



Strain impact on equine herpesvirus type 1 (EHV-1) abortion models: Viral loads in fetal and placental tissues and foals

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ABSTRACT

Equine herpesvirus-1 (EHV-1) continues to cause both sporadic and epidemic abortions despite extensive vaccination. Lack of progress in the development of protective vaccines may be hindered by the lack of equine abortion models that employ contemporary EHV-1 strains. The objective of our experiments was to compare a contemporary EHV-1 strain with a previously described challenge strain, and to quantify EHV-1 loads in various maternal and fetal tissues. Infection experiments were performed in two groups of 7 pregnant pony mares at 270–290 days of gestation with a contemporary EHV-1 strain (University of Findlay 2003 isolate – OH03) or an EHV-1 strain isolated over 30 years ago, and previously described in abortion models (Ab4). All mares in both groups exhibited nasal viral shedding and viremia. Infection with OH03 resulted in 1/7 abortion and infection with Ab4 resulted in 5/7 abortions. In the OH03 challenge, placentas of foals delivered at term showed little detectable virus, while the aborted fetus expressed high levels of virus infection in the spleen and liver, lower levels in the lung and thymus, and lowest levels in the chorioallantois. After Ab4 challenge, high viral loads were detected in fetal and placental tissues in abortions. In the two normal deliveries, the chorioallantois contained virus levels comparable with the chorioallantois of aborted foals and both foals shed EHV-1 starting on day 4 of life, but were clinically healthy. Our results demonstrate the continued importance of strain selection for abortion models, and this study is the first report of viral load quantification using contemporary methods. Extremely high EHV-1 loads in decidua from abortions illustrate the infection risk posed to other horses.

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1. Introduction

Equine herpesvirus-1 (EHV-1) continues to cause significant disease in horses throughout the world. Of greatest welfare and economic importance are sporadic outbreaks of neurological disease and the more frequent herd outbreaks of late term abortions that can have attack rates in excess of 50% [1–4]. Thus development and evaluation of vaccines against this disease outcome remains a priority.

Vaccines currently available for EHV-1 include modified live and inactivated products. However, methodological differences and limitations in the experimental designs of published studies preclude definitive conclusions or comparisons of the currently

licensed products regarding the level of vaccine efficacy, in regards to prevention of abortion. In addition, while vaccination is extensively used to protect against EHV-1 abortion, evidence of efficacy is unclear [5].

The pathogenesis of EHV-1 abortion depends on viremia, which precipitates infection of endothelial cells of the endometrium, leading to vasculitis, thrombosis, microcotyledonary infarction, perivascular cuffing, and transplacental spread of virus at the sites of vascular lesions [6]. Protection from abortion is directly correlated to high pre-infection frequencies of EHV-1 specific cytotoxic T lymphocytes (CTLs) [7], but because CTL assays are prohibitively time consuming and tedious to perform, they are impractical for testing of vaccine efficacy. Equine abortion challenge models therefore remain essential for evaluating vaccine performance.

Historically, the association of abortion with isolation of the N₇₅₂ strain versus the D₇₅₂ strain variant has been strong [8,9], however, in recent years abortions are increasingly associated with the D₇₅₂

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genotype [10] and difficulties creating an experimental model of EHV-1 abortion in horses could only be overcome when the Ab4 virus, a D₇₅₂ strain originally described in 1981, was employed [11–14]. In the past decade there have been a number of new highly pathogenic EHV-1 isolates, perhaps most notably the 2003 outbreak at the University of Findlay [15], which was caused by a D₇₅₂ strain that has been referred to as OH03 [9] or as Ky T953 [16]. It may be advantageous to investigate abortion models employing more contemporary viral isolates that represent the highly pathogenic strains currently circulating. Also, new molecular techniques are now available for quantification of EHV-1 DNA [17,18], which have never been used for characterization of viral distribution and pathogenesis in an abortion study.

Accordingly, pony mares were challenge infected in the third trimester of gestation with either the OH03 strain or the Ab4 strain following synchronization and natural breeding. Viral loads in nasal secretions, blood leukocytes and placental and fetal tissues were determined using real-time-quantitative-PCR (qPCR) analysis. This methodology is more sensitive at detecting virus in aborted fetal tissues than conventional virological methods [19–22] and offers the additional advantage of quantifying viral loads. Our goals were to evaluate the performance of a contemporary EHV-1 strain in an abortion model, and characterize viral distribution using modern molecular techniques.

