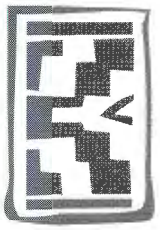

Failure to establish chronic infection of the reproductive tract of the male horse with a South African asinine strain of equine arteritis virus (EAV)

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ABSTRACT

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Eight sexually mature horse stallions were inoculated intranasally with a South African asinine strain of EAV, a strain that was isolated from the semen of a donkey carrier. All horses developed fever, with maximum rectal temperatures of 38,9–39,9 °C recorded 3–6 d post challenge. Six horses showed very mild clinical signs of equine viral arteritis and two were asymptomatic. The virus was recovered from the nasopharynxes of six horses 2–7 d after inoculation, and from buffy-coat samples of all horses, 2–11 d after inoculation. Seroconversion to EAV was detected on days 8 and 10 and peak serum-virus-neutralizing antibody titres ranging from $\log_{10}$ 1,2–1,8, on days 14–20 after challege. The titres varied from $\log_{10}$ 0,9–1,2 after about 10 weeks, when the experiment was terminated. In three stallions euthanased on days 5, 7 and 9 after challenge, virus was detected inconsistently in different parts of the reproductive tract and urine. No virus was isolated from the tissues of the reproductive tract collected from stallions on days 16, 23 and 68 after challenge.

Five stallions were bred to six seronegative mares between 13 and 34 d post challenge. No clinical signs of EAV were observed, and neither was seroconversion detected in any of the mares after mating. No virus was recovered from semen samples collected at the time of breeding.

The results of this study demonstrated that the tissues of the reproductive tracts of the stallions did not become persistently infected with a South African asinine strain of EAV.

Keywords: Asinine strain, chronic infection, EAV, equine arteritis virus, horse, reproductive tract, stallion

INTRODUCTION

Equine arteritis virus (EAV) was first identified in 1953 as the etiological agent of an influenza-like syndrome (Doll, Bryans, McCollum & Crowe 1957) which was subsequently named equine viral arteritis (EAV) owing to the characteristic medial necrosis observed in the small muscular-type arteries of infected horses (Jones, Doll & Bryans 1957). EAV is a positive-stranded RNA virus, classified in the Arterivirus genus (Cavanagh, Brien, Brinton, Enjuanes, Holmes, Horzinek, Lai, Laude, Plagemann & Siddell 1994). Only one serotype of the virus is recognized (McCollum 1969; Fukunaga, Matsumura, Sugiura, Wada, Imagawa, Kanemaru & Kamada 1994), but antigenic (Fukunaga & McCollum 1977; Kondo, Akashi, Fukunaga, Sugita, Sekiguchi, Wada & Kamada 1995) and genomic heterogeneity (Murphy, McCollum, Timoney, Klingeborn, Hyllseth, Golnik & Erasmus 1992; Chirnside, Wearing, Binns & Mumford 1994) have been shown to exist among strains of EAV from distinct geographical origins. The biological significance of this variability is yet unknown. Serosurveys

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have indicated that EAV has a worldwide distribution in different breeds of horses (Chirnside 1992), while other studies suggest a longstanding presence of the virus in donkeys in some African countries (Himeur 1976; Moraillon & Moraillon 1978; Paweska & Barnard 1993; Paweska, Binns, Woods & Chirnside 1997).

It has long been thought that EAV spreads by direct contact as droplet infection via the respiratory route (McCollum & Swerczek 1978). Investigation of the 1984 epizootic in the USA showed that a large proportion of infected stallions continued to shed virus in their semen after recovery, so that the virus was transmitted sexually to susceptible mares at breeding (Timoney 1985). Subsequently, stallions shedding EAV in the semen, were reported from many other countries (Huntington, Forman & Ellis 1990; Klingeborn, Wahlstrom, Wierup, Ballagi-Pordany & Belak 1991; Autorino, Rosati, Ferrari, Forletta, Nardi & Ammaddeo 1991; Golnik, Paweska & Dzik 1991). The virus was also isolated in South Africa from the semen of a Lippizaner stallion in 1987 (Erasmus 1988). Although this stallion originally came from eastern Europe in 1980, no oligonucleotide fingerprint homology could be demonstrated in this South African isolate, either with European isolates or with any other isolates from North America and New Zealand (Murphy *et al.* 1992).

