Pregnant rats were infected experimentally with equine herpesvirus (EHV)-9, a new neurotropic equine herpesvirus serologically similar to EHV-1, during the first and third trimesters. The inoculated dams had mild to severe neurological signs and gave birth to dead fetuses or undersized pups. Rats inoculated during the first and last trimesters had varying degrees of encephalitis as well as abnormalities of the placentas in the form of marked dilation of maternal blood sinusoids and varying degrees of atrophy and necrosis of the trophoblast cells of the labyrinth, the spongiotrophoblasts and the giant cell layer. Virus antigen was detected by immunohistochemistry in the brain and the trophoblast cells of labyrinth, the spongiotrophoblasts and giant cell layer of the placenta in rats inoculated during the first trimester. Virus antigen was detected in fetuses from rats inoculated in the first and last trimesters. Virus DNA was amplified by polymerase chain reaction from the placenta and fetuses of inoculated rats. EHV-9 may induce fetal death and abortion in pregnant dams, possibly caused by direct EHV-9 infection of the placenta and/or fetus as well as the secondary effect of vascular injury.

Keywords: equine herpesvirus-9; pathogenicity; pregnancy; rat model

Introduction
Equine herpesvirus (EHV)-9 is a newly recognized, highly neurotropic herpesvirus that was isolated during an epizootic of encephalitis in Thomson’s gazelles (Gazella thomsoni) kept in a zoological garden (Fukushi et al., 1997; Yanai et al., 1998). The natural host of EHV-9 and the complete host range are still unknown. However, a member of the family Equidae is suspected to be a reservoir host of EHV-9. Recently, serological surveys have shown that EHV-9 circulates in Burchell’s zebras (Equus burchelli) in Tanzania without any associated illness (Borchers et al., 2003), but active infection has never been documented conclusively in any equid. Theoretically, equids might be the primary host of the virus as domestic horses (Equus caballus) exhibited mild encephalitis without accompanying deaths when inoculated intranasally with EHV-9 (Taniguchi et al., 2000a). Fatal acute encephalitis has been induced experimentally by infection with EHV-9 in mice and rats (Fukushi et al., 1997), hamsters (Fukushi et al., 2000), goats (Taniguchi et al., 2000b), pigs (Narita et al., 2000) and dogs and cats (Yanai et al., 2003a and 2003b). The neurotropic features of the virus were confirmed in all of these studies. Encephalitis was induced in pigs and hamsters inoculated by different routes with EHV-9 (Narita et al., 2000; El-Habashi et al., 2010). Recently, an epidemiological survey showed that Grevy’s zebras, polar bears (Schrenzel et al., 2008;
EHV-9-induced Abortion in Rats

Viral Culture

Madin–Derby bovine kidney (MDBK) cells were used for 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) in MDBK cells. The virus was titrated by plaque formation assay on MDBK cells.

Animals and Treatments

Fourteen 10-week-old female F344/NSic rats at gestational day 3 were purchased from a breeder (SLC Inc., Hamamatsu, Japan). The animals were divided into three groups and acclimatized for 1 and 11 days for the first and third groups, respectively. The first group (n = 6) was inoculated intranasally with 50 μl of minimum essential medium (MEM) containing 2 × 10^6 plaque-forming units (PFU)/ml of EHV-9 virus during the first trimester (i.e. on day 4 of pregnancy). The second group (n = 4) acted as a control and were inoculated intranasally with MEM. The third group (n = 4) was inoculated intranasally with 50 μl of MEM containing 10^6 PFU/ml of EHV-9 virus during the third trimester (i.e. on day 14 of pregnancy). The animals were killed when they developed severe neurological signs or become comatose or at the end of pregnancy (i.e. day 23 of pregnancy). The animals were examined for clinical signs and evidence of abortion several times daily.

The animals were housed in an isolated biohazard cabinet, fed basal pellets (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and given bottled sterilized water ad libitum. The experiment was conducted in accordance with pertinent laws and related standard operating procedures on the treatment and use of laboratory animals. The experimental protocol was approved by the Animal Experiment Committee of the Faculty of Applied Biological Science at Gifu University, Japan.

