



Effect of the South African asinine-94 strain of equine arteritis virus (EAV) in pregnant donkey mares and duration of maternal immunity in foals

J.T. PAWESKA

Department of Virology, Onderstepoort Veterinary Institute
Private Bag X5, Onderstepoort, 0110 South Africa

ABSTRACT

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Clinical, virological and serological responses were investigated in five pregnant donkey mares after experimental exposure to the South African asinine-94 strain of equine arteritis virus (EAV), and the duration of maternal immunity to EAV was studied in their foals.

In four intranasally inoculated mares, fever with maximum rectal temperatures of 39,1–40,7°C was recorded 2–11 d after challenge. All the inoculated mares developed mild depression, and a serous ocular and nasal discharge; in three mares mild conjunctivitis was observed. The virus was recovered from the nasopharynx and from buffy-coat samples of all the mares 3–10 d, and 2–16 d post inoculation (p.i.), respectively. Seroconversion to EAV was detected on days 8–10 p.i. Peak serum-virus-neutralizing antibody titres of \log_{10} 1,8–2,4, and IgG ELISA OD values of 0,85–2,15 were recorded 2–3 weeks p.i. The in-contact (p.c.) control mare developed fever on days 15–19 post exposure, and showed mild clinical signs of equine viral arteritis similar to those observed in the inoculated mares. Seroconversion to EAV was detected in the p.c. mare on day 20 post exposure, and virus was isolated from nasal swabs and blood samples collected at the time of the febrile response and 1–3 d afterwards. None of the mares aborted. After they had given normal birth 45–128 d p.i. or after p.c. exposure, no virus could be isolated from their placentas. The concentration of EAV-neutralizing antibody in colostrum was two to eight times higher than in serum samples collected at the time of parturition.

All the foals born to infected mares were clinically normal at the time of birth and throughout the subsequent 1–2 months of observation. No EAV was recovered from the buffy-coat fraction of blood samples collected at birth nor from those collected on days 1, 2 and 7 after birth. Also, no virus-serum-neutralizing or IgG ELISA antibody to EAV was detected in sera collected immediately after birth before the foals started nursing. The colostrum-derived maternal antibodies against EAV gradually declined and could not be detected by either the VN test or ELISA for 2–3 months after birth.

This study demonstrates that the asinine-94 strain of EAV does not cause abortion in pregnant donkey mares. Furthermore, no carrier state could be demonstrated in foals born to mares infected at the time of pregnancy.

Keywords: Asinine strain, equine arteritis virus, experimental challenge, foals, passive immunity, pregnant donkey mares

INTRODUCTION

Equine arteritis virus (EAV) was first isolated in 1953 from foetal-lung tissue during an outbreak of abortion on a horse farm near Bucyrus, Ohio (USA) (Doll,

Bryans, McCollum & Crowe 1957a). EAV was originally classified as a Togavirus (Porterfield, Casals, Chumakov, Gaidamovich, Hannoun, Holmes, Horzinek, Mussgay, Oker-Blum, Russel & Trent 1978), but has recently been designated the prototype of the new genus Arterivirus (Cavanagh, Brien, Brinton, Enjuanes, Holmes, Horzinek, Lai, Laude, Plagemann

& Siddell 1994). Although only one serotype is thought to exist (McCullum 1969; Fukunaga, Matsumura, Sugiura, Wada, Imagawa, Kanemaru & Kamada 1994), strains of EAV have been shown to differ in their pathogenicity (Bürki 1970; McCullum & Swerczek 1978).