Two carrier states have been demonstrated in the stallion: a short-term state during convalescence, lasting 4–5 weeks, and a long-term, chronic condition which may persist for years after clinical recovery (Timoney, McCollum, Roberts & Murphy 1986; Timoney, McCollum, Murphy, Roberts, Willard & Carswell 1987). The carrier state in stallions plays a major epidemiological role in maintaining and disseminating the virus (Timoney & McCollum 1985; Timoney, McCollum & Roberts 1987). A long-term carrier state in the mature stallion appears to be testosterone dependent (Little, Holyoak, Timoney & Mc Collum 1992; McCollum, Little, Timoney & Swerczek 1994). The ampulla of the vas deferens, with other accessory sex glands, has been identified as the primary site of viral persistence (Neu, Timoney & McCollum 1988).

The shedding of EAV in the semen, and venereal transmission of the virus, have also been demonstrated in donkeys (Paweska, Volkmann, Barnard & Chirnside 1995). The susceptibility of donkeys to the equine strain KY-84 (McCollum, Timoney & Tengelsen 1995), and of horses to a South African asinine strain of EAV, was recently reported, but preliminary attempts to transmit this strain sexually from horse stallions to susceptible horse mares, were unsuccessful. However, only one stallion was infected intranasally (Paweska, Aitchison, Chirnside & Barnard 1996).

The purpose of the present study was to determine whether a chronic infection of the reproductive tract of the male horse could be established by a South African asinine strain after intranasal inoculation with a dose tenfold larger than that used previously (Paweska *et al.* 1996).

MATERIALS AND METHODS

Experimental horses

The cross-bred horses (n = 14) comprised eight 3–14-year-old stallions (numbered 1–8) and six 3–12-year-old mares (numbered 9–14). Experimental animals were obtained from the resident horse population of the Onderstepoort Veterinary Institute and the Onderstepoort Biological Product Facility. All horses were seronegative to EAV and clinically normal before inoculation with EAV.

Virus and animal inoculation

The donkey strain of EAV (asinine-94 field isolate) recovered from the semen of a naturally infected jack (Paweska, Volkmann & Barnard 1994) that had been identified as a long-term carrier and shedder of virus (Paweska *et al.* 1995), was used. A 6-mℓ inoculum comprising the supernatant of a cell-culture fluid containing $10^{4,5}$ $TCID_{50}$/mℓ of the fourth passage of virus, was administered into the nasopharynx by means of a plastic catheter (Doll 1960).

Experimental design

One of a group of five stallions (numbered 1–5) was euthanased on each of days 5, 7, 9, 16 and 23, and three stallions (numbered 6–8) were all euthanased on day 68 post challenge. A range of tissues and urine samples were collected at necropsy, for virus isolation.

Between 13 and 34 d after inoculation, stallions 4–8 were bred to mares 9–14 twice a day for 3–4 d during the same oestrus period. The schedule of test breeding inoculated stallions to unexposed mares, is shown in Table 4. After they had been mated, the mares were kept isolated and were monitored for a period of 6 weeks after the date of last service.

Clinical monitoring

Stallions and mares were observed twice daily for the appearance of the clinical signs of EAV, and rectal temperatures were recorded for a period of 3 weeks after intranasal inoculation or test breeding.

Collection and processing of specimens

Blood samples from inoculated stallions were taken 1 d before challenge and then every 2 d for 3 weeks,

and then every week, until day 68 after challenge. Blood samples from test-bred mares were taken 1 d before the first day of mating and then every week for 6 weeks after last service. Nasal swabs and heparinized blood for buffy-coat preparations were collected from stallions 1 d before challenge and than every day for 2 weeks after challenge, with additional samples taken on days 17 and 21 post inoculation. Blood and nasal swabs were collected as previously described (McCollum, Prickett & Bryans 1971). Approximately 5 mℓ of dismount semen was collected three times from each of stallions 4–8 at time of test breeding. Semen was collected as quickly after mating as possible. No antiseptics were used to wash the perineal areas of the mares and the external genitalia of the stallions before semen collection. Precautions were taken to minimize the thermal or ultraviolet exposure of samples. Mares were synchronized by use of dinoprost tromethamine [Lutalyse: Upjohn (Pty) Ltd, Isando], in accordance with manufacturer's instructions. The following tissues were collected from each of stallions 1–5 at necropsy: lung, liver, spleen, kidney, lymph nodes (submaxillary, bronchial, mesenteric), testes, epididymis, vasa deferentia, ampullae, vesicular glands, prostate gland and bulbourethral glands. Only tissues of the reproductive tract were collected from each of stallions 6, 7 and 8. Approximately 50–100 mℓ of urine was taken from each of stallions 1–7 at necroscopy. Tissues and urine were collected aseptically as soon after death 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. Wet preparations from dismount semen samples were examined microscopically to confirm that they contained sperm. Nasal swabs, buffy-coat preparations and tissues were processed for virus isolation as described by McCollum *et al.* (1971). Semen and urine samples were processed as described previously (Paweska *et al.* 1995).