Necropsy Examination, Histopathology and Immunohistochemistry

A complete necropsy examination was performed immediately after death. The nasal cavity, brain, heart, lungs, liver, spleen, kidneys, stomach, small and large intestines, uterus, placentas, fetuses and live-born pups were collected and fixed in 7% neutral buffered paraformaldehyde. Tissues were processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE). Portions of the internal organs and brains from the fetuses and pups and the placentas were collected and stored at −80°C for detection of virus DNA by PCR.

Sections of the placenta, uterus and brain of the dams, in addition to sections of the entire fetuses and live-born pups, were immunolabelled with rabbit antisera specific for EHV-9 by the avidin–biotin complex (ABC) immunoperoxidase method (Yanai et al., 1998) using ABC kits (Vector Laboratories, Burlingame, California, USA). The EHV-9 antiserum was made in the Veterinary Microbiology Laboratory (a gift from Dr. H. Fukushi) and was used at a dilution of 1 in 800. After application of the secondary antibody (biotinylated anti-rabbit IgG; Dako, Carpenteria, California, USA), liquid 3, 3′ diaminobenzenidine (DAB) substrate chromogen system (Dako) was...
used as the chromogen. The sections were counterstained with haematoxylin. Tissue sections from EHV-9-infected hamsters and substitution of primary antiserum with sera from a non-immunized rabbit and goat were used as controls.

**Immunofluorescence Labelling**

Formalin-fixed and paraffin wax-embedded (FFPE) sections of the placenta, uterus and brain from dam number 3 (a case of abortion), in addition to the fetuses and live-born pups from all dams inoculated in the first or last trimesters, were immunolabelled by indirect immunofluorescence. Rabbit antiserum specific for EHV-9 and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG secondary antibody (Sigma Aldrich, St Louis, Missouri, USA) were applied after a modification of the technique described by Robertson *et al.* (2008). Antigen retrieval was performed by incubation with proteinase K (Dako) for 5 min. The primary antibody (as above) was used at a dilution of 1 in 800 followed by application of the secondary antibody at a dilution of 1 in 500. Labelling was assessed using a Keyence Biozero fluorescence microscope (BZ-8000; Keyence, Osaka, Japan). Tissue sections from the EHV-9-infected hamsters and substitution of primary antiserum with sera from a non-immunized rabbit and goat were used as controls.

**DNA Extraction and Polymerase Chain Reaction**

Fresh tissues (including the placenta and internal organs and brains of fetuses and live-born pups) were
subjected to DNA extraction with a SepaGene kit for virus DNA detection (Sanko Junyaku Co., Tokyo, Japan). Virus DNA was detected using primers for the open-reading frame (ORF) 76-F (5'0-TTT CCC TCT CAG CGA TCA CTT TTC ACC ACC GAA GAA CAG GCC CTC ATC GG-3') and ORF76-R (5'0-GGG CTG TTG TGG GGT AAA AGG TGG TGT TAC GGA 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++ 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

#### Clinical Signs, Gross Findings and Number of Fetuses

**Inoculation of First Trimester Rats.** All animals inoculated in the first trimester of pregnancy showed neurological signs, nasal discharge and ruffled fur. One animal (number 3) also had a serosanguineous vaginal discharge and hunched posture. This animal aborted seven undersized, poorly-developed fetuses on day 13 of pregnancy, 2 days after the onset of neurological signs. The ratio of dead to live fetuses was not easily determined in two animals due to the early stage of pregnancy; the other two animals gave birth to 6:6 and 0:8 dead fetuses:live pups. The last animal in this group had full-term pregnancy and gave birth to eight live, but undersized, pups.

