Transmission of the South African asinine strain of equine arteritis virus (EAV) among horses and between donkeys and horses

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ABSTRACT


Lateral and sexual transmission of EAV among horses and lateral transmission between donkeys and horses were attempted by experimental infection with the South African asinine strain. Clinical, immunological and virological responses were evaluated.

All intramuscularly inoculated horses developed very mild clinical signs, were viraemic, shed virus from nasopharynx, and seroconverted. Lateral infection was demonstrated in one in-contact mare. Re-infection of two stallions by intranasal instillation was shown by virus recovery from buffy-coat cultures. After nasal instillation of virus, one stallion which did not become infected by in-contact exposure, showed slight serous nasal and ocular discharge, contained virus in a blood and nasopharynx and seroconverted. Attempts to transmit the virus from seropositive stallions to seronegative mares by breeding, were not successful; no virus was isolated from semen.

All inoculated donkeys and three in-contact horses showed clinical signs consistent with an EAV infection. Although virus was isolated from donkey buffy-coat preparations and the nasopharynx, and they seroconverted, no virus was isolated from the horses, and they failed to seroconvert; it was assumed that their clinical signs were due to factors unrelated to EAV.

The South African strain of EAV appears to be poorly transmissible to horses, supporting the findings of other field studies which indicate a widespread distribution and long-standing presence of the virus among South African donkeys, but a very restricted prevalence of seropositive horses.

Keywords: Asinine strain, EAV, equine arteritis virus, donkeys, horses

INTRODUCTION

Equine viral arteritis (EVA) was first recognized as a separate entity among viral infections in horses in 1953, when it's aetiological agent, equine arteritis virus (EAV) was isolated from foetal lung tissue during an outbreak of respiratory disease and abortion in standard-bred horses in the United States (Doll, Bryans, McCollum & Crowe 1957). EAV was originally classified as a Togavirus (Porterfield, Casals, Chumakov, Gaidamovich, Hannoun, Holmes, Horzinek, Mussgay, Oker-Blom, Russel & Trent 1978) and, more recently, designated a member of a newly proposed virus family, the Arteriviridae (Cavanagh, Brien, Brin ton, Enjuanes, Holmes, Horzinek, Lai, Laude, Plagemann & Sicdell 1994).
Although genetic variation (Murphy, McCollum, Timoney, Klingeborn, Hyllseth, Golnik & Erasmus 1992; Chirnside, Wearing, Binns & Mumford 1994) and differences in pathogenicities (Bürki 1970; McCollum & Swerczek 1978) have been demonstrated among virus isolates from disparate chronological and geographical origins, only a single serotype of EAV has been recognized to date (McCollum 1969; Fukunaga, Matsumura, Sugiura, Wada, Imagawa, Kanemaru & Kamada 1994). Serosurvey studies have indicated that EAV is distributed worldwide among horses, with the highest prevalence in standard-bred horses (Chirnside 1992). The clinical signs of infection are extremely variable. In acute disease, typical signs include pyrexia, anorexia, depression, leg and palpebral oedema, conjunctivitis, laceration and rhinitis. Less frequently, photophobia, skin rash, coughing, respiratory distress, diarrhoea, weakness and unsteadiness are observed. In pregnant mares, the virus may cause a high abortion rate (Mumford 1985). A fatal outcome of disease has been reported in both natural (Golnik, Michalska & Michalak 1981; Vaala, Hamir, Dubovi, Timoney & Ruiz 1992) and experimental infections (Doll et al. 1957; Coignou & Cheville 1984), but most field infections appear to be subclinical in nature (Timoney 1992). EAV is spread by respiratory (McCollum & Swerczek 1978) and venereal routes (Timoney & McCollum 1988). A high proportion of stallions exposed to virus may become persistently infected and shed EAV in semen (Timoney, McCollum, Roberts & Murphy 1986; Timoney, McCollum, Murphy, Roberts, Willard & Carswell 1987).