The two major modes of EAV transmission are by direct contact as droplet infection via the respiratory route (McCullum & Swerczek 1978) and via venereal infection from carrier stallions during mating (Timoney, McCullum, Murphy, Roberts, Willard & Carswell 1987). While exposure to EAV may result in the development of clinical infection, the vast majority of field infections appear to be inapparent. Clinical signs of equine viral arteritis (EVA) vary widely and, regardless of severity, naturally infected adult horses make uneventful recoveries (Timoney & McCullum 1991). However, mortality has been reported in naturally infected foals (Golnik, Michalska & Michalak 1981; Valla, Hamir, Dubovi, Timoney & Ruiz 1992) and in experimentally challenged horses (Doll *et al.* 1957a; Coignoul & Cheville 1984). To the horse industry EAV-related abortions are economically significant. Abortion may occur in mares from three to more than 11 months of gestation during, or shortly after, an acute or subclinical infection with EAV (Timoney & McCullum 1991).

Recent virological and transmission studies in South Africa: Paweska, Volkmann, Barnard & Chirnside 1995) have confirmed the earlier serological findings of Himeur (1976) and Moraillon & Moraillon (1978) that the natural host range of EAV includes both horses and donkeys. Serosurveys demonstrated a widespread distribution of EAV, and suggest not only a longstanding presence but also an increasing spread of the virus amongst donkeys in South Africa (Paweska & Barnard 1993; Paweska, Binns, Woods & Chirnside 1997). While no field cases of clinical disease have been reported in this species, experimental exposure to both the asinine-94 and KY-84 strains of EAV have resulted in mild clinical infection (Paweska *et al.* 1995; Paweska, Aitchison, Chirnside & Barnard 1996; McCullum, Timoney & Tengelsen 1995).

The primary objective of this study was to establish whether the South African asinine-94 strain of EAV would cause abortion in pregnant donkey mares after intranasal inoculation, and in-contact (p.c.) exposure. In the event of abortion not occurring, the second aim was to determine the duration of maternal immunity to EAV in donkey foals.

MATERIALS AND METHODS

Donkeys

Five pregnant donkey mares (numbered 1–5), clinically normal, sero-negative to EAV, 4–8 years old and

7–10 months in foal, were used. The donkeys were purchased from their owners in the North-West and Northern Provinces, and kept at the Onderstepoort Veterinary Institute throughout the trial.

Animal inoculation

The source of the asinine-94 strain of EAV was the semen of a naturally infected jackass that had been identified as a long-term carrier and shedder of virus (Paweska *et al.* 1995). A 3-ml inoculum comprising the supernatant of sonicated semen containing approximately $10^{3.5}$ TCID₅₀/ml of virus, was administered into each nostril by means of a plastic catheter (Doll 1960).

Experimental design

Mares 1–4 were inoculated intranasally (i.n.) and mare 5 was p.c. exposed. All the mares were kept together in an open pen for 4 weeks post inoculation (p.i.). Then the two mares, most advanced in pregnancy (mares 2 and 4), were moved to an adjacent pen. Mares and foals were monitored clinically throughout this experiment, and a range of samples were taken for virological and serological examination.

Clinical examination

Mares were observed for the clinical signs of EVA twice daily, and rectal temperatures were recorded at 4 p.m. for a period of 4 weeks p.i., or lateral p.c. exposure. Mares were supervised at the time of parturition. New-born foals were physically examined after delivery and then monitored for an additional 1–2 months.

Collection and processing of specimens

Blood

Blood samples for serum were taken from mares before inoculation, and then at regular intervals. The last samples were collected on the day of parturition. Heparinized blood was taken from the inoculated mares every day for the first 3 weeks p.i., and from the p.c.-exposed mare during a febrile response and 3 d afterwards. Blood samples were collected from foals immediately after birth, before they started nursing, and then at regular intervals for an additional period of 2–3 months. Heparinized blood was taken at birth, and then on days 1, 2 and 7.

Nasal swabs

Nasal swabs were taken from the inoculated mares and the p.c. control mare, along with the heparinized blood samples.

Placenta, colostrum and milk

Placenta and colostrum samples were collected as soon as possible post parturition, and milk samples were taken on days 2, 5, 7 and 14 after that.

After collection, all specimens were transported to the laboratory on freezer packs. Buffy-coat preparations, nasal swabs and tissues were processed for virological examination as described by McCollum, Prickett & Bryans (1971). Colostrum and milk samples were processed for serological testing by the method of McCollum (1976).