**Inoculation of Third Trimester Rats.** Animals inoculated in the last trimester of pregnancy showed neurological

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Cerebral lesions</th>
<th>Virus antigen</th>
<th>Spongiotrophoblasts, giant-cell layer</th>
<th>Trophoblasts in labyrinthine zone</th>
<th>Maternal blood sinuoids</th>
<th>Fetal capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+++  +  -</td>
<td>+</td>
<td>+ (congestion)</td>
<td>+ (atrophy)</td>
<td>+++ (dilation)</td>
<td>+ (congestion)</td>
</tr>
<tr>
<td>2</td>
<td>+     +  +</td>
<td>+</td>
<td>+++ (congestion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+     +  +</td>
<td>+</td>
<td>+ + + (necrosis, atrophy)</td>
<td>+ + + (necrosis, atrophy)</td>
<td>+ + + (dilation)</td>
<td>+ (congestion)</td>
</tr>
<tr>
<td>4</td>
<td>+     +  -</td>
<td>+</td>
<td>+ (congestion)</td>
<td>+ (atrophy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+++  +  +</td>
<td>+</td>
<td>+ + + (congestion)</td>
<td>+ + + (dilation)</td>
<td>+ + + (congestion)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+++  +  -</td>
<td>+</td>
<td>+ + + (necrosis, congestion)</td>
<td>+ + + (necrosis, congestion)</td>
<td>+ + + (dilation)</td>
<td>+ (congestion)</td>
</tr>
<tr>
<td>7</td>
<td>-  -  -  -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-  -  -  -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>++  +++  -</td>
<td>+</td>
<td>+ (congestion, necrosis)</td>
<td>+ (atrophy)</td>
<td>+ + + (dilation)</td>
<td>+ + + (congestion)</td>
</tr>
<tr>
<td>10</td>
<td>+++  +  +</td>
<td>+</td>
<td>+++ (congestion, atrophy)</td>
<td>+ + + (necrosis, atrophy)</td>
<td>+ + + (dilation)</td>
<td>+ + + (congestion)</td>
</tr>
<tr>
<td>11</td>
<td>+++  +  +</td>
<td>+</td>
<td>+++ (congestion, necrosis, atrophy)</td>
<td>+ + + (necrosis, atrophy)</td>
<td>+ + + (dilation)</td>
<td>+ + + (congestion)</td>
</tr>
<tr>
<td>12</td>
<td>+  +  +  +</td>
<td>+</td>
<td>+ (congestion)</td>
<td>+ (atrophy)</td>
<td>++ (dilation)</td>
<td>+ (congestion)</td>
</tr>
<tr>
<td>13</td>
<td>-  -  -  -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-  -  -  -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Severity: +, mild; ++, moderate; +++, severe.

*Animals 1 to 6 were inoculated with EHV-9 in the first trimester, animals 7 and 8 are control rats for the first trimester, animals 9 to 12 are animals inoculated with EHV-9 in the last trimester and animals 13 and 14 are control rats for the last trimester.

---

**Fig. 2.** The placenta of a rat inoculated with EHV-9 during the first trimester showing haemorrhage and separation of the chorion. HE. Bar, 30 μm.
signs, nasal discharge and ruffled fur and gave birth to 2:8, 0:7, 0:8 and 0:8 dead fetuses:live undersized pups. Control animals gave birth to 7, 8, 8 and 9 healthy normal sized pups.

**Histopathology and Immunohistochemistry**

All rats inoculated during either the first or last trimester had moderate to severe encephalitis with varying degrees of neuronal degeneration and necrosis, perivascular lymphocytic cuffing, gliosis and meningoitis. The normal histological structure of the rat placenta is shown in Figs. 1a and b. Histopathological changes induced by EHV-9 in the placenta of rat dams are summarized in Table 1.

For animals inoculated in the first trimester, the placentas showed mild to moderate atrophy and necrosis of the trophoblast cells in the labyrinth (Figs. 1c and d), congestion of the spongiotrophoblast cell layer (trophospongium) and congestion and necrosis of the giant cell layer. Moderate to severe widening of maternal blood sinusoids was also observed, together with haemorrhage of the placenta and separation of the chorion (Fig. 2). Haemorrhagic foci and inflammatory cell infiltration were observed in the lamina propria, together with epithelial necrosis and desquamation into the lumen of the cervix in the aborted case (Fig. 3). Animals inoculated in the last trimester showed mild to severe atrophy and necrosis of the trophoblast cells in the labyrinthine zone (Figs. 4a and b), together with mild to moderate atrophy, degeneration and vacuolation of the spongiotrophoblasts (Figs. 4c and d) and giant cell layer. There was moderate to severe widening of maternal blood sinusoids, together with mild to moderate disorganization of fetal capillaries compared with those in control animals at the same gestational stage (Fig. 5). The degree of placental damage differed within the same animal, where some placentas showed severe pathological changes and the others were completely normal.