Serological results reported by Himeur (1976), Moiraillon & Moraillon (1978) and, more recently, by others (Paweska & Barnard 1993; Paweska 1994), have indicated that donkeys can also be infected with EAV under natural conditions. However, the observation of natural EAV transmission, and the clinical, virological and serological findings in donkeys were first briefly reported in 1994 (Paweska, Volkman & Barnard) and then described in more detail (Paweska, Volkman, Barnard & Chirnside 1995). A high degree of genetic and antigenic homology between the asinine and equine virus isolates (Paweska et al. 1995) indicates that the donkey strain of EAV could infect horses and vice versa. Evidence that donkeys are susceptible to an equine strain of EAV (KY-84) has recently been reported (McCollum, Timoney & Tangelersen 1995). However, the susceptibility of horses to the asinine strain of EAV has not been investigated.

The objectives of this study were to evaluate clinical, immunological and virological responses in horses to the asinine isolate of EAV and to attempt lateral in-contact and venereal transmission among horses and lateral in-contact transmission from donkeys to horses in challenge experiments.

**MATERIALS AND METHODS**

**Experimental animals**

**Horses**

Clinically normal, EAV-seronegative horses were supplied by Onderstepoort Biological Products, Onderstepoort. The sixteen mixed-bred horses comprised six stallions (hS1-hS6) 3–8 years old and ten mares (hM1-hM10) 4–10 years old.

**Donkeys**

Two donkey mares (dM1-dM2), both 4 years old, and one 5-year-old stallion (dS1) were purchased. All donkeys were clinically healthy and seronegative to EAV.

**Virus inoculum**

Semen was collected from a naturally infected seropositive donkey stallion that was confirmed as a long-term shedder of EAV (Paweska et al. 1995). A 5·mℓ inoculum comprising a supernatant of sonicated semen containing 10³·5TCID₅₀/ml was administered intramuscularly (i.m.) or instilled intranasally (i.n.) into each nostril.

**Experimental design**

Animals were kept loose in their respective experimental groups in about 240 m² (experiments I and II) or 100 m² (experiment III) fenced outdoor pens with concrete floors and communal food and water troughs. The food troughs were situated against a solid brick wall with the remaining three sides of the enclosures lined with chest-high open fence.

**Experiment I**

Horses were kept in two separate groups; in group I there were three horse stallions (hS1-hS3) and in group II, four mares (hM1-hM4). The donkey stallion (dS1), used as an experimental control, was housed separately from the horses. Two animals from each horse group (Grp I, hS1 and hS2; Grp II, hM1 and hM2) and dS1 were inoculated i.m. The remaining horses in each group were exposed to the inoculated horses for a period of 6 weeks post inoculation. After this period, hS3 (which did not seroconvert) and hS1 were re-challenged i.n.; hS2 was re-challenged i.n. 14 weeks after i.m. inoculation.

**Experiment II**

Stallions hS1-hS3 were test-bred to seronegative mares twice a day for 4 d during the same oestrus period. Stallion hS1 was bred to hM5 and hM6 and hS3 to hM9 and hM10, 3 weeks after i.n. challenge or re-challenge, respectively, with hS2 to hM7 and
hM8, 9 weeks after i.m. challenge. After mating, mares were kept isolated and monitored for a period of 6 weeks after last cover.

**Experiment III**

Two donkey mares (dM1 and dM2) were inoculated i.m. and three horse stallions (hS4-hS6) placed in-contact with them for a period of 4 weeks. The donkeys were then removed and the horses monitored for clinical signs of infection and seroconversion to EAV for a further 4 weeks.

**Clinical examination**

Following inoculation, re-challenge, in-contact exposure and test breeding, the horses were monitored for clinical signs of EVA and rectal temperatures were taken twice daily for a period of 3–4 weeks.

**Sampling and processing of specimens**

**Blood**

Blood samples from inoculated animals were taken every 2–3 d for 3 weeks after challenge or re-challenge, and then every 3–5 d for a minimum of 3 more weeks. Blood samples from in-contact, exposed horses and from test-bred mares were collected every 7 days for 6–8 weeks after in-contact exposure and mares’ last cover. Heparinized blood for buffy-coat preparations was taken from inoculated animals every 2 d for the first 2 weeks and then every 3 d for a further 2 weeks. Heparinized blood from re-challenged horses was collected on days 3, 5, 7, 10 and 14 post-re-challenge and from in-contact, exposed horses during febrile response and during 3 post-febrile days.

