informa
healthcare

Experimental intranasal infection of equine herpesvirus 9 (EHV-9) in suckling hamsters: Kinetics of viral transmission and inflammation in the nasal cavity and brain

Nagwan El-Habashi,[1] El-Shaymaa El-Nahass,[1] Hideto Fukushi,[2] Daisuke Hibi,[1] Hiroki Sakai,[1] Vito Sasseville,[3] and Tokuma Yanai[1]

[1]Department of Veterinary Pathology; [2]Department of Veterinary Microbiology, Faculty of Applied Biological Science, Gifu University, Gifu, Japan; and [3]Discovery Toxicology, Bristol-Myers Squibb (VS), Boston, USA

Equine herpesvirus 9 (EHV-9), the newest member of the equine herpesvirus family, is a highly neurotropic herpesvirus that induces encephalitis in a variety of animals. To access transmission of EHV-9 in the nasal cavity and brain, a suckling hamster model was developed so that precise sagittal sections of nasal and cranial cavities including the brain could be processed, which proved useful in detecting viral transmission as well as extension of pathological lesions. Suckling hamsters were inoculated intranasally with EHV-9, and were sacrificed at 6, 12, 18, 24, 36, 48, and 60 h post inoculation (PI). Sagittal sections of the entire head, including nasal and cranial cavities including the brain, were made to assess viral kinetics and identify the progress of the neuropathological lesions. At 12 to 24 h PI the virus attached to and propagated in the olfactory epithelium, and infected adjacent epithelial cells. At 48 h PI, immunohistochemistry for EHV-9 viral antigen showed that virus had extended from the site of infection into the olfactory bulb and olfactory nerve. These results indicate that EHV-9 rapidly invades the brain via the olfactory route after experimental intranasal infection. *Journal of NeuroVirology* (2010) **16**, 242–248.

Keywords: EHV-9; neuropathogenesis; olfactory pathway; suckling hamsters

Introduction

Equine herpesvirus 9 (EHV-9), the newest member of the equine herpesvirus family, is a highly neurotropic herpesvirus firstly described in an outbreak of disease in Thomson's gazelles (*Gazella thomsoni*) that died of fulminant encephalitis (Fukushi *et al*, 1997). Serologically, EHV-9 is most closely related to the recently emergent neurotropic pathogen, EHV-1, but its DNA fingerprint is different from that of EHV-1 and other equine herpesviruses.

Recently, the EHV-9 virus and a virus serologically similar to EHV-9 were proven to have a high seroprevalence of 60% in free-living zebras in Serengeti National Park, which suggests that zebras might be a natural host of EHV-9, and that there might be a possibility of widespread exposure to the EHV-9 virus within zebra populations in the wild (Borchers *et al*, 2005).

Many experimental studies on the infectivity of EHV-9 using rodents, domestic animals, and a new-world monkey have been conducted. Emerging EHV-9 infection was considered a concern, because of the wide range of susceptible hosts and the ease of transmission by the nasal route, which is thought to be the most probable route of transmission in the field. Recently, EHV-9 was detected in a polar bear with progressive encephalitis as well as in a giraffe

EHV-9 acute nasal infection
N El-Habashi *et al*
243

(Schrenzel *et al*, 2008; Donovan *et al*, 2009; Kasem *et al*, 2008), raising fears of emerging infections in various wild and domestic animal species. Although EHV-9 was shown to infect a broad range of animals, including mice, rats (Fukushi *et al*, 1997), hamsters (Fukushi *et al*, 2000), goats (Taniguchi *et al*, 2000b), pigs (Narita *et al*, 2000), dogs and cats (Yanai *et al*, 2003a, 2003b), and common marmosets (Kodama *et al*, 2007), it remains unknown how the virus travels from the nasal cavity to the brain, or how long this progression takes. Because the Syrian hamster has been reported to be a useful model for studying the pathogenesis of EHV-9 infection by the nasal route (Fukushi *et al*, 2000), we recently conducted a study in this model to clarify acute infection of EHV-9. The results showed that it took 48 h for the virus to travel from the nasal mucosa to the olfactory bulb (data not yet published), but it was still not clear how EHV-9 moved from the olfactory epithelium, through the ethmoid turbinate and septa, to the olfactory bulb. Precise sagittal sections of the nasal cavity, the ethmoid turbinate and septa, and the olfactory bulb were needed to accurately detect and stage the kinetics of viral infection from the nasal cavity to the brain, but the calcified cranial bone in adult hamsters created significant technical obstacles. Thus, to resolve this technical difficulty, we used suckling hamsters, since they have a very thin and soft skull and bones making precise histologic sagittal sections of the nasal and cranial cavity feasible. This model provided a panoramic view of the entire head, including the nasal and cranial cavities and the brain in the same section. Suckling hamsters have been used in some studies in the past to investigate pathogenesis in various viruses. The suckling hamster was used for studying mumps virus pathogenicity and the development of hydrocephalus through intracerebral inoculation (Kilham and Overman, 1952; Takano *et al*, 1991), and Doll et al (1953) adapted EHV-1 to suckling hamsters.

