 Kinetics and Pathogenicity of Equine Herpesvirus-9 Infection following Intraperitoneal Inoculation in Hamsters

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Summary

The kinetics of infection and pathogenicity of equine herpesvirus-9 (EHV-9) was studied in a hamster model. Five-week-old Syrian hamsters and 5-day-old suckling hamsters were inoculated intraperitoneally with 10^5 and 4 x 10^4 plaque-forming units of EHV-9, respectively. EHV-9 antigens were detected by immunocytochemistry in the peritoneal macrophages, which may be the primary site of virus attachment and propagation at 6 h post inoculation (hpi). At 12 hpi, viral antigen was observed in the abdominal nerves and ganglia (mainly the coeliac ganglia). Virus antigen was detected in the dorsal root (spinal) ganglia, in parts of the spinal cord (particularly the mid-lumbar area) and in the myenteric plexuses at 36, 48 and 72 hpi, respectively. At 96 hpi, virus antigen was detected in the most caudal part of the brain. Polymerase chain reaction conducted on samples of the blood, spinal cord and brain revealed EHV-9 DNA in the spinal cord at 36 hpi and in the blood at 48 hpi and for 4 days after this initial detection. It is suggested that after initial propagation in the abdominal macrophages, EHV-9 infected the abdominal ganglia or myenteric plexuses and then travelled to the brain via the peripheral nerves and spinal cord. Examination of other organs also revealed the presence of EHV-9, suggesting that the virus might infect tissues other than those of the nervous system.

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Keywords: EHV-9; hamster model; neuropathogenesis

Introduction

Equine herpesvirus-9 (EHV-9), a varicellovirus in the subfamily Alphaherpesvirinae, is the newest member of the equine herpesviruses and is closely related to equine herpesvirus-1 (EHV-1). EHV-9 was first described in an outbreak of disease in Thomson’s gazelles (Gazella thomsoni) in a Japanese zoo (Fukushi et al., 1997) and subsequently in a giraffe (Giraffe camelopardalis reticulata) with signs of encephalitis (Borchers et al., 2005; Kasem et al., 2008). Recently, EHV-9 was detected in a polar bear with progressive encephalitis (Donovan et al., 2009), raising fears of emerging infections in various wild and zoo animal species. Emerging EHV-9 infection is also a concern with respect to domestic animals, because the virus has exhibited a wide range of susceptible hosts and has been shown to be easily transmittable via the nasal route in goats (Taniguchi et al., 2000), pigs (Narita et al., 2000), dogs and cats (Yanai et al., 2003a, b) and common marmosets (Kodama et al., 2007).

The pathogenesis of EHV-9 following nasal infection has been studied in a suckling hamster model. EHV-9 propagated in the olfactory epithelium 12–24 h post inoculation (hpi) and then gained access to the brain through the olfactory and trigeminal nerves (El-Habashi et al., 2010a).

EHV-9 may also infect hamsters by the ocular, oral and intraperitoneal routes (El-Habashi et al., 2010b). Animals infected via the oral and peritoneal routes...
exhibited milder lesions and fewer viral antigen-positive cells in the glomerular and mitral cell layers of the olfactory bulb than animals infected via the nasal route. This observation suggests that the virus most likely does not enter the brain through the olfactory nerve, but may enter through other nerves. Further investigations are needed to determine exactly how EHV-9 reaches the brain following infection by non-nasal routes and to identify the pathogenesis of EHV-9-induced encephalitis following these routes of administration. In particular, the primary site of propagation of the virus has not yet been identified, nor is it known how the virus gains access to the brain by these routes. Another unanswered question is the time required for the virus to travel to the brain. The pathogenesis of EHV-1 induced encephalitis and myelitis following intraperitoneal inoculation has been studied previously (Hasebe et al., 2002), and other neurotropic viruses, such as Theiler’s virus and poliovirus, have been studied in the CBA mouse model using different routes of infection in order to elucidate their detailed neuropathogenicity (Villarreal et al., 2006).

The aims of the present study were (1) to determine the primary site of virus propagation following intraperitoneal inoculation of hamsters with EHV-9, (2) to characterize the pathway by which EHV-9 accesses the central nervous system (CNS), (3) to define the kinetics of development of encephalitis induced by EHV-9 and (4) to determine whether EHV-9 has any effect on the internal organs of infected animals.