Cell culture and virus isolation

The RK-13 line of rabbit-kidney cells (ATCC CCL37) was cultivated in Eagle's minimum essential medium (BioWhittaker, Inc., USA) supplemented with 10% foetal-calf serum (Delta Bioproducts, South Africa) for growth, and with 1% for maintenance medium. Procedures for virus isolation and identification of EAV have been described previously (Paweska *et al.* 1996).

Serological tests

Virus-neutralization test (VN)

Determination of serum-neutralizing antibody titres to EAV were carried out with the use of a microneutralization test (VN) in RK-13 cells in the presence of 10% guinea-pig complement (Paweska & Barnard 1993). A serum was considered positive when it had a VN antibody titre $\geq \log_{10} 0,6$.

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA, with slight modifications, was used to detect IgG and IgM antibodies to EAV (Chirnside, Francis, De Vries, Sinclair & Mumford 1995). The recombinant EAV ELISA antigen was obtained from Dr Ewan Chirnside. The Animal Health Trust, UK. C-It™ immunoplates (Nunc) were used. A blocking solution consisted of 3,5% Nestlè Lactogen milk powder (Food & Nutritional Products, Randburg, RSA), 1% casein hydrolysate, and 1% Tween 20 in PBS. The same solution was used to dilute sera and affinity-purified biotin-labelled goat anti-γ chain horse IgG (KPL) and anti-μ chain horse IgM (KPL). Optical-density values were recorded with a microplate reader (Bio-Tek EL340, Bio-Tek Instruments, Winooski, Vt, USA) at a wavelength (A) of 490 nm. An $A_{490} \geq 0,135$ was taken as the cut-off point determining an ELISA-positive absorbence value (Paweska *et al.* 1996).

RESULTS

Clinical responses

All eight stallions developed a fever, six showed mild clinical signs of EAV and two remained asymptomatic after intranasal inoculation of the virus. Fever having a duration of 2–5 d (mean: 3,5 d) with maximum rectal temperatures of 38,9–39,9°C (mean: 39,2°C) was recorded 3–9 d post challenge (Table 1). The clinical signs were most prominent at time of febrile response and they included depression, serous ocular and nasal discharge, conjunctivitis and photophobia. Recovery usually occurred between 1 and 3 d after the end of pyrexia.

Virological findings

The results of virus isolation from nasopharynx, buffy-coat preparations, semen and urine, are shown in Table 1.

Nasopharynx

Virus was isolated from the nasopharynxes of six out of eight stallions between 2 and 7 d post challenge. The duration of virus shedding from the respiratory tract, excluding the two animals necropsied during this period, ranged from 3–6 d (mean: 4,2 d).

Buffy coats

Virus was isolated from buffy-coat preparations between 2 and 11 d post challenge. The duration of

TABLE 1 Clinical and immunological responses and virus isolation from nasopharynx, buffy coat, semen and urine in stallions inoculated instranasally with the asinine-94 strain of EAV

Stallion no.	Fever[a]		Clinical signs	Log_{10} VN titre[a]		Virus isolation results[a, b]			
	Max. Temp. °C	Duration		Seroconversion[c]	Peak titre	Nasopharyn x	Buffy coat	Semen	Urine
1	38,9 (4)	▼	Mild	–	▼	–	+ (3–5)	NC	+
2	39,9 (5)	▼	Mild	–	▼	+ (3–4)	+ (2–7)	NC	+
3	38,9 (4)	(4–7) ▼	Mild	0,9 (8)	▼	+(2–5)	+ (3–10)	NC	–
4	39,2 (5)	(5–9) ▼	Mild	0,9 (10)	1,8 (14–▼)	+ (2–6)	+ (3–11)	–	–
5	39,8 (6)	(4–7)	Mild	0,9 (10)	1,5 (16–20)	+ (2–4)	+ (2–9)	–	–
6	38,9 (6)	(6–7)	None	0,6 (8)	1,2 (14–20)	–	+ (2–8)	–	–
7	39,4 (5)	(4–8)	Mild	0,9 (8)	1,5 (14–22)	+ (3–7)	+ (2–11)	–	–
8	38,9 (3)	(3–5)	None	0,6 (8)	1,5 (18–20)	+ (2–5)	+ (3–8)	–	NC