Cell culture and virus isolation

Maintenance of the RK-13 line of rabbit-kidney cells (Paweska 1997), virus isolation and identification procedures, follow those previously described (Paweska *et al.* 1996).

Serological examination

Virus-neutralization (VN) test

EAV-neutralizing antibodies were detected in a complement-dependent VN test in microtitre plates with RK-13 cells and the Bucyrus isolate of EAV as antigen (Paweska & Barnard 1993). A serum was considered positive when it had a VN antibody titre $\log_{10} \geq 0,6$.

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA (Chirnside, Francis, De Vries, Sinclair & Mumford 1995), with slight modifications (Paweska 1997), was used to detect IgG antibodies to EAV. The recombinant EAV ELISA antigen was obtained from Dr Evan Chirnside (The Animal Health Trust, Newmarket, UK). An $A_{490} \geq 0,135$ reading was taken as the cut-off point determining an ELISA-positive absorbance value.

RESULTS

Clinical findings

In mares 1–4, an increase in rectal temperatures was recorded 2–11 d p.i.. Duration of fever was 4–8 d (mean 5,8 d), with maximal rectal temperatures ranging from 39,1–40,7°C (mean 39,8°C). In mare 5, fever was recorded on days 15–19 p.c. exposure, and reached a maximum temperature of 39,9°C (Table 1). All mares developed only mild depression, and a slight serous nasal and ocular discharge shortly before or during the time of the febrile response. In three of four inoculated mares, mild conjunctivitis of about 1 week's duration was observed. Two to four days after the end of pyrexia, all animals were clinically normal. None of the mares aborted. Mares 1, 2, 3 and 4 gave birth on days 128, 57, 65 and 45 p.i., respectively, and mare 5 on day 103 p.c. exposure.

All foals were clinically normal at the time of birth and during the subsequent period of observation.

Virological findings

EAV was recovered from the nasopharynx of mares 1–4 3–10 d p.i. and from buffy-coat cultures 2–16 d p.i. The mean duration of virus shedding from the respiratory tract was 4,25 d and the mean viraemia 11,25 d. In mare 5, virus was isolated from the nasopharynx and the buffy coat at the time of febrile response and 1–3 d after that. Virus was not isolated from the placentas (Table 1).

EAV was not recovered from buffy-coat fraction of blood samples taken from foals at birth, nor on days 1, 3 and 7 after that (Table 3).

Serological findings

In mares 1–4, seroconversion to EAV was detected on days 8–10 p.i., and in mare 5, on day 20 p.c. exposure. Peak VN antibody titres of \log_{10} 1,8–2,4 and IgG ELISA OD values of 0,85–2,15 (Table 1) were detected 2–3 weeks p.i. At the time of parturition, the VN antibody titres in colostrum were two to eight times higher than those in the serum samples. All milk samples collected on day 7 post partum, tested negative in the VN test; the ELISA showed only one to be low positive (Table 2).

Serum samples of foals, collected before they started nursing, were negative for EAV antibody. On day 2 after birth, VN antibody titres varied from \log_{10} 1,5–2,4 and ELISA IgG OD values, from 0,72–2,2. All foals were sero-positive on day 30, two of them were VN and ELISA positive, and one ELISA low positive on day 60. All foals were negative 90 d after birth (Table 3).

DISCUSSION

In this study, all the pregnant mares became infected with the asinine-94 strain of EAV. The clinical, serological and virological findings were similar to those previously reported in non-pregnant donkeys challenged with the same strain and dose of virus (Paweska *et al.* 1995; Paweska *et al.* 1996). Regardless of the route of exposure to the asinine-94 strain, none of the infected mares aborted; there was no evidence of premature delivery or gross abnormalities in the placentas. All foals were physically normal at the time of birth and did not show any signs of disease subsequently. The negative virus isolation results from the placentas and buffy-coat fractions of blood indicates that, at the time of birth, the virus was not present in the placental tissues nor in the white-blood cells of the new-born foals.