**Materials and Methods**

**Virus Culture**

Madin-Darby bovine kidney (MDBK) cells were used for the propagation of EHV-9. The inocula were prepared by culturing the virus from the original seed stocks of EHV-9 (P19, 5th passage in MDBK cells). The virus was titrated by plaque-forming assay on MDBK cells.

**Animals**

Four 5-week-old male and twelve 10-week-old female Syrian hamsters that were 10 days pregnant were purchased from a breeder (SLC Inc, Hamamatsu, Japan). The animals were housed in plastic cages and kept in an isolated biohazard cabinet for approximately 1 week of acclimatization. The animals were provided with a basal pellet diet (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and bottled water ad libitum. The experiment was conducted in accordance with laws and Standard Operating Procedures related to the use of laboratory animals. The experimental protocol was approved by the Animal Experiment Committee of the Faculty of Applied Biological Science at Gifu University.

**Virus Inoculation**

Four 5-week-old hamsters were injected intraperitoneally with a single dose of $10^5$ plaque-forming units (PFUs) of EHV-9 virus and were maintained until the end of the experiment (15 days post inoculation [dpi]) or the appearance of neurological signs. The 12 pregnant dams were left to give birth, after which fifty 5-day-old suckling hamsters were inoculated intraperitoneally with $4 \times 10^4$ PFU of EHV-9 virus. Of the five suckling hamsters that were killed at intermediate intervals of 6, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hpi, four were subjected to histopathological and immunohistochemical analysis and one provided samples for polymerase chain reaction (PCR) studies. Five uninfected animals were kept as a control group. The animals were checked for clinical signs at least three times daily.

**Collection of Peritoneal Cavity Cells**

Cells were collected from the peritoneal cavity as described by Goafa et al. (1996). After death, the abdominal skin was treated with 70% ethanol and then the outer skin layer was opened and 10 ml of cold phosphate buffered saline (PBS; pH 7.2, 0.01 M) containing 3% fetal calf serum was injected into the abdominal cavity using a 27-gauge needle attached to a 5 ml syringe. The abdomen was gently massaged and then the PBS solution was carefully aspirated using a 25-gauge needle attached to a 5 ml syringe. The cell suspension obtained was centrifuged at 400g for 8 min. The supernatant was discarded and the cells were suspended in PBS in order to prepare smears for immunocytochemistry (ICC).

**Pathological Investigations**

The brain, different levels of spinal cord, lungs, liver, spleen and small and large intestines were taken from the juvenile hamsters and fixed in 10% neutral buffered formalin. The suckling hamsters were bisected in the sagittal plane and were fixed similarly. Samples were processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE). The suckling hamsters were not decalcified.

Fixed tissue sections were also subjected to immunohistochemistry (IHC) by the avidin–biotin complex (ABC) method as described previously (Yanai et al., 1998). The primary antibody was a rabbit antibody specific for EHV-9 (1 in 800 dilution; Veterinary Microbiology Laboratory, Gifu University).
University) and the secondary antibody was biotinylated anti-rabbit immunoglobulin (Ig) G (DAKO Cytomation, Fort Collins, Colorado) with ‘visualization’ of the reaction by application of liquid 3,3′-diaminobenzidine substrate chromogen system (DAKO Cytomation) and haematoxylin counterstain. Tissue sections from the EHV-9-infected hamsters and sera from a non-immunized rabbit and goat were used as controls.

For ICC, cytological preparations of the abdominal cell suspensions were air dried for 30 min, fixed in cooled acetone and immunolabelled with the EHV-9 antiserum as described above.

**DNA Extraction and Polymerase Chain Reaction**

Blood and fresh tissues (including the spinal cord and brain) were subjected to DNA extraction with a Sepagene kit for virus DNA detection (Sanko Pharmaceuticals, Tokyo, Japan). Virus DNA was detected using primers for the open-reading frame (ORF) 76-F (5′-TTT CCC TCT CAG CGA TCA CTT TTC ACC GAA GAA CAG GCC CTC ATC GG-3′) and ORF76-R (5′-GGG CTG TTG TGG GGT AAA AGG TGG TGT TAC GGA AAC ACG CGT GCC AAG AA-3′).