2. Materials and methods

2.1. Animals

In Experiment 1 (OH03), eleven 2-year old female ponies were acquired in 2006, hormonally synchronized and naturally bred. At the time of challenge infection ponies were 3-years old and seven were pregnant. Ponies were group-housed in an outdoor pen with a walk-in shelter in Fort Collins, CO, throughout the experiment, and were fed twice a day with a diet of hay and pelleted concentrate and ad libitum water.

For Experiment 2 (Ab4), nine 2-year old female ponies were acquired in 2007 from the same breeding stock as Experiment 1, hormonally synchronized and naturally bred. At the time of challenge infection ponies were 3-years old and seven were pregnant. Ponies were group housed in Fort Collins, CO and fed as described above, until approximately 6 months of gestation, and then moved to a similar facility in Fort Dodge, IA for the remainder of the experiment.

All experiments were performed in accordance with the Animal Care and Use Committee guidelines of Colorado State University (experiment 1) and Fort Dodge Animal Health (experiment 2). The IACUC approvals were obtained prior to initiating any experimental manipulations with animals. Colorado State University is AAALAC-Intl accredited and holds all appropriate assurances and licenses relative to animal experimentation.

2.2. Estrus synchronization

For both experiments, the mares were naturally bred after synchronization of ovulation by administration of oral altrenogest (0.044 mg/kg; Regu-Mate® Solution, Merck Animal Health) administered according to the manufacturer's recommendations, followed by an I.M. injection of PGF2-alpha (1 ml/450 kg; Estrumate, Schering Plough Animal Health) on the last day of altrenogest administration. Stallions were moved in with the mares on day three post-altrenogest.

In experiment 1, synchronization and pregnancy was determined by weekly progesterone measurements and a day 75 post-altrenogest Pregnant Mare Serum Gonadotropin (PMSG)

measurement >79 IU/ml using Bluegrass Embryo Transfers (BET) Reproductive laboratories (Lexington, KY) that indicated that 7/11 mares were pregnant.

In experiment 2, all mares were checked for pregnancy via ultrasound on days 23 and 58 after stallions were introduced and seven of the 9 mares were determined to be pregnant (six with the first ultrasound and one at the time of second ultrasound (mare #4).

Pregnancies in both experiments were confirmed by total estrogen determination at 4–5 months of gestation and showed values greater than 750 pg/ml (BET lab).

2.3. Serum neutralization titers

Serum neutralization (SN) titers for both experiments were determined throughout pregnancy and on days–1, 7, 14 and 21 post challenge infection as previously described using Ab4 as a reference strain [23].

2.4. Equine herpesvirus viruses

Experiment 1 used the neuropathogenic EHV-1 strain Ab4, which is a native equine strain that was originally isolated in 1980 from quadriplegic mare and is known to cause respiratory disease, abortions and neurological disease. Experiment 2 used the neuropathogenic EHV-1 strain Ohio'03 that was isolated from the 2003 outbreak at the University of Findlay. Both viruses were provided at low passage by the Osterrieder laboratory. The nucleotide sequence identity of OH03 to Ab4 is 98% in contrast to 99.5% with V592 a strain of lesser neuropathogenicity (unpublished data: Li and Balasuriya, 2012). To our knowledge, most differences between OH03 and Ab4 are located in the ORF68 gene which is longer in Ab4 but no further data is available at this point that would warrant any major conclusions on differences between the strains used in the experiments (personal communication Dr. Osterrieder).

2.5. EHV-1 challenge infection

Pregnant mares in each experiment were simultaneously challenge-infected with 5×10^7 PFU of the respective viruses in a total volume of 10 ml administered via nasopharyngeal instillation producing an aerosol as previously described [24], at approximately 270–290 days of gestation (see Fig. 2).

2.6. Clinical monitoring, blood and nasal swab collection

For both experiments, physical exams were performed daily from day–2 through day 22 post-challenge infection by the attending veterinarian.

Blood for detection of viremia was collected on days–2, 1 through 14, and every other day from day 14 to day 21 after challenge infection (day 0), and peripheral blood mononuclear cells (PBMC) were separated as previously described [25].

Nasal swabs for detection of EHV-1 viral shedding were collected as previously described [25] at the same times as blood samples. Prior to storage at -80°C , swabs were filtered using a $0.45\ \mu\text{m}$ syringe filter for experiment 2, and stored without filtering in experiment 1.

2.7. Necropsy and tissue collection

For both experiments, aborted fetuses and fetal membranes were subjected to complete gross post-mortem examination. Two $1000\ \text{mm}^3$ samples of placental and fetal tissues were collected, placed in 2 ml Eppendorf tubes, flash frozen in liquid nitrogen and

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