[a] Days after intranasal inoculation
[b] Virus isolated on RK-13 cells
[c] Serum-neutralizing antibody titre $log_{10} \geq 0,6$
NC Sample not collected
▼ Stallions 1, 2, 3 and 4 were euthanased on days 5, 7, 9 and 16, respectively

TABLE 2 Results of virus isolation from the tissues of reproductive tract of stallions inoculated intranasally with the asinine-94 strain of EAV

Days after inoculation	5	7	9	16	23	68
Stallion no.	1	2	3	4	5	6, 7, 8
Testis	–	+	–	–	–	–
Caput epididymis	–	+	–	–	–	–
Cauda epididymis	–	+	+	–	–	–
Ductus deferens	–	+	+	–	–	–
Ampulla	–	+	+	–	–	–
Vesicular glands	+	+	–	–	–	–
Prostate	–	–	+	–	–	–
Bulbourethral gl.	+	+	–	–	–	–

+ = Virus isolated on RK-13 cells

TABLE 3 Results of virus isolation from organs and lymph nodes of stallions inoculated intranasally with the asinine-94 strain of EAV

Days after inoculation	5	7	9	16	23
Stallion no.	1	2	3	4	5
Lung	+	–	–	–	–
Liver	+	–	–	–	–
Spleen	+	+	–	+	–
Kidney	–	+	–	–	–
Lymph nodes					
Submaxillary	+	+	+	–	–
Bbronchial	+	+	+	+	–
Mesenteric	+	+	+	–	–

+ = Virus isolated on RK-13 cells

viraemia, excluding the three animals necropsied during this period, ranged from 6–11 d (mean: 8 d).

Semen

No virus was recovered from semen of stallions 4, 5, 6, 7 and 8 between 13 and 34 d post challenge.

Urine

EAV was detected in urine of stallions 1 and 2, collected at the time they were euthanased on days 5 and 7 after inoculation.

Tissues

EAV was isolated from various tissues of the reproductive tract of stallions 1, 2 and 3, sampled on days 5, 7 and 9, but it was not recovered from the tissues of the reproductive tract taken from stallions 4, 5, 6, 7 and 8 on days 16, 23 and 68 post challenge. In the three animals tested on days 5, 7 and 9, virus was recovered from vesicular and bulbourethral glands of stallion 1, from all the tissues of the reproductive tract (except prostate) collected from horse 2, and from the cauda epididymis, ductus deferens, ampulla, and prostate of horse 3 (Table 2).

All organs and lymph nodes tested of horse 1, were infected with EAV. Similar results were obtained from horse 2, except in lung and liver, from which virus was not recovered. In stallion 3, virus was isolated from all lymph nodes tested, and in stallion 4, it was detected only in the spleen and bronchial lymph nodes. No virus was recovered from the organs and lymph nodes of horse 5 (Table 3).

Serological findings

The first detectable virus-serum-neutralizing (VN), IgM and IgG antibody to EAV was demonstrated in samples collected from stallions on days 8 and 10 post challenge. At this time VN antibody titres ranged from log_{10} 0,6–0,9, and ELISA absorbence values (OD) for IgM varied from 0,17–0,43, and for IgG from 0,14–0,22. Peaks of IgM were detected on days 12–14 (OD: 0,43–0,56), and of IgG (OD: 0,52–0,72) and of VN (1,2–1,8) antibody, on days 14–20 post inoculation.

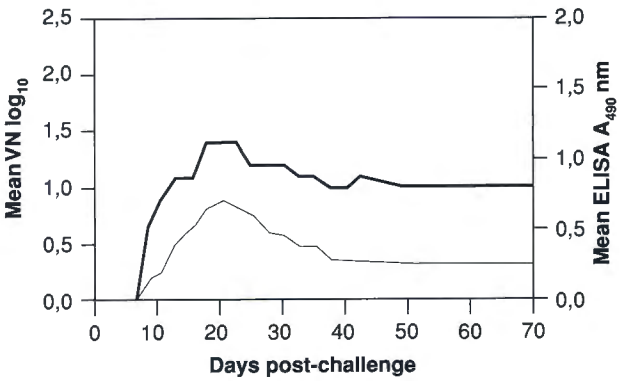

FIG. 1 Mean serological response in a group of three stallions inoculated intranasally with the asinine-94 strain of EAV