In the present study, we used the suckling hamster to enable sagittal sectioning of the head, including the nasal cavity, the ethmoid turbinate and septa, and the brain. The panoramic view obtained from sagittal sectrions enabled a focused study on the dynamic kinetics of EHV-9 from nasal infection to induction of acute encephalitis.

Results

The majority of inoculated hamsters showed various degrees of clinical signs that included depression and uncoordinated movement starting at 48 h post inoculation (PI). By 60 h PI, the end of the in-life portion of the study, all of the animals had severe incoodination of the movement and depression.

Histopathology
Sagittal sections of suckling hamster made it easy to view the nasal cavity and brain in a single histologic section (Figure 1). Histopathological changes in the olfactory epithelium and the brain in EHV-9 inoculated animals are summarized in Table 1 and as follows:

- At 6 h PI: There were no significant findings except for detachment of the superficial microvillus in some areas, along with a few infiltrations of inflammatory cells, including neutrophils and lymphocytes, between olfactory epithelial cells and in the lumen of the nasal cavity.
- At 12 h PI: There were varying degrees of necrosis observed in the columnar olfactory epithelial cells. In addition, there was a slight degree of irregularity on the surface of the olfactory epithelium, as well as varying degrees of ablation of the superficial microvillus.
- At 18 h PI: Prominent necrosis of the olfactory epithelial cells was observed, as well as vacuolization of the olfactory receptor neurons and infiltration of the lamina propria by inflammatory cells, including neutrophils and lymphocytes.
- At 24 h PI: The surface of the olfactory epithelium had become more irregular, with frequent necrosis of the olfactory epithelial cells, which became desquamated into the lumen in some areas. A complete ablation of the microvillus was often observed. In and around the olfactory mucosa of the nasal cavity, there were frequent clusters of neutrophils, some of which were desquamated into the lumen.
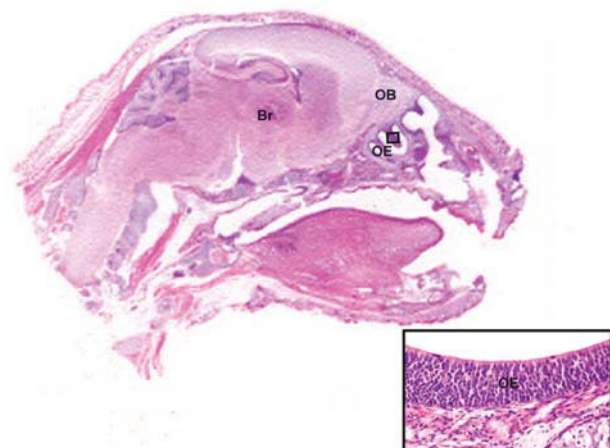

Figure 1 Sagittal section showed the head from the nose to the whole brain, it is easy to check the connection between olfactory bulb (OB), brain (Br), and olfactory epithelium (OE) and to examine the cranial nerves and ganglion. *Inset*: Higher magnification showing normal intact olfactory epithelium (OE) of control uninfected animals.

Table 1 Histopathological changes observed in the olfactory epithelium and brain in EHV-9–inoculated animals

Tissue	Lesion	h PI (*n*)*						
		6 (3)	12(3)	18(3)	24 (3)	36(3)	48 (3)	60(3)
Olfactory epithelium	Irregularity	−#	+	++	++	+++	+++	+++
	Necrosis	−	+	+	++	+++	+++	+++
	Infiltration of inflammatory cells	+	+	++	++	++	+++	+++
	Erosion	−	−	−	+	++	++	+++
Brain	Encephalitis	−	−	−	−	−	++	+++
	(Perivascular cuffing, gliosis, neuronal necrosis)							

*h PI = hour post inoculation; *n* = number of sacrificed animals at this hour post inoculation.#Histopathologic score: −, none; +, slight; ++, moderate; +++, severe.