**Nasal exudate**

Approximately 1 ml of nasal exudate was collected directly into sterile tubes from inoculated animals every second day for 2 weeks post inoculation and from re-challenged stallions and in-contact, exposed horses at the same time as heparinized blood was taken for buffy-coat preparations.

**Semen**

Approximately 5–10 ml of dismount semen samples were collected from each stallion three times after test mating. Mares were synchronized in oestrus with the use of dinoprost tromethamine [Lutalyse; Upjohn (Pty) Ltd, Isando], in accordance with the manufacturer’s instructions.

After collection, samples for virus isolation were transported with a minimum of delay to the laboratory on frozen freezer packs and processed for inoculation into cell culture as described previously (Paweska et al. 1995). Wet preparations from dismount semen samples were examined microscopically to confirm that they contained sperm.

**Virus isolation**

Virus isolation procedures followed those as described previously (Paweska et al. 1995), with slight modifications. One-day-old monolayers of RK-13 cells (ATCC CCL 37) in 25-cm² culture flasks were used. These were inoculated with 0.5 ml of each sample. After absorption for 2 h at 37°C, the monolayers were overlaid with maintenance medium, incubated at 37°C, and examined daily for 7 d for virus cytopathic effect (cpe). In the absence of cpe, cultures were frozen at -20°C, thawed and passaged four times, after which, in the absence of cpe, the samples were considered to be EAV-negative. The identity of EAV was confirmed by neutralization of its infectivity in a virus neutralization test in the presence of 10% guinea-pig complement and specific horse antiserum (Fukunaga, Wada, Matsumura, Anzai, Imagawa, Sugliura, Kumanomido, Kanemaru & Kamada 1992).

**Serology**

Antibodies to EAV were detected by a complement-dependent virus neutralization test (VN) in microtitre plates with RK-13 cells (Paweska & Barnard 1993) and in an enzyme-linked immunosorbent assay (ELISA) based on recombinant EAV antigen (Chirnside, Francis, De Vries, Sinclair & Mumford 1995).

**RESULTS**

**Lateral spread between horses**

The clinical, virological and immunological results of this experiment are shown in Table 1. Horses (hS1, hS2, hM1, hM2) and the control donkey (dS1), inoculated i.m., became febrile between days 2 and 9, post inoculation, with maximal temperatures between 38.8 and 40.1°C. Clinical signs of infection were very mild in the horses, but more severe in dS1, and included depression, serous nasal and ocular discharge and conjunctivitis. The clinical signs were most prominent during the febrile response and all the horses recovered 3–5 d after the end of the febrile response. The clinical signs exhibited by in-contact hM3, resembled those in inoculated horses, but neither of the remaining two in-contact horses (hS3 and hM4) showed any signs of EAV infection. When hS1 and hS2 were re-challenged intranasally, no fever and only mild conjunctivitis was observed; hS3, which had not been infected during the initial in-contact period with hS1 and hS2, became febrile on days 3–5, with maximal rectal temperature (39.2°C) on day 4, and showed a slight nasal and ocular dis-
TABLE 1  Clinical, immunological and virological responses in horses and a donkey inoculated intramuscularly with the asinine strain of EAV and that in in-contact horses