Transplacental transmission of EAV to the foetus, accompanied by abortion, has been documented (Doll

TABLE 1 Clinical, virological and serological responses of five pregnant donkey mares exposed to the South African asinine-94 strain of EAV

Mare ure	Route of exposure	Approx. age of foetus at the time the mare was exposed	Fever		Abortion	Delivery ^a	Virus isolation ^{a,b}			Sero-conversion ^{a,c}	Peak VN titre	Peak ELISA IgG
			Max. temp. °C	Duration ^a			Nasopharynx	Buffy coat	Placenta			
1	i.n.	7-8 m	40,7	4-11	No	128	4-10	3-12	-	8	1,8	0,85
2	i.n.	8-9 m	39,1	2-5	No	57	6, 8	2-9	-	8	2,1	1,63
3	i.n.	8-9 m	39,9	4-10	No	65	3-8	3-16	-	10	2,4	1,92
4	i.n.	9-10 m	39,4	3-5, 7-8	No	45	5, 8	2-14	-	8	2,1	1,75
5	contact	7-8 m	39,9	15-19	No	103	1-20	15-22	-	20	2,4	2,15

^a Days after inoculation or in-contact exposure

^b Virus isolated on RK-13 cells

^c VN antibody titre $\log_{10} \geq 0,6$ and ELISA OD values at $A_{490} \geq 0,135$

i.n. = Intranasal inoculation

m = Age of foetus in months

- = Negative virus isolation on RK-13 cells

TABLE 2 Correlation between VN antibody titres and IgG ELISA OD values in serum, colostrum and milk samples of donkey mares infected with the asinine-94 strain of EAV

Mare no.	Serological test	Serum ^a	Colostrum ^a	Milk			
				Days after delivery			
				2	5	7	14
1	VN ^b	1,50	2,10	0,60	< 0,600	< 0,600	< 0,600
	ELISA ^c	0,75	1,85	0,17	< 0,135	< 0,135	< 0,135
2	VN	1,80	2,40	1,20	0,900	< 0,600	< 0,600
	ELISA	0,92	2,20	0,54	0,160	0,150	< 0,135
3	VN	2,10	2,70	0,90	< 0,600	< 0,600	< 0,600
	ELISA	1,62	2,62	0,24	0,150	< 0,135	< 0,135
4	VN	1,80	2,70	0,60	< 0,60	< 0,600	< 0,600
	ELISA	0,75	2,53	0,15	0,140	< 0,135	< 0,135
5	VN	1,80	2,40	0,90	0,600	< 0,600	< 0,600
	ELISA	0,86	2,32	0,16	0,160	< 0,135	< 0,135

^a Serum and colostrum samples collected at the parturition

^b VN test positive, \log_{10} titre $\geq 0,6$

^c ELISA positive, OD value at $A_{490} \geq 0,135$

TABLE 3 Results of virus isolation from buffy coat and duration of passive humoral immunity in foals born to donkey mares infected with the asinine-94 strain of EAV

Foal no.	Virus isolation from buffy coat				VN ^a and ELISA ^b antibody in serum					
	O ^c	Days after birth			O ^c	Days after birth				
		1	2	7		2	7	30	60	90
1	- ^d	-	-	n.t.	< 0,600 < 0,135	1,50 0,72	1,20 0,37	0,60 0,18	< 0,600 < 0,135	n.t. n.t.
2	n.t.	-	-	-	n.t. n.t.	2,10 1,54	1,50 0,65	1,20 0,48	< 0,600 < 0,135	< 0,600 < 0,135
3	-	-	-	n.t.	< 0,600 < 0,135	2,10 1,73	1,80 0,81	1,20 0,52	0,600 0,160	< 0,600 < 0,135
4	-	-	-	-	< 0,600 < 0,135	2,40 2,20	2,10 1,64	1,50 0,65	0,600 0,180	< 0,600 < 0,135
5	-	-	-	-	< 0,600 < 0,135	1,80 0,80	1,50 0,73	0,90 0,18	< 0,600 0,140	< 0,600 < 0,135

