peripheral nervous systems, focal edema and demyelination of the nerve fibers were observed in the rami labiales superiores of the trigeminus nerve and in the ischiadicus nerve (Fig. 10).

In other organs and tissues, no histopathological lesions including an inclusion body were found in either of the horses.

Examination of the CNS by the immunoperoxidase method: Immunoperoxidase procedure by the ABC method could not detect any EHV-1 antigen in the CNS.

Discussion
During the epizootic of the EHV-1 infection among racehorses at the Ritto Training Center of the Japan Racing Association between January and February, 1989, 7 of 132 horses with fever showed incoordination and paralysis in the hind limbs, when 2 of the 7 horses with paralysis were necropsied and examined pathologically. Histopathological
Fig. 3. Arteriolitis on the pia mater spinalis of 18th thoracic cord. Horse No. 1.
HE stain. ×160.

Fig. 4. Fibrinoid thrombus in an arteriole of the pars basalis rhinencephali. Horse No. 1.
PTAH stain. ×160.

Fig. 5. A malacia of the pons. Horse No. 1. HE Stain. ×40.

Fig. 6. Ballooning of the axon in 9th thoracic cord. Horse No. 1. HE stain. ×280.
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Fig. 7. Small mononuclear perivascular cuffing in the pia mater cranialis. Horse No. 2. HE stain. ×160.

Fig. 8. Perivascular cuffing of many small mononuclear cells and a multinucleate giant cell. Horse No. 2. HE stain. ×160.

Fig. 9. Demyelination in the funiculus lateralis of the 7th thoracic cord. Horse No. 2. LFB stain. ×40.

Fig. 10. Edema and demyelination in the rami labiales superiores of the trigeminus nerve. Horse No. 2. Alcian blue stain. ×160.
observation revealed sporadic vasculitis and multifocal malaciae in the CNS. Because histopathological findings were consistent with descriptions in literatures\textsuperscript{2,5,17,18}, they were pathologically diagnosed as myeloencephalopathy or meningoencephalomyelitis.

Although remarkable lesions under a light microscope were present in the CNS in both of the 2 horses, there were some differences probably due to the stage of the disease between them. Vasculitis and malacic foci observed in the CNS of horse No. 1 were regarded as those in the acute or subacute stage. On the other hand, perivascular cuffings of predominant small mononuclear cells in the brain and demyelination in the white matter of the spinal cord were still observed even after the 236th day of the onset of illness. These results suggested that histopathological findings of myeloencephalopathy associated with EHV-1 infection remained for a long term. Although the reason why those findings remains for such a long term is still unknown, it was suggested that they were irreversible changes in the CNS.

There are some forms of encephalitis associated with herpesviruses, such as herpes simplex encephalitis in man, infectious bovine rhinopneumonitis encephalitis in calves and pseudorabies in piglets. In these diseases, each herpesvirus has neurotropism and multiplicates in the neurons and glia cells, resulting in their destruction. In contrast, there have been no evidence that EHV-1 multiplicates in the neuron and glia cells. Also, in this study, the immunoperoxidase method could not detect any antigens associated with EHV-1 in the CNS. Therefore, it is possible that the lesions of the CNS for this disease are thought to be caused by an infarction of the neural tissue subserved by the thrombosed vessels\textsuperscript{20}.

It seems that EHV-1 has a predilection for endothelium of the blood vessels, especially those of the CNS\textsuperscript{20}. To think of vasotropism of the herpesvirus, it is full of suggestions that canine herpesvirus has also it in the puppies\textsuperscript{21}. Necrotizing vasculitis occurred in the lobar arteries of the kidneys as a result of the virus multiplication in the cells of the tunica intima, media and adventitia of the vessels. In addition to the viral multiplication, an EHV-1 specific immunological interaction appeared to play an important role in the development of the vascular lesions. Further an investigation will be needed before pathogenesis of the vascular lesions can be clarified.

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Literature Cited

6) Saxegaard, F. 1966. Isolation and identification


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**要 約**

ウマヘルペスウイルス1型の感染による脊髄障害の病理的所見：和田隆一, 兼丸 卓, 横田 貞夫, 南部 実, 松村富夫 (日本中央競馬会競走馬総合研究所姫木支所, 日本中央競馬会馬事部防疫課)——1989年栗東トレーニング・センターの競走馬群にウマヘルペスウイルス1型感染症が流行した際, 症状を呈した2症例をそれぞれ急性期と慢性期に病理解剖的観察をした結果, 症例1は急性期に剖検され, 左側頸髄および脳幹血管に微小軟化変を認めた。病理組織学的には脳および脊髄の細・中等静脈における血栓形成を伴う血管炎とその支配領域における脳血管炎でなかった。症例2は慢性期に剖検され, 病変の分布は症例1と同じであったが, 脳血管における主病変は小円形球に包まれた網状格子形浸潤像であった。脊髄では軽度の脱髓維が頸髄から仙側にいたるまで多発性に認められ, 末梢神経病では, 亀類神経上脇枝と坐骨神経の神経周間に被われた神経線維束に軽度と脱髓が認められた。