Immunohistochemically, EHV-9 antigen was detected in the brains of all dams inoculated in the first or last trimesters (Fig. 6) and in the trophoblast cells of the placental labyrinth (Figs. 7a and b), the spongiotrophoblasts and giant cell layer of the placentas in the dams inoculated at the first trimester (animals 2, 3 and 5), as well as in the cervix of the aborted case (Fig. 8). In addition, EHV-9 antigen was detected in the alveolar epithelium and macrophages in the lungs, as well as macrophages in the skin of fetuses and live-born pups, from all dams inoculated in the last trimester (Figs. 9 and 10).

Viral antigen was detected by immunofluorescence in the trophoblasts of the placental labyrinth (Fig. 11) and in the brain of dam number 3 and the lungs of fetuses and live-born pups (Fig. 12) from dams 1, 2 and 5 inoculated in the first trimester and all fetuses and live-born pups from dams inoculated in the last trimester.

**Fig. 3.** The cervix of rat number 3, inoculated with EHV-9 during the first trimester, showing degeneration and sloughing of the epithelium of the lumen of the cervix together with subepithelial haemorrhage. HE. Bar, 30 μm.

**Fig. 4.** (a) The placenta of a rat inoculated with EHV-9 during the last trimester. HE. Bar, 30 μm. (b) Higher magnification showing atrophy and necrosis of the trophoblast cells in the labyrinth (arrow heads) and congestion and widening of the maternal blood sinusoids (S). HE. Bar, 60 μm. (c) Higher magnification showing congestion, atrophy and degeneration of the spongiotrophoblast layer. HE. Bar, 60 μm. (d) Higher magnification showing vacuolation of the spongiotrophoblast layer. HE. Bar, 60 μm.
EHV-9 DNA was amplified by PCR from samples of placentas from all dams and from those fetuses and live-born pups from dams 1, 2, 3 and 5 inoculated in the first trimester and from the placentas of all dams inoculated in the last trimester as well as their fetuses and live-born pups.

Discussion

EHV-1 infection is of great significance to the thoroughbred industry and is a common cause of abortion and neonatal death in the horse (Allen and Bryans, 1986). EHV-9 has a close immunological relationship to EHV-1, so the present study investigated the effects of EHV-9 infection on pregnant rats. The virus induced abortion, placental abnormalities and fetal deaths in rats. The presence of EHV-9 viral particles in placentas and fetuses was not confirmed in a previous study of pregnant mice and hamsters (El-Habashi et al., 2011). In the present study, virus antigen was detected by immunohistochemistry (IHC) in the brain and the trophoblast cells of the placental labyrinth, as well as in the spongiosotrophoblast cells and giant cell layer of the placenta. Virus proliferation was confirmed by PCR in the placenta and fetuses and live-born pups from rats inoculated in the first and last trimesters of pregnancy.

In respect of the pathogenesis of EHV-1-induced abortion in the horse, two hypotheses have been proposed. Firstly, abortion may be due to fetal and/or placental infection with EHV-1. Secondly, abortion may be due to placental infarction resulting from virus-induced endothelial damage. The second hypothesis arose from observations on experimental infection of EHV-1 in ponies where viral antigen could not be detected in aborted fetuses and placentas (Smith et al., 1992, 1993). Edington et al. (1991) reported abortion following EHV-1 infection without virus recovery from the fetus, suggesting that some abortions might be due to endometrial damage without fetal infection. However, the majority of EHV-1-induced abortions in horses are associated with virus isolation from a wide variety of organs and tissues with reliably demonstrated changes in tissues (Bryans et al., 1977; Hartley and Dixon, 1979).