TABLE 4 The results of test breeding five stallions inoculated intranasally with the asinine-94 strain of EAV to susceptible mares

Stallions		Mares			
No.	D post inoculation when test bred	No.	Clinical signs	Seroconversion	
				VN[a]	ELISA[b]
4	13, 14, 15	9	None	< 0,6	< 0,135
5	18, 19, 20	10	None	< 0,6	< 0,135
6	14, 15, 16, 17	11	None	< 0,6	< 0,135
7	16, 17, 18, 19	12	None	< 0,6	< 0,135
8	16, 17, 18	13	None	< 0,6	< 0,135
	31, 32, 33, 34	14	None	< 0,6	< 0,135

[a] Serum-neutralizing titre $\log_{10} \geq 0,6$
[b] ELISA IgG OD value at $A_{490} \geq 0,135$

The IgM started to decline rapidly after 2–3 weeks post challenge and reached preinfection OD values after 4–5 weeks. All three horses monitored until day 68 remained seropositive, with VN titres ranging from $\log_{10}$ 0,9–1,2, and IgG OD values from 0,19–0,34. The mean VN $\log_{10}$ titres and OD IgM and IgG values in sera from the group of stallions 6, 7 and 8, are shown in Fig 1.

Venereal transmission

No clinical signs of EAV were seen in mares during a period of 3 weeks' observation, and seroconversion was not demonstrated in any serum samples collected within 6 weeks after mating to the experimentally infected stallions (Table 4).

DISCUSSION

All stallions in this study became infected after intranasal inoculation with the asinine-94 strain. Most of them developed only very mild clinical signs of EAV, similar to those reported previously (Paweska *et al.* 1996), except for photophobia. Virus was isolated from nasopharynx and buffy-coat preparations during the acute phase of the infection, and seroconversion was detected by both VN and ELISA on days 8 and 10 after challenge. The severity of clinical signs was milder and the mean duration of viraemia and virus shedding from the nasopharynx and in the urine was shorter than was reported in the majority of sexually mature stallions inoculated by the same route and with a similar dose of equine isolates of EAV (Neu *et al.* 1988; Autorino, Cardeti, Rosati, Ferrari, Vulcano, Ammaddeo, McCollum & Timoney 1994).

In this study, the asinine-94 strain was inconsistently recovered from various parts of the reproductive tract during a period of 5–9 d after challenge. Since the virus was isolated from the reproductive tract during a period of viraemia only, and no quantitative or other assays were undertaken to verify viral replication in these tissues, it could be hypothesized that circulating infected white-blood cells were also responsible for positive virus isolation from the reproductive tract during the acute phase of infection. Nevertheless, the lack of a detectable amount of the virus in the reproductive system from 16–68 d, and in semen samples collected from 13–34 d post inoculation, and the failure of sexual transmission of the infection to a group of seronegative mares, indicate that, after clinical recovery, none of the stallions developed a chronic infection of the reproductive tract with virus shedding in the semen. The clearance of the asinine-94 strain from internal organs, but especially from lymph nodes, was also more rapid than was reported in horses infected with equine isolates of EAV (McCollum *et al.* 1971; Neu *et al.* 1988; Fukunaga, Wada, Matsumura, Anzai, Imagawa, Sugiura, Kumanomido, Kanemaru & Kamada 1991).

Virus isolation or test mating is used to detect virus shedding in semen and to confirm the carrier state in stallions seropositive to EAV due to natural or experimental infection. Both methods are highly effective and of equal sensitivity (Timoney *et al.* 1987). Duration of the carrier status can range from several weeks to a period of years, if not for life, in certain individuals (Timoney *et al.* 1986). The carriers shed EAV continuously in the semen and there is no evidence to indicate intermittent shedding by seropositive stallions (Timoney *et al.* 1987; Timoney, McCollum & Murphy 1991).

The challenge virus used in this study, was successfully transmitted by the sexual route from a carrier jackass to two susceptible jennies. Of a total of five naturally infected jacks, two (40%) were identified as shedders of the virus in the semen (Paweska *et al.* 1995). The results of a seroepidemiological study indicated a high correlation between the number of positive donkey males and females in some areas of South Africa. In contrast, in areas where very limited