PCR amplification was performed in 50 μl volumes containing DNA (100 ng), 8 μl of each dNTP, 0.5 μl of each primer, 25 μl LA Taq Buffer (Mg +2 plus) and 0.5 μl Takara LA Taq™ DNA polymerase (Takara, Kyoto, Japan). The PCR conditions were: 5 min at 94°C (initial denaturation), 30 cycles of 5 sec at 98°C, 30 sec at 68°C, 90 sec at 72°C and finally 7 min at 72°C (final extension). The PCR product was separated on an agarose gel (0.9%) and stained with ethidium bromide.

**Results**

**Five-week-old Syrian Hamsters**

**Clinical Findings.** One of the four adult hamsters inoculated intraperitoneally developed clinical signs compatible with those reported previously in EHV-9

![Image](image_url)
infection. These included weight loss, crouching posture, nasal and lachrymal discharges and salivation. By the second day, this animal became hyper-reactive to stimuli and displayed occasional tremors associated with uncoordinated movements of the limbs.

**Pathological Findings.** At necropsy examination, none of the inoculated animals exhibited gross abnormalities. Microscopically, all of the hamsters inoculated with EHV-9 had lymphocytic meningomyelitis and meningoencephalitis characterized by neuronal degeneration and necrosis, varying degrees of gliosis, perivascular aggregates of lymphocytes, plasma cells and neutrophils, with mild focal or diffuse lymphocytic infiltration of the meninges. The affected neuronal cells displayed severe chromatolysis or karyorrhexis. The degenerate neuronal cells often had intranuclear inclusion bodies. No abnormalities were detected in other organs except for moderate interstitial pneumonia in the animals inoculated with EHV-9. The pulmonary changes consisted of diffusely thickened alveolar septa with varying degrees of macrophage and neutrophil infiltration.

**Immunohistochemistry.** EHV-9 antigen was detected in the pseudounipolar neurons of the dorsal root (spinal) ganglia (Fig. 1a) and their synapses within the spinal cord (Fig. 1b) and in neurons in the grey matter of the spinal cord (Fig. 1c). The presence of EHV-9 antigen was confirmed at most levels of the spinal cord in all inoculated animals. Strong positive immunolabelling was detected in the medulla oblongata, pons and cerebellum. There was moderate intensity of labelling in the hippocampus, cerebral cortex and granular and mitral layers of the olfactory bulb and weak labelling of the glomerular layer of the olfactory bulb.

**Suckling Hamsters**

**Clinical Signs.** All of the inoculated suckling hamsters had varying degrees of clinical signs that consisted of depression and uncoordinated movements starting at 96 hpi. At 168 hpi all animals exhibited markedly uncoordinated movement.

**Pathological Findings.** At necropsy examination, no gross abnormalities were observed in any of the animals examined. Sagittal sections of the suckling hamsters enabled examination of the entire body in a single histological section. Microscopical findings in suckling hamsters inoculated with EHV-9 are summarized in Table 1.

At 6 hpi there were no significant abnormalities except for mild neutrophil and lymphocyte infiltration of the peritoneal cavity.

At 12 hpi there was mild degenerative change and vacuolation of the abdominal and peripheral ganglia (particularly the coeliac ganglia). There was also mild perineuritis and neuritis with mild neutrophil and lymphocyte infiltration around the axons of nerve fibres within the abdominal cavity (Fig. 2).

At 24 hpi, lymphocytic infiltration was observed in the peripheral ganglia and nerves in the abdominal cavity, in addition to early vacuolation of the pseudounipolar neurons of the dorsal root ganglia.

At 36 hpi there were more prominent degenerative changes and necrosis of the peripheral ganglia as well as the pseudounipolar neurons of the spinal ganglia.

**Table 1**

<table>
<thead>
<tr>
<th>Tissue</th>
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–, negative; +, positive; ND, not detected.
but there were no microscopical abnormalities in the spinal cord except for mild focal lymphocytic menignitis. There were degenerative changes in hepatocytes near Glisson’s capsule in the liver.

At 48 hpi there was frequent necrosis of the neurons in the spinal ganglia. In the grey matter of the spinal cord, the most characteristic finding was multifocal meningoeyelitis, which presented as neuronal degeneration accompanied by gliosis and perivascular lymphocyte aggregation. There was multifocal hepatocellular degeneration and necrosis. Within the myenteric plexuses of the intestine there was degenerative change accompanied by moderate infiltration of lymphocytes and plasma cells.