- At 36 h PI: A severe irregularity of the olfactory epithelium surface was noticed, as well as frequent focal necrosis and erosion of the olfactory epithelium.
- At 48 h PI: Animals with clinical signs had varying degrees of lymphocytic meningoencephalitis in the olfactory bulb, which consisted of neuronal necrosis, mixed-cell perivascular aggregates, gliosis, intranuclear inclusion bodies, and diffuse lymphocytic infiltrates in the meninges. In addition, the olfactory epithelium showed severe and wide erosion in the mucosa as well as significant neutrophil infiltration (Figure 2). The trigeminal ganglion showed neuronal necrosis and neuronophagia of the trigeminal ganglion cells (Figure 3) in addition to a few infiltrations of neuroglial cells and lymphocytes around the trigeminal ganglion cells.
- At 60 h PI: There was complete erosion in the nasal mucosa (Figure 4); in addition, the encephalitis was progressing to the frontal and temporal lobes of the cerebral cortex of the brain as well as the pons and medulla oblongata.

No abnormalities were found in other organs except for moderate interstitial pneumonia (48 h PI). The pulmonary changes consisted of diffusely thickened alveolar septa with varying numbers of macrophages and neutrophils. Also, there was bronchitis/bronchiolitis characterized by multifocal mucosal necrosis, along with degeneration and desquamation of the epithelial cells with abundant inflammatory cell infiltrates within the mucosa and in airway lumen.

Immunohistochemistry
Immunohistochemical staining with EHV-9 antigen was used to determine viral kinetics of transmission from the site of inoculation to the brain. Immunohistochemical detection of EHV-9 in the olfactory epithelium and the brain are summarized in Table 2 and as follows:
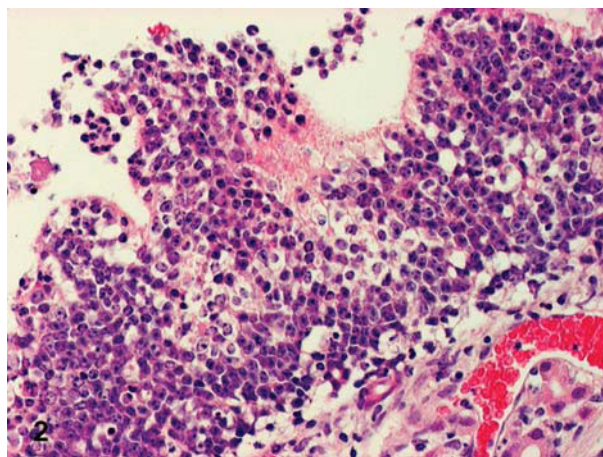

Figure 2 At 48 h PI, the olfactory epithelium showed irregularity, necrosis, and erosion in the mucosa, together with desquamation of clusters of neutrophils in the lumen. H&E stain.
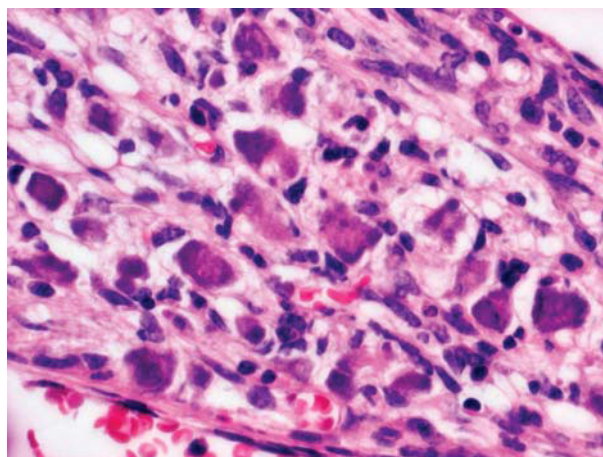

Figure 3 At 48 h PI, The trigeminal ganglion showed neuronal necrosis, neuronophagia of the trigeminal ganglion cells. H&E stain.

