

## Experimental exposure of pregnant mares to the asinine-94 strain of equine arteritis virus

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### ABSTRACT

Clinical, virological and serological responses were evaluated in 10 pregnant mares after different challenge exposures to the asinine-94 strain of equine arteritis virus (EAV). The outcome of maternal infection on the progeny was also investigated. Mares were inoculated intranasally ( $n = 4$ ), intramuscularly ( $n = 2$ ), intravenously ( $n = 1$ ), or contact-exposed ( $n = 3$ ). All inoculated mares developed pyrexia, 5 showed mild clinical signs related to EAV infection and 2 remained asymptomatic. Viraemia was detected in all the inoculated animals and shedding of virus from the respiratory tract occurred in 6. Five mares were re-challenged intranasally 7 and 15 weeks after inoculation. Clinical signs of the disease in these mares were limited to mild conjunctivitis. After re-challenge, virus was recovered from buffy coat cultures of 2 mares 2–6 days after re-infection. EAV was not recovered from colostrum and milk samples during the 1st week *post partum*. All inoculated mares seroconverted to EAV 8–12 days post inoculation and also seroconverted after re-challenge. No clinical signs of EAV infection were observed in the 3 mares kept in close contact during the post-inoculation and re-challenge periods. Serum neutralising antibody to the virus was detected in 1 in-contact mare only, while a detectable concentration of specific IgG was found by ELISA in the colostrum of 1 of the other in-contact mares. Eight of the mares gave birth to clinically normal foals, although 1 was born prematurely. Shortly after birth, 7 foals developed fever and variable clinical signs; 5 foals became septicaemic and 3 of them died 2–5 days after birth, while the remaining 2 were euthanased at 1 month of age. EAV was not recovered from the placenta, from buffy coat fractions of blood collected from foals immediately after birth and 1–3 days later, or from a range of tissues taken from the 3 foals that died and 2 that were euthanased. Virus was not isolated from tissues collected from 1 mare and her foetus 3 weeks after this mare was re-challenged.

**Key words:** asinine-94 virus strain, equine arteritis virus, experimental exposure, pregnant mares, progeny, responses.

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### INTRODUCTION

Equine viral arteritis is an infectious disease of horses first recognised and characterised after an outbreak of respiratory disease and abortion in Standard-bred horses in the United States in 1953<sup>6</sup>. The causative agent, equine arteritis virus (EAV), is a small, enveloped, positive-stranded RNA virus classified in the genus *Arterivirus*<sup>1</sup>. Although antigenic<sup>8,9</sup>,

genomic<sup>3,24</sup> and pathogenic differences<sup>7,17</sup> among strains of the virus have been demonstrated, only 1 major serotype of EAV has been recognised<sup>11,15</sup>. The significance of the antigenic and genomic variation among EAV isolates in disease pathogenesis, diagnosis and vaccine development is not known. The virus is transmitted by the respiratory<sup>17</sup> and venereal routes<sup>32</sup> and to a lesser extent by indirect contact with contaminated fomites<sup>30</sup>. A long-term carrier state is established in a high proportion of stallions<sup>32</sup> but has not yet been demonstrated in mares or foals. EAV has a worldwide distribution in different breeds of horses<sup>31</sup>, while serological surveys have also revealed longstanding presence of the virus in donkeys in some African countries<sup>23,25,27</sup>. Susceptibility of

donkeys to a horse isolate<sup>20</sup>, and conversely of horses to a donkey isolate of EAV<sup>28</sup>, has recently been demonstrated.