seroprevalence of EAV infection was found amongst jacks, the incidence of EAV infection in jennies was also shown to be very limited, or it could not be detected at all. Additionally, the seroprevalence of EAV infection among South African donkeys less than two years old, was found to be a quarter of that in older animals (Paweska 1994; Paweska *et al.* 1997). However, only limited data are available concerning the incidence of a carrier state in EAV-seropositive jacks, but the results obtained so far, together with the findings of a seroepidemiological study in South Africa, indicate that venereal transmission might, as in horses, be one of the chief modes of virus spread in asses. It is rather surprising, therefore, that none of the stallions in the present and in an earlier study (Paweska *et al.* 1996) displayed either a long- or a short-term carrier state after experimental infection with the asinine-94 strain of EAV. This is in sharp contrast with the findings by Neu *et al.* (1988), who demonstrated a long-term carrier state in five of eight (62,5%) mature stallions in which the KY-84 strain of EAV was detected in the reproductive tract up to 148 d after challenge, when the experiment was terminated. Furthermore, a high incidence of the carrier state was also demonstrated among horse stallions naturally infected with EAV (Timoney, McCollum & Murphy 1991).

The mechanism of the persistence of EAV is determined by factors which are as yet not completely defined. It has been shown that the concentration of testosterone plays an essential role in the establishment and maintenance of a long-term carrier state (Little *et al.* 1992; McCollum *et al.* 1994). However, EAV infection of the reproductive tract, lasting from about 1–3 months in 83,3% of six prepubertal colts and from 4–6 months in 33,3% of six additional prepubertal colts, was demonstrated when they were inoculated with the KY-84 strain. This indicates that this strain at least has a strong tropism for the tissues of the reproductive tract in the absence of testosterone or other specific factors that may be present in the mature stallions (Holyoak, Little, McCollum & Timoney 1993a). The cells of the reproductive tract in which EAV replicates and persists, have not as yet been identified. The presence of essentially plasma cells in the inflammatory infiltrates found in the ampulla of the vas deferens of infected young colts, suggests that the cells may play a role in persistence and may also boost the systemic anti-EAV VN antibody titres found in these animals (Holyoak, Giles, McCollum, Little & Timoney 1993b). One has to stress, however, that the KY-84 isolate is the only equine strain of EAV that has so far been used to study the mechanism of viral persistence in the reproductive tract of the male horse. Although antigenic (Kondo *et al.* 1995) and genomic (Murphy *et al.* 1992; Chirnside *et al.* 1994) diversity among strains of EAV has been demonstrated, exact factors affecting the pathogenicity displayed by particular strains are still unknown. Comparison between sequential isolates recoverd at regular intervals from the same naturally infected stallions, revealed ongoing oligonucleotide variation which may be important for the establishment of the carrier state (Murphy, Timoney & McCollum 1991). Other possible mechanisms which EAV may utilize in the establishment of persistent infection, include changes in viral epitope expression by down-regulation of viral glycoprotein production or antibody binding of viral antigens presented on host-cell surfaces, thus masking them and allowing the progress of infection. These changes were also discussed by Holyoak *et al.* (1993a).

The presence of VN antibody to EAV in horse sera, indicates that an animal has been infected, but does not prove that it is a carrier. In a carrier stallion, however, the VN antibody titre is usually maintained at a moderate or high level, suggesting that humoral immunological interference with viral replication within the tissues of the reproductive system is minimal or non-existent (Holyoak *et al.* 1993a). Comparison of the VN antibody titre and isolation of EAV from semen, suggests that carriers would have a VN titre of $\log_{10}$ 1,5 or greater, and those with a titre of $\log_{10}$ 1,2 or less, may not be carriers. High VN antibody titres were also found in stallions when EAV was not recovered from their semen (Huntigton, Forman & Ellis 1990). Therefore the predictability of the carrier state, based on magnitude of antibody titre, has to be studied futher. Nevertheless, it is worthy of mention that the asinine-94 strain was isolated from two jacks with high VN antibody titres ($\log_{10}$ 2,1 and 2,7) which persisted (when tested at regular intervals) for a period of 1–2 years (J.T. Paweska, unpublished data 1996). In the present study, all stallions examined about 10 weeks after intranasal inoculation, had VN titres of less than $\log_{10}$ 1,2. Similar serological results were obtained previuosly in two intramuscularly exposed stallions and one exposed intranasally, and here also,the asinine-94 strain could neither be isolated from their semen nor be sexually transmitted to susceptible mares (Paweska *et al.* 1996). However, more detailed comparative studies on the pathogenicity of the asinine-94 strain are needed. Available experimental data (Paweska *et al.* 1995, 1996) indicate that in donkeys, the humoral immunological response is stronger than in horses infected with the asinine-94 strain. Furthermore, the duration of viraemia in horses infected with this isolate, appears to be shorter than that in horses infected with other isolates of EAV (McCollum *et al.* 1971; Neu *et al.* 1988; Fukunaga, Imagawa, Tabuchi & Akiyama 1981; Fukunaga *et al.* 1991; Autorino *et al.* 1994). This suggests a distinct replication pattern of the asinine-94 strain in horses, and could be the reason for the differences in the kinetics of the immunological response, or it could even play a role in the successful establishment of a carrier state with this particular strain. It has been shown that individual horses may harbour EAV in cells of the