^a VN test positive, \log_{10} titre $\geq 0,6$

^b ELISA positive, OD value at $A_{490} \geq 0,135$

^c Serum collected before foal nursed

^d Negative virus isolation on RK-13 cells
n.t. = Sample not available or not tested

et al. 1957b; Golnik & Michalak 1979), but microscopic lesions were rarely seen in the foetus or placenta (Jones, Doll & Bryans 1957; Coignoul & Chevile 1984; Cole, Hall, Gosser, Hendricks, Pursell, Senne, Pearson & Gipson 1986). The diagnosis of abortion due to infection with EAV is largely dependent on isolation of virus from the placenta or foetal tissues (Huntington, Ellis, Forman & Timoney 1990; Michalska & Golnik 1992). The mechanism of abortion following EAV infection is not precisely understood. It has been suggested that abortion is a result of myometrial necrosis and oedema, leading to placental detachment and foetal death (Coignoul & Chevile 1984).

There is no evidence of a congenitally acquired carrier state in foals born to horse mares pregnant at the time of EAV infection (Timoney *et al.* 1987). In this study, the colostrum-derived, maternal immunity to EAV was not detectable in foals at 2–3 months after birth. Similar results were reported by McCollum (1976) in foals born to EAV-immune horse mares. In this study, the high correlation obtained between the VN test and ELISA results, indicates that the potential application of the recombinant ELISA EAV antigen in the serological monitoring of colostrum and milk samples, has promise.

In the first recorded outbreak of EVA, 31 of 60 pregnant mares exposed to virus, aborted. Abortions occurred 10–34 d after exposure; the age of the aborted foetuses varied from 5–10 months. Among the mares that aborted, 20 developed clinical signs of EVA before or at the time of abortion; 11 aborted without apparent signs of disease (Doll, Knappenberger & Bryans 1957b). Between 1953 and 1989, 40 confirmed outbreaks of EVA were reported worldwide; abortions occurred in 15 of these. The incidence of abortion varied from < 10–60% (Timoney & McCollum 1991). In an experimental study of six pregnant mares, three aborted 9–12 d post i.n. inoculation and, of seven additional control mares, two aborted on days 19 and 28 after p.c. exposure (McCollum & Timoney 1984). In another study, ten (71.4%) of 14 mares aborted 23–57 d after contact with mares that had been infected after mating with carrier stallions (Cole *et al.*).

Serological surveys have demonstrated that EAV infection is distributed worldwide among horses. However, clinical infections have only infrequently been reported (Timoney & McCollum 1991). The most severe outcome of natural EAV infection, i.e. abortion in pregnant mares or death in foals, has to date been reported in Europe only, and mainly in Poland (Golnik & Michalak 1979; Golnik *et al.* 1981; Golnik *et al.* 1986; Golnik 1992; Nowotny 1992; Michalska & Golnik 1992; Michalska & Golnik 1993; Eichorn, Heilmann & Kaaden 1995) and in North America, mainly in the USA (Doll *et al.* 1957b; Carman, Rae & DuBovi 1988; Johnson, Baldwin, Timoney & Ely 1991; Timoney & McCollum 1991; Valla *et al.* 1992). It is worth mentioning that the

phylogenetic analysis of the EAV M gene suggests that the USA strains of EAV diverged from the Polish group during the late 19th century. Moreover, this analysis suggests that the evolutionary patterns of EAV appear to be geographically restricted (Sugita, Kondo, Sekiguchi, Yamaguchi, Kamada, Nerome & Fukunaga 1995). Although more comparative studies on pathogenicity are needed, strains of EAV have been shown to differ in their virulence, which may explain why not all of them are abortogenic. Seroepidemiological studies indicate that EAV is endemic in the South African donkey population (Paweska & Barnard 1993; Paweska *et al.* 1997). However, no field occurrences of EAV-induced disease, abortion or mortality have been reported in any species of Equidae in South Africa.

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