The clinical outcome of EAV infection varies widely, ranging from overt signs of disease to very mild, often subclinical infection. Typical cases of disease may present with any combination of the following signs: pyrexia, anorexia and depression, lacrimation, conjunctivitis, oedema of the periorbital region, legs, genitals and abdomen, serous nasal discharge and congestion of the nasal mucosa. Less frequently photophobia, coughing, respiratory distress, diarrhoea, general weakness and unsteadiness are observed. In its most severe form, EAV infection causes abortion in pregnant mares and infrequently death amongst young foals. Abortion may occur in mares from 3 to over 11 months of gestation during, or shortly after, an acute or subclinical infection. EAV-induced abortion has been reported in 15 of the 40 confirmed outbreaks in North America and Europe with the abortion rate varying from less than 10 % to between 50 and 60 %<sup>30,31</sup>. Sporadic cases of EVA-induced abortions were observed on 1 stud over a period of several years<sup>21</sup>. Under experimental conditions, abortion rates of 38 % and 71 % have been reported<sup>5,18</sup>. Three possible mechanisms for abortion following EAV infection have been suggested. Abortion may result from lethal foetal infection<sup>7</sup>, from impaired utero-placental blood flow due to generalised vascular necrosis<sup>14</sup>, or occurs as a result of myometrial necrosis and oedema leading to placental detachment and foetal death, with or without foetal infection<sup>4</sup>. Usually the foetuses aborted in natural or experimental cases of EVA have no significant lesions<sup>4,6,14</sup>. EVA in foals is frequently complicated by secondary bacterial infection, making diagnosis difficult<sup>22</sup>.

The aim of this study was to determine clinical, virological and serological responses in pregnant mares following different methods of exposure to the asinine-94 strain and to determine the effect of maternal infection on their progeny.

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## MATERIALS AND METHODS

### Mares

Ten clinically normal, EAV-seronegative mares in the 5th to 11th month of gestation were used. They included 2 ponies, 5 and 7 years old (Nos 1 and 2) and 8 cross-bred horses between 6 and 10 years old (Nos 3–10).

### Virus and animal inoculation

The 2 sources of the asinine-94 strain of EAV were semen collected from a donkey carrier stallion and the 4th passage of the virus on the RK-13 line of rabbit kidney cells<sup>28</sup>. A 6 ml inoculum of sonicated semen supernatant containing  $10^{3.5}$ TCID<sub>50</sub>/ml (inoculum A) was administered intramuscularly (i.m.) or intranasally (i.n.) and 6 ml of the cell culture supernatant fluid containing  $10^{4.5}$ TCID<sub>50</sub>/ml (inoculum B) was administered i.n. or intravenously (i.v.).

### Experimental design

Mares were kept in 3 separate groups in open pens approximately 20 × 10 m. Group I consisted of 4 mares (Nos 1–4) at 5–7 months of gestation. The mares were initially infected with inoculum A. Nos 1 and 3 were inoculated i.n. and Nos 2 and 4 i.m. Fifteen weeks later they were all re-challenged i.n. using inoculum B. Group II contained 4 mares (Nos 5–8) at 5 to 9 months of gestation. Nos 5 and 6 were challenged i.n. with inoculum A and 7 weeks later they were re-challenged i.n. with inoculum B. Nos 7 and 8 were exposed by contact with Nos 5 and 6 during the post-inoculation and post-challenge periods. No. 6 was euthanased 3 weeks after re-challenge and her foetus removed from the uterus. Group III consisted of 2 mares at 10–11 (No. 9) and 7–8 (No. 10) months of gestation. No. 9 was inoculated i.v. with inoculum B and No. 10 used as in-contact control (Table 1). Mares were monitored for the clinical signs of EVA and rectal temperatures were recorded twice daily at approximately 08:00 and 16:00.

### Virological procedures

Heparinised blood for buffy coat preparations was collected from mares every 2nd day for the 1st 3 weeks after infection and for the 1st 2 weeks after re-challenge, and from foals (Nos 1–5 and 7–10) immediately after birth, and on days 1 and 3 thereafter. Nasal swabs were taken from mares every 2nd day for the 1st 2 weeks post challenge. Colostrum was collected at parturition, and milk samples on days 2, 4 and 6 *post partum* from all the mares. Specimens of placenta were collected

from all the mares, and uterus, amnion and amniotic fluids, mammary glands and lymph nodes from No. 6; also lung, spleen, kidney and liver and lymph nodes from 3 dead (Nos 3, 7, 9) and 2 euthanased (Nos 4, 8) foals and from the foetus from mare No. 6. Tissues were collected aseptically as soon after death or euthanasia as possible.

After collection, all specimens were placed on freezer packs and, with a minimum of delay, transported to the laboratory, where they were either processed immediately or frozen at –20 °C.