At 72 hpi there were more diffuse and prominent inflammatory aggregates in the spinal ganglia, as well as intranuclear inclusions in the pseudounipolar neurons. Other findings included extension of meningoeyelitis in the spinal cord (Fig. 3) and extension of the affected areas of the liver.

At 96 hpi there was more severe meningoeyelitis extending to the anterior segments of the spinal cord, in addition to marked necrosis of the dorsal root ganglia (Fig. 4). There was meningoencephalitis affecting the brain and this was particularly prominent in the posterior area of the brain at the level of the pons, medulla oblongata and cerebellum. These lesions were characterized by neuronal necrosis, mixed cell perivascular aggregation and gliosis. Intranuclear inclusions were identified in the hepatocytes (Fig. 5) and there was increased severity of degenerative change and inflammatory cell infiltration of the myenteric plexuses.

At 120 hpi, severe meningoencephalitis was present. This was characterized by neuronal necrosis, gliosis and perivascular lymphocyte aggregation. Intranuclear inclusions were observed in the brain. These changes extended to the more anterior parts of the cerebral cortex and hippocampus.

At 144 and 168 hpi there was severe meningoeyelitis and meningoencephalitis affecting most of the CNS, including the olfactory bulb, and severe necrosis and inflammation of the hepatic parenchyma.

No abnormalities were observed in organs other than the CNS and liver except for moderate interstitial pneumonia in the lungs (at 36 hpi). The pulmonary changes consisted of diffusely thickened alveolar septa with infiltration of these by varying numbers of macrophages and neutrophils. In addition to the presence of bronchitis/bronchiolitis with multifocal mucosal necrosis, findings included degeneration and desquamation of the epithelial cells with abundant inflammatory cell infiltration of the mucosa extending to the lumina of airways.

**Immunohistochemistry.** The distribution of EHV-9 antigens in the different parts of the body and the CNS is summarized in Table 2. A representative sagittal section is shown in Fig. 6.

At 6 hpi, EHV-9 antigen was observed in the peritoneal macrophages (Fig. 7) and this labelling became more intense in cells collected at 12 hpi. Virus antigen was also detected in peripheral nerves within the abdominal cavity at 12 hpi (Fig. 8). At 24 hpi there was immunoreactivity in the pseudounipolar nuclei of the peripheral ganglia of the abdominal cavity (particularly the coeliac ganglia; Fig. 9) and in the axons of the peripheral nerves. At 36 hpi, virus antigen was detected in the dorsal root ganglia and there was light labelling of the meninges of the spinal cord. At 48–72 hpi, EHV-9 antigen was found in most of the neurons (either in the cytoplasm or in the nuclei).

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**Equine Herpesvirus-9 Infection of Hamsters**

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**Table 2.**
in the spinal cord (Fig. 10), and frequent inclusions were present in the neurons of the dorsal root ganglia, with early detection of EHV-9 in the myenteric plexuses at 72 hpi.

At 96 hpi, virus antigen was observed in nearly all parts of the spinal cord and in the more posterior parts of the brain, particularly the medulla oblongata, pons and cerebellum (Fig. 11). In addition, virus antigen was detected in the myenteric plexuses of the intestine (Fig. 12). At 120 and 144 hpi, EHV-9 antigen was detected in the more anterior parts of the brain and at 168 hpi, intense expression of virus antigen was observed in all parts of the brain, including the olfactory bulb.

Immunohistochemical detection of EHV-9 antigen within the liver is summarized in Table 2. Antigen was found within hepatocytes near Glisson’s capsule of the liver at 36 hpi. From 48 hpi until the end of the experiment, virus antigen was detected in the hepatic parenchyma and intranuclear inclusions were present within hepatocytes (Fig. 13).

**Polymerase Chain Reaction**

Detection of EHV-9 DNA by PCR is summarized in Table 3. EHV-9 DNA was detected in the spinal cord at 36 hpi and continued to be present in this tissue until the end of the experiment. DNA was first detected in brain samples at 96 hpi and was then present in this tissue until the end of the experiment. Virus DNA was detected in blood samples at 48 hpi and for 4 days thereafter.