<table>
<thead>
<tr>
<th>Experiment I</th>
<th>Challenge exposure</th>
<th>Fevera</th>
<th>Sero- conversionb</th>
<th>Virus isolationc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max. temp.</td>
<td>Duration</td>
<td>Buffy coat</td>
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<tr>
<td>Group I</td>
<td>Stallions</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>hS1</td>
<td>i.m.</td>
<td>38,9 °C</td>
<td>5-6</td>
<td>8</td>
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<tr>
<td>hS2</td>
<td>i.m.</td>
<td>39,8 °C</td>
<td>2-7</td>
<td>10</td>
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<tr>
<td>hS3</td>
<td>contact</td>
<td>-</td>
<td>-</td>
<td>n.a.</td>
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<tr>
<td>Group II</td>
<td>Mares</td>
<td></td>
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<tr>
<td>hM1</td>
<td>i.m.</td>
<td>38,8 °C</td>
<td>6-7</td>
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<tr>
<td>hM2</td>
<td>i.m.</td>
<td>39,5 °C</td>
<td>2-6</td>
<td>10</td>
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<tr>
<td>hM3</td>
<td>contact</td>
<td>40,1 °C</td>
<td>15-18</td>
<td>16</td>
</tr>
<tr>
<td>hM4</td>
<td>contact</td>
<td>-</td>
<td>-</td>
<td>n.a.</td>
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<tr>
<td>Control donkey</td>
<td></td>
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<tr>
<td>dS1</td>
<td>i.m.</td>
<td>40,5 °C</td>
<td>2-9</td>
<td>8</td>
</tr>
</tbody>
</table>

| a | Days after inoculation or in-contact exposure |
| b | Not attempted |

TABLE 2  Results of in-contact exposure of three horses to two donkey mares challenged with asinine strain of EAV

| Experiment III | Challenge exposure | Fever | Sero-conversionb | Virus isolationc |
|               |                    | Max. temp. | Duration |          |               |
| Donkeys       |                    |         |        |          |               |
| dM1           | l.m.               | 39,4 °C | 4-9    | +        | +             |
| dM2           | l.m.               | 39,9 °C | 6-6    | +        | +             |
| Horses        |                    |         |        |          |               |
| hS4           | contact            | 39,9 °C | 12-14  | -        | -             |
| hS5           | contact            | 40,1 °C | 18-22  | -        | -             |
| hS6           | contact            | 39,9 °C | 19-21  | -        | -             |

| a | Days after inoculation or in-contact exposure |
| b | Seroconversion detected by VN and ELISA |
| c | Virus isolated from buffy coats and nasal exudate |

Charge on days 3–6 after intranasal challenge. EAV was recovered from buffy coats and nasal discharges of all the i.m.-inoculated horses and dS1. After i.n. challenge, the virus was isolated from buffy coats from hS3 during days 4–10, and from the respiratory tract on day 8. EAV was recovered from buffy coats during days 4–6 post i.n. challenge from re-challenged horses hS1 and hS2. Between days 8 and 10, all inoculated horses seroconverted to EAV, and VN antibody titres reached a peak of \( \log_{10} 1,2–1,5, 16–18 \) d post infection. These animals remained sero-positive for 6–14 weeks. The induction kinetics of VN antibody correlated with those for ELISA IgG antibody post inoculation and was not sex dependent. Increased levels of IgM detected by ELISA on day 8, peaked on days 12–14 and decreased to pre-infection levels within 3–6 weeks. The peak antibody levels in the donkey inoculated i.m., were four times higher than the levels in horses challenged in the same way (Fig. 1). Antibody levels in hS1 and hS2 which were re-challenged with the asinine virus isolate, were also lower than those of the control donkey.
Immune response of horses and a donkey after experimental infection with the asinine strain of EAV. (a) and (b) intramuscularly inoculated and intranasally re-challenged horse stallions hS1 and hS2; (c) intranasally inoculated horse stallion hS3; (d) intramuscularly inoculated donkey stallion dS1

\[ \text{---} \text{VN Ab} \quad \text{---} \text{IgG ELISA} \quad \text{---} \text{IgM ELISA} \]

**FIG. 1** Immune response of horses and a donkey after experimental infection with the asinine strain of EAV.

- **Venereal spread between horses**
  - No clinical signs of infection or seroconversion to EAV were observed in any of the horse mares (hM5–hM10) after they had been successively mated to seropositive hS1, hS2 or hS3. No virus was recovered from semen samples collected from seropositive stallions at time of test breeding.