Buffy coat preparations, nasal swabs and tissues were collected and processed for virus isolation as described previously<sup>19</sup>. Ten ml of colostrum or milk was sonicated, then centrifuged for 15 min at 5000 g, and the supernatant inoculated onto cell cultures. The RK-13 line of rabbit kidney cells (ATCC CCL37) was used for virus isolation. Maintenance of cell cultures and virus isolation and identification procedures followed those previously described<sup>26</sup>.

### Serological procedures

Blood samples for serology were collected from mares at regular intervals throughout the experiment, and from the foals immediately after birth before they were nursed or bottle-fed (day 0), and on days 7, 30, 60 and 90 after birth. Colostrum was collected as soon as possible *post partum* and milk samples every 2 days for 2–3 weeks thereafter. Colostrum and milk samples were processed for serological examination by the method of McCollum<sup>16</sup>. Antibodies to EAV were detected by a virus neutralisation test (VN)<sup>25</sup> and by an enzyme-linked immunosorbent assay (ELISA)<sup>2</sup>.

### Bacteriological examination

Samples from spleen, liver, kidney, lung and brain from each of the necropsied foals were collected for bacteriological examination. Colostrum, milk samples and vaginal swabs collected irregularly from mares *post partum* and ocular swabs taken from the foal of mare No. 2 on day 12 were examined.

Samples from mares and foals were cultured on blood tryptose agar and MacConkey agar under aerobic conditions at 37 °C for at least 72 hours. Significant isolates were identified according to standard methods<sup>13,29</sup>.

### Pathological examination

Foals that died (Nos 3, 7, 9) or were euthanased (Nos 4, 8), and the foetus from mare No. 6, were all necropsied and various tissues were fixed in 10 % buffered

formalin for histology. These included brain, conjunctiva, thymus, lungs, myocardium, liver, spleen, kidney, adrenal glands, intestines, mesenteric lymph nodes, skeletal muscle and skin. Specimens from each foal were routinely processed and stained with haematoxylin and eosin (HE). A peripheral blood smear from each foal was routinely examined for parasites. Placenta from each mare was examined.

## RESULTS

### Clinical signs

All 7 inoculated mares developed a fever of 2–4 days duration (mean, 3 days), with maximum rectal temperatures from 38.7 °C to 39.5 °C (mean 39.1 °C) recorded 2–9 days after inoculation (Table 1). Nos 2, 3, 5, 6 and 9 developed mild depression, conjunctivitis and serous ocular and nasal discharges. In Nos 3 and 5 slight periorbital and ventral trunk oedema was observed. Most clinical signs were present on days 3–7 after inoculation, except for oedema of the periorbital area which developed 4–10 days p.i., and oedema of the abdomen, which was observed 6–12 days p.i. No pyrexia and only slight conjunctivitis were observed in mares 5–9 days post-challenge. No clinical signs or fever were noted in Nos 7, 8 and 10, contact-exposed to inoculated mares. No abnormal conditions of birth were observed and all mares delivered normally. No clinical evidence of mastitis or abnormal lactations were present.

The main clinical findings in foals in the 1st weeks after birth are summarised in Table 2.

All foals were physically normal at the time of delivery except one (No. 9), which was premature. Shortly after birth, 7 foals (Nos 1–4, 8, 9) developed fever and 5 of them (Nos 3, 4, 7, 8, 9) exhibited a variety of clinical signs including depression, general weakness, anorexia, reduced suckling reflex, swelling and pain of joints associated with skin ulcerations, convulsions and coma. Three foals (Nos 3, 7, 9) died within 2–5 days and 2 (Nos 4, 8) were euthanased 4 weeks after birth.

### Virological findings

EAV was recovered from buffy coat preparations of all inoculated mares on days 2–10, and from nasal swabs of 6 out of 7 mares on days 2–8 p.i. After re-challenge, virus was detectable only in buffy coat cultures from mares Nos 1 and 3, on days 2 and 4–6 respectively. EAV was not recovered from placentas, colostrum and milk samples or from buffy coat

Table 1: Clinical and serological findings in pregnant mares inoculated with the asinine-94 strain of EAV and in-contact pregnant mares.