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Numerous studies have suggested that infection with EHV-9 may be an emerging infectious disease because the virus produces a fulminant encephalitis that is easily transmitted via nasal inoculation in various animal species including mice and hamsters (Fukushi et al., 2000), goats (Taniguchi et al., 2000), dogs and cats (Yanai et al., 2003a, b) and common marmosets (Kodama et al., 2007). In addition, natural infections with EHV-9 have been reported recently in a polar bear (Donovan et al., 2009) and a giraffe (Schrenzel et al., 2008).

Previous studies have shown that EHV-9 encephalitis may be induced experimentally in hamsters following inoculation of virus by the ocular, oral or intraperitoneal routes. However, these investigations have not defined the primary site of virus attachment and propagation or the detailed pathogenesis of infection (El-Habashi et al., 2010b).

In the present study, we first inoculated young adult Syrian hamsters intraperitoneally with EHV-9 to study the infectivity and pathogenesis, but it was difficult to examine the spinal cord and the associated peripheral nerves and ganglia because of the calcified vertebrae and skull. Therefore, we used a suckling hamster model, which had been the subject of a previous study elucidating the pathogenesis of EHV-9 infection following nasal inoculation (El-Habashi et al., 2010a).

To confirm virus propagation at the site of inoculation, peritoneal macrophages were collected at 6–24 hpi. These cells were shown to express EHV-9 antigen by ICC and this was interpreted to mean that these cells may be the primary site of virus attachment and propagation after intraperitoneal inoculation. Moreover, the virus may then spread from the peritoneal macrophages to the peripheral nerves and ganglia in the abdominal and pelvic cavities. The role of peritoneal macrophages in the transmission of viruses has been described in studies of herpes simplex virus type 1 (Hirsch et al., 1970; Letterio et al.,

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### Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Virus antigen labelling pattern</th>
<th>6hpi (4)</th>
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<td>+</td>
<td>ND</td>
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<tr>
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<tr>
<td>Spinal cord</td>
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<td>ND</td>
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<tr>
<td>Brain</td>
<td>Migration</td>
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<td>Liver</td>
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–, negative; +, positive; ND, not detected.

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Fig. 6. Sagittal section of the entire body of a suckling hamster from the neck to the tail showing all internal organs. Note marked antigen expression in the liver. IHC. Bar, 10 mm.

Fig. 7. Expression of EHV-9 antigen in peritoneal macrophages (arrow) at 6 hpi. ICC. Bar, 100 μm.
After this virus replicated in the peritoneal macrophages it extended to the gut plexuses (mainly the myenteric plexuses) and was then transmitted to the CNS (Letterio et al., 1983). In the present study, EHV-9 was similarly transmitted via the neuronal, rather than the haematogenous, route.

Based on the findings of the present study, it is possible that after initial propagation in macrophages, EHV-9 infects axons of the peripheral nerves and the coeliac plexuses, which connect to ganglia located at the level of the first lumbar vertebra. Infection of the coeliac plexuses, which include a number of smaller plexuses such as the hepatic, splenic, gastric, pancreatic and renal plexuses, may facilitate the spread of infection to most of the abdominal cavity.

In comparison with EHV-1, which was transmitted to the CNS via either the haematogenous or the neuronal route (Doll et al., 1953; Awan et al., 1990), the present study has shown that EHV-9 DNA is not found in the blood until 48 hpi. Moreover, in the present study, virus DNA was found in the spinal cord by 36 hpi and there was immunohistochemical expression of virus antigen in the peripheral nerves and ganglia by 12 hpi. These findings support the hypothesis that EHV-9 follows a neuronal pathway to gain access to the brain via the motor or sensory nerves, rather than spreading by the haematogenous route. In addition, the dorsal root ganglia showed degenerative change or necrosis at the time of immunohistochemical expression of EHV-9 antigen (36 hpi), which also suggests neuronal, rather than

Fig. 8. Expression of EHV-9 antigen in the peripheral nerve (arrowhead) adjacent to the adipose tissue surrounding the kidney at 12 hpi. IHC. Bar, 50 μm.

Fig. 9. Expression of EHV-9 antigen in the nucleus and cytoplasm of neurons (arrowheads) in abdominal ganglia at 24 hpi. IHC. Bar, 100 μm.

