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EXPERIMENTALLY INDUCED DISEASE

# Pathological Findings in Equine Herpesvirus 9-Induced Abortion in Rats

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## Summary

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.*, 2005), 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;

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Donovan *et al.*, 2009) and a giraffe (*Giraffa camelopardalis reticulata*) (Kasem *et al.*, 2008) were susceptible to infection by EHV-9. The natural host range of EHV-9 has extended to six species in three mammalian orders (Schrenzel *et al.*, 2008). Serologically, EHV-9 is closely related to the recently emergent neurotropic pathogen, EHV-1; however, cleavage of the EHV-9 DNA fingerprint by restriction enzymes was found to be different from that of EHV-1 and other equine herpesviruses (Fukushi *et al.*, 1997). Abortion is one of the major complications of EHV-1 infection in the horse and a major cause of loss to the thoroughbred industry (Allen and Bryans, 1986).

Abortion in a Persian onager (*Equus hemionus onager*) in San Diego Zoological Park was shown to be caused by EHV-9. The onager fetus was aborted after the dam was in close contact with a Grevy's zebra in the same park. EHV-9 was recovered from the tissues of the aborted fetus. Polymerase chain reaction (PCR) and DNA sequencing showed that the onager had an EHV-9 identical to that found in the polar bear (Schrenzel *et al.*, 2008). The extended host range for EHV-9 and the close immunological relationship to EHV-1 led us to investigate the impact of EHV-9 on pregnant animals. Intranasal inoculation of EHV-9 into pregnant mice and hamsters induces placental abnormalities, abortion and fetal deaths (El-Habashi *et al.*, 2011); however, infection of fetuses has not been shown for mice and hamsters, owing to failure of virus isolation from the placentas and fetal tissues as well as shortage of samples for virus detection by PCR. The presence of the virus in the placenta and fetal tissues has therefore not been confirmed.

The aims of this study were to determine the effects of EHV-9 infection during the first and last trimesters of pregnancy in the rat and to determine whether viral antigen and DNA could be detected in the placentas and fetuses of the infected rats.

## Materials and Methods

### *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 $2 \times 10^6$ 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 antiserum 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, Carpinteria, California, USA), liquid 3, 3′ diaminobenzidine (DAB) substrate chromogen system (Dako) was