Mare No.	Method of initial inoculation and time of re-exposure		Approximate period of gestation at time of initial inoculation (months)	Fever		Abortion	Time of seroconversion <sup>a</sup> (days p.i. / re-challenge)	Antibody to EAV at time of parturition			
	Inoculation and route	Re-challenge (days p.i.) and route		Max. temp. (°C)	Duration (days p.i.)			Serum VN	Serum ELISA <sup>b</sup>	Colostrum VN	Colostrum ELISA
<b>Group I</b>											
1	i.n.	105 i.n.	6–7	38.7	4–5	No	10 / 14	1.5	0.74	2.1	1.32
2	i.m.	105 i.n.	5–6	39.5	3–6	No	8 / 12	1.2	0.40	1.8	0.85
3	i.n.	105 i.n.	6–7	39.1	7–9	No	12 / 10	1.8	0.85	2.7	1.63
4	i.m.	105 i.n.	6–7	38.8	5–6	No	10 / 10	1.2	0.37	2.1	1.13
<b>Group II</b>											
5	i.n.	49 i.n.	7–8	38.9	3–5	No	10 / 12	1.2	0.45	1.8	0.91
6	i.n.	49 i.n.	7–8	39.3	4–5, 7–8	*	12 / 14	*		*	
7	contact	contact	5–6	—		No	28	0.6	0.18	0.9	0.24
8	contact	contact	8–9	—		No	—	<0.6	<0.135	t.e.	0.16
<b>Group III</b>											
9	i.v	—	10–11	39.1	2–5	No	8	1.5	0.62	2.4	1.45
10	contact	—	7–8	—		No	—	<0.6	<0.135	<0.6	<0.135

<sup>a</sup>Virus-serum neutralisation titre  $\log_{10} \geq 0.6$  and 4-fold increase in antibody titre after re-exposure; <sup>b</sup>ELISA IgG positive: OD value  $A_{490} \geq 0.135$ ; \*Mare No. 6 was euthanased 3 weeks after re-challenge; t.e. = toxic effect of colostrum at dilution  $\log_{10} 0.6$ ; p.i. = post inoculation.

Table 2: Clinical, bacteriological and pathological findings in foals and a foetus from mares experimentally exposed to the asinine-94 strain of EAV.

Foal or foetus  No.	Major clinical signs			VN <sup>a</sup> and IgG ELISA <sup>b</sup> antibody to EAV (days after birth)					Bacteriology	Gross pathology
	Fever		Others							
	Max. temp. (°C)	Duration (days after birth)		0 <sup>c</sup>	7	30	60	90		
Group I										
1	39.2	2, 3, 5	Hyperaemia of conjunctiva on days 2–5; recovered	<0.6 <0.134	1.2 0.5	0.9 0.18	<0.6 <0.135	<0.6 <0.135	ND	ND
2	39.7	2, 3, 4	Conjunctivitis on days 3–21, diarrhoea on days 14–15; recovered	ND	1.2 0.42	0.9 0.21	0.6 0.16	<0.6 <0.135	<i>Actinobacillus equuli</i> from ocular swab	ND
3	40.5	3, 4	Lethargy, reduced suckling reflex, convulsion, coma; died on day 5	<0.6 <0.135	ND	ND	ND	ND	<i>Streptococcus zooepidemicus</i> from various organs	Septicaemia, cellulitis and necrotic myositis
4	40.2	2–28	Depression, anorexia, lameness, swelling and painful joints with associated skin ulcerations, altered behaviour, icterus, hypothermia; euthanased day 30	<0.6 <0.135	1.5 0.65	1.2 0.38	ND	ND	<i>Salmonella typhimurium</i> and <i>Staphylococcus aureus</i> from various organs	Septicaemia, icterus and anaemia, cellulitis and skin ulceration, <i>Babesia equi</i> parasitaemia
Group II										
5	—	—	No clinical signs	<0.6 <0.135	1.5 0.68	1.2 0.45	0.9 0.2	ND *	ND	ND
6*	—	—	*	*	*	*	*	*	ND	Subcutaneous and intermuscular haemorrhages overlying the metacarpal and hip joints
7	—	—	Weakness, difficulty in standing, reduced suckling reflex, convulsion, coma; died on day 2	<0.6 <0.135	ND	ND	ND	ND	<i>Actinobacillus equuli</i> from various organs	Septicaemia, icterus, oedema of ventral abdomen
8	40.5	2–30	Depression, lameness, swelling and painful joints with associated skin ulcerations; euthanased day 31	<0.6 <0.135	<0.6 <0.135	ND	ND	ND	<i>Streptococcus zooepidemicus</i> and <i>Staphylococcus aureus</i> from various organs	Septicaemia, focal arthritis with cellulitis and skin ulceration
Group III										
9	39.8	2	Reduced strength of suckling, convulsion, coma; died day 3	<0.6 <0.135	ND	ND	ND	ND	<i>Actinobacillus equuli</i> from various organs	Septicaemia, oedema of legs, ulcerative stomatitis
10	—	—	No clinical signs	ND	<0.6 <0.135	<0.6 <0.135	<0.6 <0.135	ND	ND	ND