buffy coat for a long time. For example, Fukunaga *et al.* (1981) isolated the Bucyrus strain from the buffy-coat fraction on day 36, and Neu *et al.* (1988), the KY-84 strain on day 110 after experimental challenge. The nature of the infection and the type of blood leukocytes infected with EAV have not yet been determined. However, equine macrophage cultures have been shown to support the replication of the Bucyrus strain of EAV (McCollum 1978, cited by Neu *et al.* 1988).

The status of EAV infection in the South African horse and donkey populations is rather intriguing, particularly with regard to the long coexistence of both species in the country. The disease seems to be endemic in donkeys (Paweska & Barnard 1993; Paweska 1994) but not in horses (Erasmus 1988; P.G. Howell, unpublished data 1996). The failure to establish a carrier state in the male horse after infection with the asinine-94 strain of EAV in this and in an earlier study, which also indicated the low lateral in-contact transmission of this strain among horses, and failed to demonstrate its lateral spread from experimentally infected donkeys to susceptible horses (Paweska *et al.* 1996), contribiutes to a better understanding of the EAV epidemiological situation in South Africa.

The potential epidemiological significance of *Equus asinus* in maintaining EAV in different endemic areas, has still to be determined. In the light of the carrier state established in donkey stallions, the intensity of mule production in some countries could play a role in the dissemination of the virus or affect the virulence of EAV.

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REFERENCES

AUTORINO, G.L., ROSATI, R., FERRARI, G., FORLETTA, R., NARDI, A. & AMMADDEO, D. 1991. Equine arteritis virus isolation from stallions in Italy, in *Proceedings of the Sixth International Conference on Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.F. Wade. R & W Publications (Newmarket): 303.

AUTORINO, G.L., CARDETI, G., ROSATI, R., FERRARI, G., VULCANO, G., AMMADDEO, D., McCOLLUM, W.H. & TIMONEY, P.J. 1994. Pathogenic and virological properties of an Italian strain of equine arteritis virus in stallions following intranasal challenge with infective raw semen, in *Proceedings of the Seventh International Conference on Equine Infectious Diseases*, edited by H. Nakajima & W. Plowright. R & W Publications (Newmarket): 335–336.

CAVANAGH, D., BRIEN, D.A., BRINTON, M., ENJUANES, L., HOLMES, K.V., HORZINEK, M.C., LAI, M.M.C., LAUDE, H., PLAGEMANN, P.G.W. & SIDDELL, S. 1994. Revision of the taxonomy of the Coronavirus, Torovirus and Arterivirus genera. *Archive of Virology*, 135:227–237.

CHIRNSIDE, E.D. 1992. Equine arteritis virus: an overview. *British Veterinary Journal*, 148:181–197.

CHIRNSIDE, E.D., WEARING, C.M., BINNS, M.M. & MUMFORD, J.A. 1994. Comparison of M and N gene sequences distinguishes variation amongst equine arteritis virus isolates. *Journal of General Virology*, 75:1491–1497.

CHIRNSIDE, E.D., FRANCIS, P.M., DE VRIES, A.A.F., SINCLAIR, R. & MUMFORD, J.A. 1995. Development and evaluation of an ELISA using recombinant fusion protein to detect the presence of host antibody to equine arteritis virus (EAV). *Journal of Virological Methods*, 54:1–13.

DOLL, E.R., BRYANS, J.T., McCOLLUM, W.H. & CROWE, M.E. 1957. Isolation of a filterable agent causing arteritis of horses and abortion by mares. Its differentiation from the equine abortion (influenza) virus. *Cornell Veterinarian*, 47:3–41.

DOLL, E.R. 1960. A catheter for nasal inoculation of large animals. *American Journal of Veterinary Research*, 21:518–519.

ERASMUS, B.J. 1988. Equine viral arteritis. *South African Racehorse*, September:100.

FUKUNAGA, Y. & MCOLLUM, W.H. 1977. Complement fixation reactions in equine viral arteritis. *American Journal of Veterinary Research*, 38:2043–2046.