EHV-1 is reported to cross the placenta and invade the fetus in the mare (Allen and Bryans, 1986). In addition, the abortion is partly due to viraemia during acute infection and infection of endothelial cells of the small and medium-sized blood vessels of the placenta. The pathogenesis of experimental EHV-1-induced abortion in mice is associated with changes in the placenta rather than direct virus transmission to fetuses. Such pronounced vascular changes have been observed in murine placentae (Walker et al., 1999). Moreover, the proposed mechanism of abortion in equine viral arteritis infection was suggested to relate to impairment of the uterine blood supply, resulting in expulsion of the fetus (Jones et al., 1957), or a direct effect of the virus on fetuses, causing lethal infection and subsequent expulsion (Doll et al., 1957). EHV-9 was recovered from the tissues of the aborted fetus from the Persian onager and confirmed by PCR and DNA sequencing analyses (Schrenzel et al., 2008).

Fig. 5. Normal histological structure of the placenta of a rat in the last trimester showing the labyrinth, the spongiosotrophoblast (ST) layer and the giant cell layer (G). Bar, 30 μm. Inset shows the placenta of a rat in the control group in the last trimester, with normal distribution of size of the trophoblast cells (arrow heads) in the middle layer interspersed with free spaces and maternal blood sinusoids in the labyrinthine zone. HE. Bar, 60 μm.

Fig. 6. A positive reaction for EHV-9 is seen in neurons in the brain of an animal inoculated with virus in the last trimester. IHC. Bar, 60 μm.
In pregnant mice and hamsters, EHV-9 virus was not recovered from the placentas or fetuses from pregnant mice and hamsters inoculated in the first and last trimesters; however, several changes were observed in the placenta of inoculated dams and the viral antigen was demonstrated by IHC in the placenta of some dams (El-Habashi et al., 2011). In the present study, EHV-9 virus was demonstrated by immunofluorescence, IHC and PCR in the placenta as well as the lung of fetuses, suggesting that abortion may be due to placental damage as well as fetal infection similar to horses infected by EHV-1 (Bryans et al., 1977; Hartley and Dixon, 1979).

The histopathological changes observed in these rats included atrophy and focal necrosis of the placental trophoblast layer and congestion and dilation of the maternal blood sinusoids, as well as congestion and necrosis of the spongiotrophoblast cell layer and the giant cell layer. These changes were similar to those reported in mice infected experimentally with EHV-1 (Walker et al., 1999) and mice and hamsters infected experimentally with EHV-9 (El-Habashi et al., 2011), as well as in horses infected naturally and experimentally with EHV-1 (Smith et al., 1992). The histological changes described in EHV-1-infected mice, as well as EHV-9-infected mice and hamsters, were restricted to the placenta (Walker et al., 1999; El-Habashi et al., 2011), similar to the findings of the present study. However, the histological changes in horses infected naturally or experimentally by EHV-1 were similar to those observed in the present study, but they were restricted to the uterus rather than the placenta (Smith et al., 1992).

In the present study, the degree of placental changes varied between animals and also within the same animal. In addition, all animals inoculated in the first or last trimesters showed cerebral lesions, but no virus antigen was detected in the fetuses and live-born pups and/or placentas of some of these cases. Similarly, in mice infected with EHV-1, no clear relationship was found between the presence or absence of respiratory and systemic signs in dams and perinatal mortality. Some of the dams remained healthy and yet gave birth to dead fetuses (Walker et al., 1999). Edington et al. (1991) and Smith et al. (1992) reported EHV-1-induced abortion in mares with endometritis and infarction and necrosis of the uterus following thrombosis, but were unable to detect viral antigen in any of the fetal tissues, similar to the reports in

Fig. 7. (a) Expression of EHV-9 antigen in trophoblast cells (arrow heads) in the middle layer of the labyrinth of the placenta in rats 2, 3 and 5 inoculated in the first trimester. IHC. Bar, 30 μm. (b) Higher magnification of the placenta shown in (a) revealing expression of EHV-9 in trophoblast cells in the middle layer of the labyrinth. IHC. Bar, 60 μm.