<sup>a</sup>Virus serum-neutralisation test positive result  $\log_{10} \geq 0.6$ ; <sup>b</sup>ELISA IgG positive OD value  $A_{490} \geq 0.135$ ; <sup>c</sup>Sample collected immediately after birth before foal was nursed or bottle-fed; \*foetus collected from uterus 3 weeks after mare was re-challenged i.n.; ND = not done.

Table 3: Virological findings in pregnant mares inoculated with the asinine-94 strain of EAV, and in the progeny from inoculated and in-contact exposed mares.

Mare and foal No.	Mares														Placenta, colostrum and milk	Foals			
	Nasopharynx					Buffy coat						Buffy coat (days after birth) 0-3	Lymph nodes and organs						
	Days post inoculation					Days post inoculation								Days post re-challenge					
	2	4	6	8	10-14	2	4	6	8	10	12-22			2		4	6	8-14	
Group I																			
1	+	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	ND	
2	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	ND	
3	-	+	+	+	-	+	+	+	+	+	-	-	+	+	-	-	-	-	
4	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	
Group II																			
5	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	ND	
(6)*	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	- <sup>c</sup>	ND	-
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
Group III																			
9	+	+	+	-	-	+	+	+	+	+	-	ND	ND	ND	ND	-	-	-	-
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-	ND

+ = positive virus isolation on RK-13 cells.

- = negative virus isolation on RK-13 cells.

\* = foetus collected from uterus 3 weeks after mare was re-challenged.

c = colostrum and milk not tested.

ND = not done.

fractions of blood from foals, or from a range of tissues collected from foals, or from mare No. 6 and her foetus at necropsy (Table 3).

### Serological findings

All inoculated mares seroconverted to EAV 8–12 days p.i. Peak levels of VN ( $\log_{10}$  1.2–1.8) and IgG antibodies (OD 0.47–0.79) were detected 13–22 days p.i. Seroconversion to EAV was detected only in 1 in-contact mare (No. 7) on day 28 with peak levels of VN ( $\log_{10}$  1.2) and IgG antibodies (OD 0.39) on day 40 after exposure. In the re-challenged mares, peak antibody concentrations of VN ( $\log_{10}$  1.5–2.1) and IgG (0.67–1.25) were detected 10–13 days after re-infection. During a period of post re-challenge exposure, No. 7 had stable titres of VN and IgG antibody, while No. 8 tested negative consistently. The concentrations of VN and IgG ELISA antibodies in serum and colostrum collected at the time of parturition are shown in Table 1. The colostrum sample from No. 8 proved to be toxic in the VN test at a dilution of  $\log_{10}$  0.6, and it was negative at higher dilutions, but tested low positive for IgG ELISA antibody (OD 0.16). The VN titres and ELISA IgG OD absorbance values in milk samples collected from Nos 1, 2, 5 and 7 on days 2 and 5 *post partum* varied from  $\log_{10}$  0.6 to 1.2 and 0.150 to 0.47 respectively. Samples taken from these mares on day 7 all tested negative in the VN test, 3 were negative but 1 from No. 5 was low positive in ELISA

(OD 0.135). VN and IgG concentrations in milk of Nos 3, 4 and 9 varied from  $\log_{10}$  1.2 to 1.5 and from 0.39 to 0.55 on day 2; from  $\log_{10}$  0.6 to 1.2 and 0.170 to 0.51 on day 14, respectively. Samples taken on day 21 from No. 4 tested negative but samples from Nos 3 and 9 were positive, with a concentration of VN antibody of  $\log_{10}$  0.9 and 1.2 and IgG antibody of 0.18 and 0.45 respectively. Milk samples from Nos 8 and 10 tested negative.