- At 12 h PI: Positive reactions in a small number of neurons scattered through the olfactory mucosa. Other cells of the olfactory epithelium and lamina propria showed no immunoreactivity.
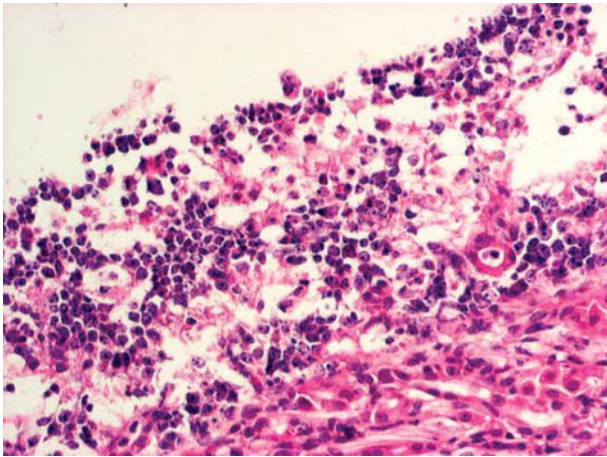
Figure 4 At 60 h PI, the olfactory epithelium showed complete erosion of the mucosa, together with desquamation of clusters of neutrophils in the lumen. H&E stain.

- At 18 h PI: There was frequent immunoreactivity in the neurons in the olfactory epithelium.
- At 24 h PI: There was frequent and more intense immunoreactivity in the neurons in the olfactory epithelium. No positive reactions were observed in other cells in the olfactory epithelium, lamina propria, or olfactory bulbs.
- At 48 h PI: EHV-9 antigen was found in most of the neurons in the olfactory epithelium. There were frequent intense positive reactions in the cell debris in the nasal cavity, as well as the central processes of the olfactory epithelial neurons, olfactory nerve, and the olfactory bulb (Figure 5A, B, C). The trigeminal ganglia, as well as the trigeminal nerve, showed mild reactions for viral antigen.
- At 60 h PI: EHV-9 antigen was found in most of the neurons in the olfactory epithelium. The central processes of the olfactory epithelial neurons, olfactory nerve, and olfactory bulb showed strong positive reactions. In addition, the viral antigen was detected in the frontal and temporal lobes of the cerebral cortex. One of the most striking findings

was the presence of the viral antigen in the connection of the trigeminal sensory nerve root to the brain stem (Figure 6), the pons and the medulla oblongata, as well as weak positive reactions in the trigeminal nerve. Distribution of the viral antigen in brain sagittal sections after 48 h and 60 h PI are depicted in Figures 7 and 8, respectively. The trigeminal ganglia showed strong reactivity for viral antigen.

Discussion

The possibility of EHV-9 becoming an emerging infection has been demonstrated by previous experimental infectious studies by nasal inoculation in many different animals species, including mice, rats (Fukushi *et al*, 1997), hamsters (Fukushi *et al*, 2000), goats (Taniguchi *et al*, 2000b), pigs (Narita *et al*, 2000), cats and dogs (Yanai *et al*, 2003a, 2003b), and common marmosets (Kodama *et al*, 2007). Recently, natural infection by EHV-9 was observed in a polar bear with progressive encephalitis as well as in a giraffe (Schrenzel *et al*, 2008; Donovan *et al*, 2009; Kasem *et al*, 2008). EHV-9 was proved to have a broad range of hosts, as well as being a possible emerging infection in various wild and domestic animal species.

In a previous study in which the authors examined the acute pathogenicity of EHV-9 in the nasal cavity and the brain using adult hamsters, the time-course histopathological alterations induced in the nasal cavity and brain within 48 h PI were investigated (data not published), but it was unclear how the virus gained access to the brain or which routes the virus takes after leaving the olfactory epithelium and passing through the ethmoid turbinate and septa to the brain. However, it was very difficult to observe the cranial nerves in the ethmoid turbinate and septa, as well as the trigeminal ganglion, because of hard calcified cranial bones in adult hamsters. The panoramic view through the olfactory epithelium, olfactory nerve, and the brain was enabled by using suckling hamster model. By using the present model, it became possible to detect the kinetics of viral

Table 2 Viral detection by immunohistochemistry in the olfactory epithelium (viral attachment and migration), olfactory nerve, and brain in EHV-9–inoculated animals

Tissue	Viral staining pattern	h PI (*n*)*						
		6 (3)	12(3)	18(3)	24 (3)	36(3)	48 (3)	60(3)
Olfactory epithelium	Attachment	− [#]	+	+	+	+	+	−
	Migration, propagation	−	−	+	++	++	+++	+++
Olfactory nerve	Migration	−	−	−	−	−	++	++
Brain	Intranuclear inclusions, migrations							
Olfactory bulb		−	−	−	−	−	++	+++
Cerebral cortex		−	−	−	−	−	−	++
Pons, Medulla oblongata		−	−	−	−	−	−	++