Fig. 10. Expression of EHV-9 antigen in neurons of the spinal cord of a suckling hamster at 72 hpi. IHC. Bar, 200 μm.

Fig. 11. (a) Expression of EHV-9 antigen in neurons of the posterior area of the brain at 96 hpi. IHC. Bar, 10 mm. (b) Expression is predominantly localized to the medulla oblongata, pons and cerebellum (arrowheads). IHC. Bar, 500 μm.
haematogenous transmission of the virus. Spinal cords from hamsters infected with EHV-9 exhibited varying degrees of meningomyelitis, which appeared from 36 hpi until termination of the experiment. Infection in the spinal cord was characterized by lymphocytic meningitis and perivascular lymphocyte aggregation, as well as neuronal degeneration and gliosis (mostly found in grey matter), starting from the lumbar and sacral regions and then extending to all parts of the spinal cord. Meningomyelitis affecting the spinal cord has been reported in association with infection by other neurotropic viruses, such as rabies virus [Kojima et al., 2009], pseudorabies virus [Rinaman et al., 1993] and Borna disease virus [Carbone et al., 1989], while others are transmitted via the haematogenous route (among them are rabies virus [Kojima et al., 2009], pseudorabies virus [Rinaman et al., 1993] and Borna disease virus [Carbone et al., 1989], while others are transmitted via the haematogenous route (e.g. EHV-1 [Tyler and Field, 1996] and myxovirus [Takano et al., 1991]). A neuroadapted strain of EHV-1 (NHH1) and pseudorabies virus was found to have been transmitted in anterograde fashion to reach the brain after intraperitoneal inoculation and to induce encephalitis. In other experimental viral infections (e.g. with herpes simplex virus type 1), virus antigen was detected in myenteric plexuses following intraperitoneal inoculation or natural infection, suggesting that spread of the virus progresses through abdominal plexuses to reach the CNS (Ezura et al., 1995; Hasebe et al., 2002). These findings are consistent with the results of the present study, in which detection of virus antigen in myenteric plexuses at 72 hpi indicated virus replication. These plexuses, consisting of numerous ganglia and connecting fibres, lie between the longitudinal and circular muscle layer of the gastrointestinal tract from the oesophagus to the anus. Thus, based on the present results, we postulate that the virus reached these plexuses, in particular the intestinal myenteric plexus, by the aid of peritoneal macrophages, and that this was followed by virus replication and spread to the brainstem via the vagus nerve. In the present cases, no significant microscopical lesions or EHV-9 antigens were found in the mucosal epithelium.

In the present study, the potential effects of EHV-9 on internal organs other than the nervous system were examined. EHV-1 has been detected in the internal organs of neonatal foals by PCR [Bilge et al., 2005] and bovine herpesvirus-4 [Gaetano et al., 2006] and

<table>
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<th>Sample</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brain</td>
<td>/C0/C0/C0/C0/C0/C0/C0/C0/C0/C0</td>
<td></td>
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Table 3
EHV-9 DNA detection by PCR in sucking hamsters

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bovine herpesvirus-2 have been shown to produce lymphohistocytic inflammation in the kidney and liver (Erica et al., 2009).

Focal hepatocyte degeneration and necrosis, as well as intranuclear inclusions, were found in the liver of the hamsters in the present study. Immunohistochemical labelling in the same areas suggested that the liver is a possible target organ for EHV-9 when inoculated via the intraperitoneal route. Previous studies of suckling mice have shown that the liver plays an important role in virus replication, especially during the initial stages of infection (Plummer et al., 1973).

In conclusion, the present study has characterized the pathogenesis of EHV-9 infection following intraperitoneal inoculation of the virus into young adult and suckling hamsters. After inoculation, EHV-9 may have propagated in the peritoneal macrophages before being transmitted to the peritoneal peripheral nerves. The virus may then gain access to the abdominal ganglia and the dorsal root (spinal) ganglia, where it invades the spinal cord, resulting in meningoencephalitis. At the same time, the virus appears to be transmitted to the myenteric plexuses in the intestine. In the final stage of infection, EHV-9 reaches the brain (where it induces encephalitis) by means of ascending transmission via the spinal cord. The present study is also the first to describe localization of EHV-9 in the liver following inoculation of hamsters by the intraperitoneal route.

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Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other persons or organizations that could inappropriately influence or bias the content of the paper.

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