Sera collected from foals immediately after birth, before they were nursed or bottle-fed, tested negative for antibody against EAV both in the VN test and ELISA. The highest concentration of maternal antibodies was at 1 week of age; they were not detectable 3 months after birth (Table 2).

### Pathological findings

No evidence of gross lesions was present in the placentas. Gross and histological lesions in the 5 foals that were necropsied supported the diagnosis of septicaemia. Gross lesions are summarised in Table 2. Icterus was noted in foals Nos 4, 7 and 8. In foals Nos 3, 4 and 8, acute focally-extensive cellulitis associated with serofibrinous arthritis, necrotic myositis or skin ulceration occurred; 1 or 2 joints of the front or hind legs were affected. Other macroscopical lesions included *Babesia equi* parasitaemia (<5 %) and anaemia in foal No. 4; oedema of the ventral abdominal wall in foal No. 7; moderate ascites and umbilical abscessation in foal No. 8, and

subcutaneous and intermuscular oedema of some limbs and focal ulcerative stomatitis in foal No. 9. Evidence of arteritis was not observed histologically in tissues from the foals. Subcutaneous and intermuscular haemorrhages overlying the metacarpal and hip joints were evident in the foetus from mare No. 6. There were no significant lesions in the placentas.

### Bacteriological findings

*Actinobacillus equuli* was isolated from the vagina of mare No. 7 on day 1 after foaling. *Streptococcus zooepidemicus* was isolated from the vagina of 3 mares. Mare No. 3 yielded *S. zooepidemicus* from 3 consecutive swabs taken a month after foaling, mare No. 8 from 3 subsequent swabs taken 2 weeks *post partum*, and mare No. 9 from a swab collected on day 17 *post partum*. *Staphylococcus aureus* was isolated from 2 mares. Mare No. 2 yielded *S. aureus* from vaginal swabs on 4 occasions, taken on days 2, 3, 5 and 12 *post partum*. Mare No 4 yielded *S. aureus* from the vagina and milk 1 month *post partum*.

*Actinobacillus equuli* was isolated from 3 foals. Two foals (Nos 7 and 9) were septicaemic, and No. 2 only yielded *A. equuli* from an ocular swab collected 12 days after birth. *Streptococcus zooepidemicus* was isolated from all the organs submitted at necropsy from foals 3 and 8. Foal No. 8 was also infected with *Staphylococcus aureus*. *S. aureus*, together with *Salmonella typhimurium*, occurred septicaemically in foal No. 4 (Table 2).

## DISCUSSION

In this study all the mares that were directly inoculated with the asinine-94 strain of EAV became infected, as evidenced by related clinical signs, seroconversion and virus recovery from buffy coat cultures and from nasal swabs. Infected animals developed only mild clinical signs or remained asymptomatic, with mean viraemia and virus-shedding from the nasopharynx similar to those recently reported in non-pregnant mares and stallions experimentally infected with the asinine-94 strain<sup>26</sup>. The only difference compared to previous clinical findings in horses experimentally infected with the asinine-94 strain consisted of slight oedema of the periorbital fossa and of the ventral trunk region observed in 2 mares in the present experiment. None of 3 mares exposed by contact with experimentally infected mares developed fever or other clinical signs, and seroconversion to EAV could be detected only in serum samples collected from 1 mare (No. 7; Table 1). There was no significant effect of the different virus challenge routes on the clinical, virological and serological responses in inoculated pregnant mares.