*h PI = hour post inoculation; *n* = number of sacrificed animals at this hour post inoculation.[#]Presence of the viral antigen: −, absent; +, few (<5/40× field); ++, moderate (5–30/40× field); +++, numerous (>30/40× field).
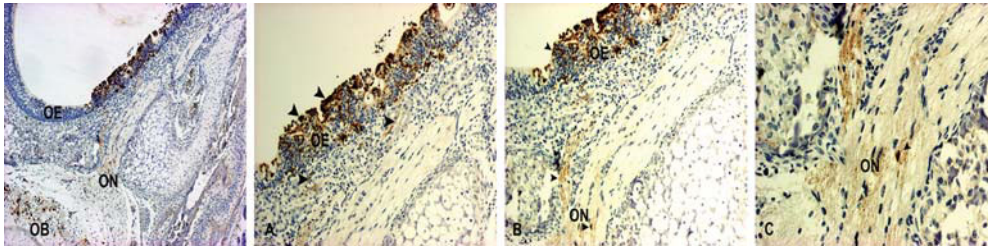
Figure 5 (*Left panel*) Results of immunolabeling with EHV-9 antibody at 48 h PI. Positive reaction was detected in most of neurons, the central processes of the olfactory epithelial neurons in the olfactory epithelium (OE), olfactory nerve (ON) and glomeruli of the olfactory bulb (OB). ABC method. (**A**) Results of immunolabeling with EHV-9 antibody at 48 h PI. Positive reaction (*arrow*) was found in most of neurons as well as the central processes of the olfactory epithelial neurons in the olfactory epithelium (OE). ABC method. (**B**) Results of immunolabeling with EHV-9 antibody at 48 h PI. Positive reaction (*arrow*) was found in most of neurons, the central processes of the olfactory epithelial neurons in the olfactory epithelium (OE) as well as the olfactory nerve (ON). ABC method. (**C**) Results of immunolabeling with EHV-9 antibody at 48 h PI. Higher magnification of the left panel, showing positive reactivity (*arrow*) in the olfactory nerve (ON) at its entry to olfactory bulb. ABC method.
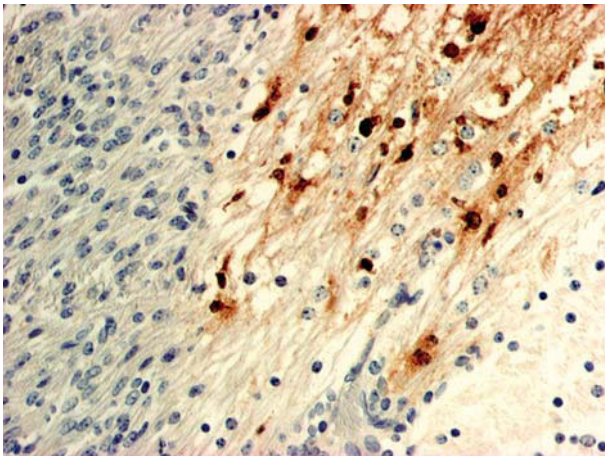

Figure 6 Results of immunolabeling with EHV-9 antibody at 60 h PI. Presence of the viral antigen in the connection of the trigeminal sensory nerve root to the brain stem. ABC method.
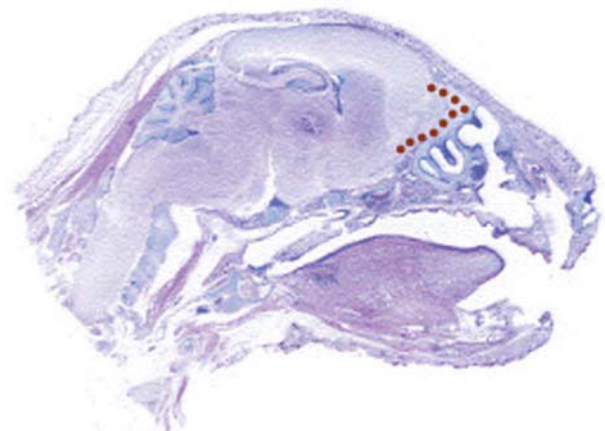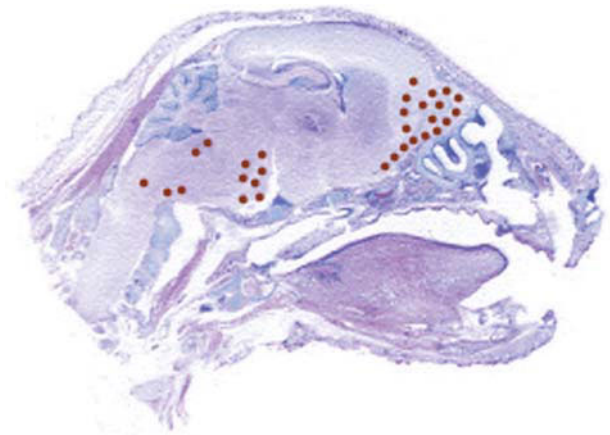