Fig. 8. Expression of EHV-9 antigen in epithelial cells of the cervix of rat number 3 inoculated in the first trimester. IHC. Bar, 60 μm.
mice (Awan et al., 1995). In general, the results of virus recovery from fetuses and placentas in animals infected with EHV-1 are inconsistent with the reported pathological changes; for example, fetuses that were virus positive did not come from dams whose placentas were virus positive (Kukreja et al., 1998).

The lungs are one of the predominant target organs of EHV-1 infection (Whitwell and Blunden, 1992). Similar histological changes were observed in the lungs of aborted mouse fetuses (Awan et al., 1995). EHV-1 infection was demonstrated in the lung tissue of seven naturally aborted mare fetuses by immunohistochemical labelling and PCR. Moreover, positive hybridization signals were observed in the cytoplasm of trophoblasts of all seven placentas (Mukaiya et al., 2000). These findings suggest that transplacental infection plays an essential role in EHV-1-induced abortion. In the present study, EHV-9 antigen was detected by immunofluorescence and IHC in the lungs of fetuses and live-born pups from some of the rat dams inoculated in the first trimester and from all rat dams inoculated in the last trimester. Additionally, EHV-9 DNA was successfully amplified from samples of placentas and fetuses and live-born pups of inoculated rats except for animal number 4.

Trophoblast epithelium supports EHV-1 replication in vitro (Smith et al., 1993) and makes an interdigital contact with endometrial cells in the microcotyledon (Samuel et al., 1975), representing the site of closest

Fig. 9. Expression of EHV-9 antigen in the alveolar epithelial cells and alveolar macrophages in the lungs of a fetus from a rat inoculated in the last trimester. IHC. Bar, 60 μm.

Fig. 10. Expression of EHV-9 antigen in macrophages in the dermis of a fetus from a rat inoculated in the last trimester. IHC. Bar, 30 μm.

Fig. 11. Expression of EHV-9 antigen in trophoblast cells in the middle layer of the labyrinth in rat number 3 inoculated in the first trimester. Immunofluorescence labelling. Bar, 60 μm.

Fig. 12. Expression of EHV-9 antigen in the lung of a fetus from rat number 2 inoculated in the first trimester. Immunofluorescence labeling. Bar, 30 μm.
contact between the endometrium and the fetal placenta. In the EHV-1-infected mouse, in-situ hybridization has demonstrated virus in chorionic epithelium and endothelium and placental trophoblasts (Awan et al., 1995). Viral infection of trophoblasts has been reported with other herpesviruses, such as herpes simplex virus (Nørskov-Lauritsen et al., 1992), cytomegalovirus (Sachdev et al., 1990) and canine herpesvirus (Hashimoto et al., 1982). In the present study, viral antigen was detected by immunofluorescence and IHC in trophoblasts in the placent al labyrinth, suggesting that transplacental infection plays an essential role in EHV-9-induced abortion.

The present study has confirmed the presence of EHV-9 antigen and DNA in the placenta and lungs of fetuses and live-born pups by IHC, immunofluorescence labelling and PCR, suggesting similarity of EHV-9 to EHV-1 with respect to infection of the placentas and fetuses and further suggesting that EHV-9 is an abortogenic virus for pregnant rodents. The death of the fetuses may be due to direct fetal infection or be secondary to vascular injury of the placenta.

Close homology between EHV-1 and EHV-9 infections was seen in terms of the histopathological changes induced in the pregnant animals and the proposed causes of abortion. Further studies are required in order to investigate the infectivity and pathogenicity of EHV-9 in the fetus and placenta of pregnant domestic animals.

Acknowledgments

This study was supported in part by a grant-in-aid (Emerging Diseases) (No. H-22-E-G-010) for scientific research from the Ministry of Health, Labor and Welfare, Japan and a grant from Ono Pharmaceutical Co., Ltd in 2013. We also thank Miss C. Swift for proofreading the manuscript.

Conflict of Interest Statement

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

References


Received, June 2nd, 2014
Accepted, August 13th, 2014