The positive ELISA results in the colostrum from mare No. 8 (Table 1) may indicate infection from lateral in-contact spread of the virus. The discrepancy in ELISA results in the serum and colostrum from this mare could be due to different concentrations of antibodies to EAV in both samples. As is the case in this study, a much higher concentration of EAV antibody was demonstrated by McCollum<sup>16</sup> in the colostrum than in serum. Among horses immunised with an EAV inactivated vaccine, 39 % tested ELISA positive but VN negative. Moreover, based on ELISA findings in field samples, it was postulated that VN antibody responses are not always induced by natural infection or that the titres drop below VN test sensitivity. The other reason for differences in sensitivity between the 2 tests may relate to the fact that the ELISA detects antibodies that bind recombinant EAV glycoprotein, of which those neutralising virus *in vitro* are a subpopulation. To date only the demonstration of circulating VN antibodies is generally accepted as evidence of infection<sup>2</sup>. The toxic effect of the colostrum in mare No. 8 prevented direct comparison by both VN test and ELISA, and the possibility that this mare was exposed to challenge virus could not be definitely established.

The directly inoculated and in-contact pregnant mares did not abort in the present study. Negative virological findings in the placenta and tissues from

the foals indicated that at least at the time of birth neither the mares nor the foals were infected or shedding the challenge virus. In a study on the pathogenicity of the KY-84 strain of EAV, of 6 susceptible mares inoculated i.n., 3 aborted, and of 7 in-contact exposures, 2 aborted, while virus was recovered from 4 of the aborted foetuses<sup>18</sup>. In another study, 10 of 13 mares aborted after in-contact exposure to infected mares and virus was isolated from foetal tissues or placenta<sup>5</sup>.

In cases of EAV-induced abortion, foetal tissues and placenta can be heavily infected with virus and can serve as direct sources of infection for susceptible in-contact animals or an indirect source of virus dissemination by contaminated personnel, equipment and the environment<sup>4</sup>. Although some degree of humoral antibody production can be demonstrated in prepartum foals, the diffuse epitheliochorial placenta of the mare does not allow the transfer of immunoglobulins to the foetus *in utero*. The newborn foal is immunologically naive but fully immunocompetent<sup>12</sup>. All foals in this study were born seronegative, and passive immunity to EAV was not detectable 1–3 months after birth. Similar results were reported by McCollum in foals born by mares immunised with the Bucyrus strain of EAV<sup>16</sup>.

In 4 cases of septicaemia in foals during this experiment, the same bacterial isolate was shed by the mare, indicating that the urinogenital tract of the mother was the primary source of infection, which most probably took place at the time of parturition. Septicaemia is known to be the leading cause of neonatal foal morbidity and mortality associated with infection by *Escherichia coli*, *Actinobacillus* sp., *Pasteurella* sp., *Klebsiella* sp., *Salmonella* sp., *Streptococcus* sp. and *Staphylococcus* sp. The main factors predisposing to neonatal septicaemia include environmental factors, perinatal stresses and disorders of the neonatal immune system. Prematurity, which can also be induced by viral infection, predisposes to neonatal septicaemia<sup>34</sup>. Pregnant mares in this study were kept in large open camps under normal management and in warm weather conditions. Mortality due to EAV infection has only been observed in horses experimentally infected with the unmodified Bucyrus strain of EAV<sup>4,6</sup> and infrequently in naturally infected foals a few days to a few months of age<sup>10,11,21,33</sup>. Of 26 foals dying between 1980–1990 on some English Thoroughbred and also Halfbred studs in the Silesia district in Poland, EAV was recovered in 13 cases. In about 79 % of these foals, viral infection

was complicated by secondary bacterial infection<sup>21</sup>. In contrast, bacterial cultures from 8 aborted foetuses following EAV infection were negative<sup>5</sup>.

Whatever the cause of elevated susceptibility to bacterial infection observed in foals in this study, the failure to induce abortion in pregnant mares following different challenge exposures to the South African asinine-94 strain indicates that this isolate of EAV is not abortogenic and does not cause a carrier state in foals born to mares infected at the time of pregnancy. Although seroepidemiological studies showed that EAV is endemic in the South African donkey population<sup>25,27</sup>, field incidences of clinical disease or abortion and mortality in foals due to EAV infection have never been reported in any species of Equidae in the country.

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