Figure 8 Distribution of the viral antigen in brain sagittal section at 60 h PI. Positive reaction was found in the olfactory bulb as well as the brain stem.

Figure 7 Distribution of the viral antigen in brain sagittal section at 48 h PI. Positive reaction was found in the olfactory bulb.

transmission in the cranial nerves involved in the hard cranial bones in EHV-9–infected hamsters, and this model may be useful in investigating other neuropathogenic viruses.

EHV-9 antigen was detected by immunohistochemistry in the olfactory receptor neurons at 12 h PI. At 48 h PI, it was found in most of the olfactory receptor neurons as well as in the central processes of the olfactory epithelial neurons, olfactory nerve, and olfactory bulb. The olfactory neuroepithelium may be the most probable portal of virus entry based on time-course transition of the lesions and distribution of the virus antigen at the sagittal view.

After infecting the olfactory receptor neurons, the virus moves to the olfactory bulb along the central processes of the olfactory epithelial neurons to the olfactory nerve. An initial infection of neuronal cells is essential for induction of encephalitis. A similar transneural passage was suggested after intranasal infection of hamsters and pigs with EHV-9 and mouse with EHV-1 as well as Bornavirus and rabies

virus infection (Fukushi *et al*, 2000; Narita *et al*, 2001; Gosztonyi *et al*, 2009, 1993).

Previously, EHV-9 antigen was found in the neurons and neural fibers but not in the glial cells in the brain, indicating that neurons are the susceptible cells to EHV-9 in the central nervous system of hamsters (Fukushi *et al*, 2000). However, other neurotropic herpesviruses such as herpes simplex virus 1 (HSV-1) and pseudorabies virus (PRV) are known to infect glial cells as well as neurons (Johnson, 1998). In the present study, there was necrosis of some of trigeminal ganglion cells as well as detection of the viral antigen in the same ganglion and in the connection between trigeminal sensory nerve root and the brain stem at the level of the pons and medulla oblongata.

Based on previous findings in hamsters and the present experiment, the neurotropism might be the most characteristic property of EHV-9, differentiating it from other neurotropic herpesviruses. In the present study, the EHV-9 primarily gained access to the brain through the olfactory pathway (olfactory nerve) in addition to the trigeminal nerve through its first and second branches; the maxillary nerve, which carries sensory information from the nasal mucosa; the roof of the pharynx, the maxillary, ethmoid, and sphenoid sinuses; and the ophthalmic nerve, which carries sensory nerve fibers to the nasal mucosa, and this explains how the virus gains access from the trigeminal nerve to the pons and medulla oblongata.

The olfactory neuroepithelal cells offer direct free surface on the internal lining of the nasal cavity, whereas the terminal nerve endings of the maxillary branch of the trigeminal nerve lie in the submucosa and could be only infected with the virus if the epithelial surface is damaged and consequently the axons are directly exposed to the virus; this was suggested from our experiment by late access of the virus to the trigeminal nerve, pons, and medulla oblongata, at 60 h PI, after the presence of complete erosions of the olfactory epithelium. Similar transneural passage was suggested after intranasal infection with the infectious bovine rhinotracheitis (IBR) virus.

Trigeminal ganglionitis, consisting of focal aggregations of the neuroglia cells along with lymphocytic perivascular cuffing, was observed in the medulla oblongata of calves inoculated with IBR virus and is the most characteristic lesion of intranasal infection of IBR virus. The involvement of the trigeminal ganglion is suggested as the result of centripetal spread of the virus along the peripheral nerve from the nasal mucosa (Narita *et al*, 1979). For the neurotropic herpesvirus, several routes of virus entry into the central nervous system have been considered, which include the neural, olfactory, and hematogenous routes (Johnson, 1998). Other neurotropic herpes viruses, including HSV-1 and PRV, may enter the central nervous system via other routes, including intravenous, intramuscular, and intraperitoneal inoculations (Johnson, 1998).