- **Lateral spread from donkeys to horses**
  - The results obtained from exposing naïve horses to i.m.-infected donkeys are summarized in Table 2. Two infected donkeys were febrile between days 4–9 post inoculation, exhibiting maximal temperatures of 39.4 and 39.9°C. Pyrexia lasting 3 or 5 d, was recorded in the three in-contact horses between 12 and 22 d post exposure. Febrile animals all suffered mild depression, conjunctivitis and serous rhinitis, with horses additionally having mild diarrhoea lasting 2–4 d. The donkeys were viraemic during days 2–14 after inoculation, shed virus from their respiratory tracts during days 4–10 and seroconverted to EAV on day 8 post virus inoculation. Although clinical signs were observed in the in-contact horses, virus was not recovered from the respiratory tract and blood, neither were seroconversions evident in these animals. Serological tests for recent infection with *Trypanosoma equiperdum*, *Babesia equi*, *Babesia caballi*, African horsesickness virus, equine encephalitis virus and Equid herpesvirus 1 and 4, were serologically negative (results not shown).

**DISCUSSION**
- Although the first serological evidence indicating that donkeys can be naturally infected with EAV was published almost 20 years ago (Himeur 1976), to date, field occurrences of clinical disease have been reported only in horses (Chirnside 1992). A high prevalence of EAV infection in Morocco, reported both in donkeys and in different breeds of horses (Moraillon & Moraillon 1978), and demonstration of
specific antibodies in sera among Moroccan and South African mules (Paweska 1995) suggest, however, that different species of Equidae may play a role in maintaining EAV in nature. There is no information regarding outbreaks of EVA due to the spread of virus from donkeys to horses, and no clear evidence for interspecies cross-infection has been reported.

The susceptibility of horses to the asinine strain of EAV after direct i.m. or i.n. inoculation, was demonstrated by observation of related clinical signs, seroconversion and virus recovery from buffy coats and respiratory tract. Febrile responses were detected in horses on days 2–7 and seroconversion was detected on days 8–10 post inoculation. This is in accordance with other reports of an incubation period, appearance of detectable levels of antibodies, with fever and the development of other clinical signs after EAV infection (Chirnside 1992; Mumford 1994). The clinical signs in horses after either inoculation or in-contact exposure, were very mild or subclinical, compared with those observed in horses experimentally infected with equine isolates of EAV (Doll et al. 1957; McCollum, Prickett & Bryans 1971; Jaksch, Sibalin, Taussig, Pichler & Bürki 1973; Neu, Timoney & Mc Collum 1988; Autorino, Cardeti, Rosati, Ferrari, Vulcano, Amaddeo, McCollum & Timoney 1994). It has been demonstrated that the severity of clinical signs is dependent on many factors, including the virulence and challenge dose, animal age and physical state, and different environmental factors (Timoney & McCollum 1991). Under field conditions, the vast majority of horses appear to undergo inapparent infection and severe clinical signs are rarely reported (Timoney 1992). The direct comparison of febrile, clinical and immunological responses to experimental inoculation with an asinine strain, suggests that in horses the infection is milder than that observed in donkeys. This seems to be in contrast to the responses noted in donkeys that had been infected with KY-84 strain, in which the symptoms were less severe than those produced in horses (McCollum et al. 1995), and it may indicate variation in the response of both species against different strains of EAV. It is worth emphasizing that if both inoculated horses and a control donkey had not been monitored throughout the course of this study, the clinical signs would probably not have been seen. The virological response to the infection with asinine strain paralleled that of horses and donkeys to equine strains (McCollum et al. 1971; Jaksch et al. 1973; Neu et al. 1988; Fukunaga, Imagawa, Tabuchi & Akiyama 1981; Autorino et al. 1994; McCollum et al. 1995). The results obtained after intranasal re-challenge of horses, indicate that initial infection with asinine strain leads to reduced clinical and virological responses after re-exposure. Similar findings were reported from studies on EVA live vaccine (Doll, Bryans, Wilson & McCollum 1968; McCollum 1969; Paweska 1991). A significant finding from the present study was the rather low rate of lateral infection from successfully infected to seronegative horses and the absence of sexual infection of the seronegative group of mares covered by experimentally infected stallions.

