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Subclinical infection and periodic shedding of equid herpesvirus 3

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Abstract

The temporary disruption of reproductive activities due to equine coital exanthema (ECE), caused by equid herpesvirus 3 (EHV-3), at thoroughbred breeding facilities and embryo transfer centres, has an appreciable economic impact. The aim of the present study was to estimate the prevalence of excretion of EHV-3 in mares without clinical symptoms under field conditions and the re-excretion patterns of the virus in two seropositive (presumably latently infected) mares maintained in isolation for 11 mo. The EHV-3 virus was detected in perineal-vaginal swabs by real time PCR in 14 (6%) of 220 thoroughbred mares without clinical symptoms at the time of breeding. In the two isolated mares, re-excretion of EHV-3 was demonstrated on two occasions, 3 mo apart (each for a 3 d interval) in one mare, and on only 1 d in the other mare. Antibodies against EHV-3 were identified by seroneutralization in 105 (48%) of the thoroughbred mares, and during the entire period in the two isolated mares. Therefore, the present study provided evidence of EHV-3 shedders in a healthy mare population under both field and isolation conditions. Furthermore, at least two periods of spontaneous EHV-3 reactivation and re-excretion in the presence of serum antibodies occurred in one mare in an 11 mo interval. These findings could assist in the design and implementation of measures to minimize the spread of EHV-3 and control ECE outbreaks.

Keywords: Equid herpesvirus 3; Equine coital exanthema; Latent infection; Viral excretion

1. Introduction

Equine coital exanthema (ECE), caused by equid herpesvirus 3 (EHV-3), is an acute, infectious, venereal disease resulting in the formation of papules, vesicles, pustules, and ulcers on the penis and prepuce of stallions, and on the vaginal and vestibular mucosa and perineum of mares. The virus is highly contagious, but causes only a local infection and clinical signs are relatively benign. The primary negative impact of ECE on equine breeding enterprises is the forced, temporary disruption of mating activities. In intensively managed stud operations with heavily-scheduled breeding dates for stallions, such disruptions may translate into notable end-of-season decreases in the number of mares bred by affected stallions [1–3]. The infection has an additional negative impact at artificial insemination and embryo transfer facilities, due to the extra time and precautions required to manage both donor and recipient mares and stallions in the face of an outbreak of ECE [4].

Virus reactivation from latently infected mares was recently reported, confirming speculation regarding the

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existence of clinically normal infected carrier animals [1,5]. It has been postulated that infected horses without clinical symptoms could transmit the virus to their breeding partners [1–3]. Epidemiological data suggest that either an infected visiting mare brought onto the stud farm for breeding, or a virus reactivated from a member of the resident stallion or mare population, may be the viral source of ECE outbreaks [1,6].

The basis for controlling the impact of outbreaks of ECE in breeding establishments is to prevent the spread of infection by immediate cessation of mating activities of clinically affected stallions and mares, heightened vigilance for early recognition of new clinical cases, and strict adherence to breeding shed hygiene procedures designed to eliminate mechanical transmission of the virus [1].

The aim of the present study was to estimate the prevalence of excretion of EHV-3 in mares without clinical symptoms under field conditions and to characterize re-excretion patterns of the virus in seropositive (presumably latently infected) mares maintained in isolation. It was expected that these data would contribute to assessing the risk of EHV-3 infection during mating in the absence of detectable ECE lesions.

2. Materials and methods

2.1. Animals and clinical examination

2.1.1. Field study

This study was carried out in a horse facility, La Mission, San Andres de Giles, Buenos Aires province, Argentina, where eight shuttle stallions from the USA, Ireland, and France were brought for the 2007 breeding season. A total of 1080 mares from 69 breeding farms visited the facility to be bred (natural mating) by shuttle stallions.

All mares booked at the stallion station were clinically evaluated before mating; those with systemic or local clinical signs or lesions suspicious of infectious diseases were segregated and rejected for breeding.

2.1.2. Isolation study

Two EHV-3 seropositive polo mares (Mares A and B), which had been included in a previously described EHV-3 reactivation study [5], were used to investigate the spontaneous reactivation and re-excretion of the virus over the time. To this end, they were kept isolated from other horses from August 2007 to June 2008. Rectal temperature, general conditions, and careful observation of the genital and perineal regions were recorded daily. All mares were kept in accordance with the international standards regarding animal welfare [7], and the protocol was approved by the institutional (INTA) animal care and use committee.

2.2. Clinical samples

Samples for virus detection and titration consisted of swabs from the perineal and vaginal regions (PVS) in viral transport media (Minimum Essential Medium, GIBCO BRL, Grand Island, NY, USA), supplemented with 5% fetal calf serum and 8% penicillin-streptomycin [8]. Serum samples were also collected for antibody detection and quantification. In the field study, PVS and serum samples were obtained from 220 mares immediately before preparation (i.e., washing genitalia and wrapping the tail) for breeding. The sampling time frame was from August to November 2007. In the two isolated mares, PVS were collected daily and serum samples once a week, from August 2007 to June 2008. The PVS and serum samples were stored at −80 and −20 °C respectively, pending laboratory studies.

2.3. Virus detection and measurement of virus excretion by quantitative real time PCR

To detect and quantify EHV-3 in both the field and isolation studies, a quantitative real time PCR assay targeting the gG gene was conducted, as previously described [5], on DNA extracted from PVS.

2.4. Seroneutralization test

Antibodies against EHV-3 in blood serum samples were detected by the seroneutralization test, as previously described [5].

3. Results

3.1. Field study

Shedding of EHV-3 was demonstrated in 14 (6%) of the 220 mares without clinical symptoms, by real time PCR, whereas EHV-3 specific antibodies were detected by a seroneutralization test in 105 (48%). In six of the 14 mares, EHV-3 shedding was detected in the absence of detectable antibodies, whereas in the remaining eight, the virus was present in PVS simultaneously with serum antibodies. In 97 mares, no virus shedding but antibodies were detected, and in 109, no antibodies and no virus were found. The amount of excreted virus, as quantified by real time PCR, ranged between 1.2 and 4.6 $\log_{10}$ tissue culture infectious dose 50% ($TCID_{50}$) equivalents, whereas the antibody titer ranged from negative to 2.4 ($\log_{10}$ reciprocal of 1:256 serum dilu-