Previously, EHV-9 induced encephalitis in hamsters through different routes of inoculation,

including the oral, ocular, and peritoneal routes; however, the intravenous route failed to induce encephalitis and there were differences in the distribution of antigen-positive cells and in the location and severity of the cerebral lesions, indicating the possibility of transmission of the virus through regional nerves (non-olfactory route), after initial propagation at the site of viral entry (El-Habashi *et al*, 2010). Further investigation using the sagittal section model of the suckling hamsters will be beneficial in explaining how the virus gains access to the brain through different inoculation routes.

The present study provides important information on the pathogenesis of EHV-9 induced encephalitis by nasal inoculation. This sagittal section animal model may be useful for studying the pathogenesis and forms of neurovirulence in various neurotropic viruses as well as EHV-9, as it enables follow-up of the viral dynamics in the body as a whole.

Materials and methods

Animals
Seven Syrian hamsters (*Mesocricetus auratus*) at gestional day 10 were purchased from a commercial breeder (SLC, Hamamatsu, Japan). The animals were housed in an isolated biohazard cabinet and were fed a basal pellet diet (Oriental MF, Oriental Yeast, Tokyo, Japan) and bottled water *ad libitum* until they gave birth, after which they were left to rear the pups until the end of the experiment. This experiment was conducted in accordance with the pertinent laws and regulations on the treatment and use of laboratory animals. The experiment protocol was approved by the Animal Experiment Committee for Animal Welfare in the Faculty of Applied Biological Science at Gifu University.

Inoculation of virus
Twenty-six 9-day-old suckling hamsters were inoculated intranasally with 15 μl (10^6 plaque-forming units [PFU]) of EHV-9 virus solution. Three animals each were sacrificed at 6, 12, 18, 24, 36, 48 and 60 h post inoculation (PI) or when they were moribund. Five uninfected animals were kept as a control group. The animals were checked for clinical signs at least twice daily, in the morning and afternoon.

Necropsy, histopathology, and immunohistochemistry
A necropsy animals were bisected on the sagittal plane from rostrum to tail and then the bisected bodies were fixed in 10% neutral-buffered formalin. After fixation, the head and body were separated, dehydrated, and embedded in paraffin wax by routine methods, and then sectioned at 5 μm, stained with hematoxylin and eosin (H&E), and examined by light microscopy. The bones, including the skulls, of the

suckling hamster were soft enough to prepare histo-logical slides with good quality and no decalcifica-tion. In addition to histopathologic examination of the nasal cavity and brain, the following organs/tissues were also examined: lung, liver, kidneys, small and large intestines, and spleen.

Paraffin-embedded sections of the head were immunolabeled with EHV-9 rabbit antiserum by the avidin-biotin-complex (ABC) immunoperoxidase method, as described previously (Yanai *et al*, 2003b), with ABC kits (Vector Laboratories, Burlingame, CA, USA). The primary antibody was EHV-9 antiserum (1:800, Veterinary Microbiology Laboratory at Gifu University) followed by application of a secondary antibody (biotinylated anti-rabbit immunoglobulin G [IgG], DAKO Cytomation, USA) with Liquid DAB Substrate Chromogen System (DAKO) used as the chromogen and hematoxylin counterstain. Tissue sections from the EHV-9–infected hamsters and sera from a nonimmunized rabbit and goat were used as controls.

Declaration of interest: The authors report no con-flicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Borchers K, Wiik H, Frolich K, Ludiwig H, East ML (2005). Antibodies against equine herpesviruses and equine arteritis virus in Burchell's zebra (*Equus Burchelli*) from the Serengeti ecosystem. *J Wildl Dis* **41:** 80–86.

Doll ER, Richards MG, Wallace ME (1953). Adaptation of the equine abortion virus to suckling syrian hamsters. *Cornell Vet* **42:** 551–558.

Donovan TA, Schrenzel MD, Tucker T, Pessier AP, Bicknese B, Busch MD, Wise AG, Maes R, Kiupel M, McKnight C, Nordhausen RW (2009). Meningoenceph-alitis in a polar bear caused by equine herpesvirus 9 (EHV-9). *Vet Pathol* **46:** 1138–43.