The existence of a long-term carrier state in a high proportion of EAV seropositive stallions is of major epizootiological importance in virus maintenance and spread (Timoney & McCollum 1991). A transmission rate of almost 100% was demonstrated when mares seronegative to EAV were bred to long-term carrier stallions (Timoney et al. 1987; Golnik, Paweska & Dzik 1991). Lateral aerosol spread is generally a minor route of virus transmission, except in circumstances where clinical respiratory disease or abortion provides a substantial infectious aerosol and close prolonged contact takes place (Huntington, Forman & Ellis 1990). Indirect contact through contaminated fomites is not regarded as very important in disease spread (Timoney & McCollum 1987). During the first outbreak of EVA in the United Kingdom, some horses remained seronegative despite being stabled in the same airspace and not more than two meters from others which showed severe clinical signs of disease (Wood, Chirnside, Mumford & Higgins 1995). Under stud conditions monitored over a period of 2 years, of the 27 cases of EAV infection, 24 (85.2%) followed venereal transmission (Golnik, Paweska & Dzik 1992). This would indicate that lateral aerosol spread of virus depends on some still unknown conditions, and extremely close contact is required at least in the case of these particular strains of EAV. In this study, none of the horses infected with the asinine strain, developed coughing or abnormal sneezing which might have significantly increased dissemination of air-borne infections. The fact that animals were kept in relatively large outdoor pens under sunny and warm weather conditions may also have contributed to the reduction of aerosol spread of challenge virus.

Although, a limited number of horse stallions were tested, the negative results of both test breeding and virus isolation show that the challenge virus was not shed via semen during the post inoculation or re-challenge test period. The virus used in this study was isolated from semen of a naturally infected donkey stallion and was sexually transmitted from this stallion to seronegative donkey mares (Paweska et al. 1995). Failure to achieve venereal transmission of this strain among horses and to recover it from semen samples, raises the question of possible association between the species-mediated susceptibility and different strains of EAV in establishing the carrier state in stallions.

After three horses had been exposed to infected donkeys, they all developed fever and clinical signs at times and under circumstances which would suggest that lateral in-contact spread of challenge virus had occurred. Most of the clinical signs in horses were similar to those observed in donkeys, except that the
horses also developed mild diarrhoea. The latter sign was noted less frequently and usually accompanied other severe signs (Mumford 1985). Therefore, the failure to demonstrate seroconversion, the negative virus-isolation results and the absence of diarrhoea in all the inoculated animals, indicate that when the horses were exposed to the donkeys, they may have become infected with an agent other than the challenge virus.

The results of serological surveys conducted regularly between 1961 and 1987 suggested that EAV was not present in South Africa. However, in 1987 the virus was isolated from the semen of a Lippizaner stallion that had been imported for breeding purposes in 1980. It was subsequently established that all the mares served by this stallion were seropositive to EAV. Of 200 thoroughbred stallions tested in 1988, about ten had antibody to EAV, but no persistent shedder was detected at that time (Erasmus 1988). Less than 2% of the approximately 2 000 thoroughbreds tested in South Africa in 1989 were seropositive to EAV (Mumford 1994). Among sera collected from an equal number of horses in different regions of South Africa during 1995–1996, and sent to Onderstepoort Veterinary Institute for various other tests, about 1.4% were seropositive for EAV antibody (J.T. Paweska, unpublished data 1996). Nevertheless, it still remains to be established whether the virus is circulating in the country’s horse population or whether the seropositives represent only vaccinated animals that have been imported.

Under the conditions of this experimental study, the South African asinine strain of EAV was found to be of very low transmissibility and pathogenicity for horses. Our findings support the results of field studies which indicate a widespread distribution and long-standing presence of virus among South African donkeys (Paweska & Barnard 1993; Paweska 1994) and very restricted prevalence of seropositive horses (Erasmus 1988; Mumford 1994; J.T. Paweska, unpublished data 1996).

Further investigation needs to be undertaken to study the possible lack of target receptors for the asinine strain in the reproductive tract of horse stallions, to determine the clinical and virological protection given by the asinine strain against more virulent EAV strains, and to establish the potential epizootiological significance of EAV interspecies cross-infection.

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Transmission of the asinine strain of equine arteritis virus