El-Habashi N, Murakami M, EL-Nahass E, Hibi D, Sakai H, Fukushi H, Sasseville V, Yanai T (2010). Study on the infectivity of equine herpesvirus 9 (EHV-9) by different routes of inoculation in hamsters. *Vet Pathol*: In press.

Fukushi H, Taniguchi A, Yasuda K, Yanai T, Masegi T, Yamaguchi T, Hirai K (2000). A hamster model of equine herpesvirus 9 induced encephalitis. *J NeuroVirol* **6:** 314–319.

Fukushi H, Tomita T, Taniguchi A, Ochiai Y, Kirisawa R, Matsumura T, Yanai T, Masegi T, Yamaguchi T, Hirai K (1997). Gazelle herpesvirus 1: a new neurotropic her-pesvirus immunologically related to equine herpesvirus 1. *Virology* **227:** 34–44.

Gosztonyi G, Dietzschold B, Kao M, Rupprecht CE, Ludwig H, Koprowski H (1993). Rabies and Borna disease: a compar-ative pathogenetic study of two neurovirulent agents. *Lab Invest* **68:** 285–295.

Johnson RT (1998). *Viral infections of the nervous system*, 2nd ed. Philadelphia: Lippincott-Raven Publishers.

Kasem S, Yamada Souichi, Kiupel Matti, Woodruff Mary, Ohya Kenji, Fukushi Hideto (2008). Equine herpesvirus type 9 in giraffe with encephalitis. *Emerg Infect Dis* **14:** 1948–1949.

Kilham L, Overman JR (1953). Natural pathogenicity of mumps virus for suckling hamsters on intracerebral inoculation. *J Immunol* **70:** 147–151.

Kodama A, Yanai T, Yomemaru K, Sakai H, Masegi T, Yamada S, Fukushi H, Kuraishi T, Hattori S, Kai C (2007). Acute neuropathogenicity with experimental infection of equine herpesvirus 9 in common marmosets (*Callithrix jacchus*). *J Med Primatol* **36:** 335–342.

Kritas SK, Pensaert MB, Mettenleiter TC (1994). Invasion and spread of single glycoprotein deleted mutants of Aujeszky's disease virus (ADV) in the trigeminal nervous pathway of pigs after intranasal inoculation. *Vet Microbiol* **40:** 323–334.

Narita M, Inui S, Namba K, Shimizu Y (1976). Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with infectious bovine rhinotracheitis virus. *J Comp Pathol* **86:** 93–100.

Narita M, Ishikawa Y, Inui S (1979). Neural changes in calves intranasally inoculated with two strains of infec-tious bovine rhinotracheitis virus. *J Comp Pathol* **89:** 489–494.

Narita M, Uchimura A, Kimura K, Tanimura N, Yanai T, Masegi T, Fukushi H, Hirai K (2000). Brain lesions and transmission of experimental equine herpesvirus type 9 in pigs. *Vet Pathol* **37:** 476–479.

Schrenzel MD, Tucker TA, Donovan TA, Martin DM, Busch MDM, Wise AG, Maes RK, Kiupel M (2008). New hosts for equine herpesvirus 9. *Emerg Infect Dis* **14:** 1616–1619.

Takano T, Ohno M, Yamano T, Shimada M (1991). Exper-imental Hydrocephalus in suckling hamster induced by myxovirus infection: pathogenesis of hydrocephalus caused by mumps virus. *Cong Anom* **31:** 115–128.

Taniguchi A, Fukushi H, Matsumura T, Yanai T, Masegi T, Hirai K (2000a). Pathogenicity of a new neurotropic equine herpesvirus 9 (gazelle herpesvirus 1) in horses. *J Vet Med Sci* **62:** 215–218.

Taniguchi A, Fukushi H, Yanai T, Masegi T, Hirai K (2000b). Equine herpesvirus 9 induced lethal encepha-litis in experimentally infected goats. *Arch Virol* **145:** 2619–2627.

Yanai T, Fujishima N, Fukushi H, Hirata A, Sakai H, Masegi T (2003a). Experimental infection of equine herpesvirus 9 in dogs. *Vet Pathol* **40:** 263–267.

Yanai T, Tsujioka S, Sakai H, Fukushi H, Hirai K, Masegi T (2003b). Experimental infection with equine herpesvirus 9 (EHV-9) in cats. *J Comp Pathol* **128:** 113–118.