FUKUNAGA, Y., IMAGAWA, H., TABUCHI, E. & AKIYAMA, Y. 1981. Clinical and virological findings on experimental equine viral arteritis in horses. *Bulletin of the Equine Research Institute*, 18:110–118.

FUKUNAGA, Y., WADA, R., MATSUMURA, T., ANZAI, T., IMAGAWA, H., SUGIURA, T., KUMANOMIDO, T., KANEMARU, T. & KAMADA, M. 1991. An attempt to protect against persistent infection of equine viral arteritis in the reproductive tract of stallions using formalin inactivated-virus vaccine, in *Proceedings of the Sixth International Conference on Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.W. Wade. R & W Publications (Newmarket): 239–244.

FUKUNAGA, Y., MATSUMURA, T., SUGIURA, T., WADA, R., IMAGAWA, H., KANEMARU, T. & KAMADA, M. 1994. Use of the serum neutralisation test for equine viral arteritis with different virus strains. *The Veterinary Record*, 28:574–576.

GOLNIK, W., PAWESKA, J. & DZIK, W. 1991. Ogier potencjalnym zrodlem zakazenia wirusem zapalenia tetnic koni. *Medycyna Weterynaryjna*, 48:52–54.

HIMEUR, D. 1976. Incidence in Morocco of equine infectious anaemia, rhinopneumonitis and viral arteritis. *Veterinary Bulletin*, 46:11.

HOLYOAK, G.R., LITTLE, T.V., McCOLLUM, W.H. & TIMONEY, P.J. 1993a. Relationship between onset of puberty and establishment of persistent infection with equine arteritis virus in the experimentally infected colt. *Journal of Comparative Pathology*, 109:29–46.

HOLYOAK, G.R., GILES, R.C., McCOLLUM, W.H., LITTLE, T.V. & TIMONEY, P.J. 1993b. Pathological changes associated with equine arteritis virus infection of the reproductive tract in prepubertal and peripubertal colts. *Journal of Comparative Pathology*, 109:281–293.

HUNTINGTON, P.J., FORMAN, A.J. & ELLIS, P.M. 1990. The occurrence of equine arteritis virus in Australia. *Australian Veterinary Journal*, 67:432–435.

JONES, T.C., DOLL, E.R. & BRYANS, J.T. 1957. The lesions of equine viral arteritis. *Cornell Veterinarian*, 47:52–68.

KLINGEBORN, B., WAHLSTROM, H., WIERUP, M., BALLAGI-PORDANY, A. & BELAK, S. 1991. Prevalence of antibodies to equine arteritis virus in Sweden, shedding of the virus in semen from Swedish breeding stallions and evaluation of its possible

influence on fertility, in *Proceedings of the Sixth International Conference on Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.F. Wade. R & W Publications (Newmarket): 319–320.

KONDO, T., AKASHI, H., FUKUNAGA, Y., SUGITA, S., SEKIGUCHI, K., WADA, R. & KAMADA, M. 1995. Production and characterisation of monoclonal antibodies against structural proteins of equine arteritis virus, in *Proceedings of the Seventh International Conference on Equine Infectious Diseases*, edited by H. Nakajima & W. Plowright. R & W Publications (Newmarket): 21–26.

LITTLE, T.V., HOLYOAK, G.R., TIMONEY, P.J. & McCOLLUM, W.H. 1992. Output of equine arteritis virus from persistently infected carrier stallions is testosterone dependent, in *Proceedings of the Sixth International Conference of Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.F. Wade. R & W Publications (Newmarket): 225–229.

McCOLLUM, W.H. 1969. Development of a modified virus strain and vaccine for equine viral arteritis. *Journal of American Veterinary Association*, 2:293–299.

McCOLLUM, W.H., PRICKET, M.E. & BRYANS, J.T. 1971. Temporal distribution of equine arteritis virus in respiratory mucosa, tissues and body fluids of horses infected by inhalation. *Research in Veterinary Science*, 12:459–464.

McCOLLUM, W.H. & SWERCZEK, T.W. 1978. Studies on an epizootic of equine viral arteritis in racehorses. *Journal of Equine Medicine and Surgery*, 2:293–299.

McCOLLUM, W.H., LITTLE, T.V., TIMONEY, P.J. & SWERCZEK, T.W. 1994. Resistance of castrated male horses to attempted establishment of the carrier state with equine arteritis virus. *Journal of Comparative Pathology*, 111:383–388.

McCOLLUM, W.H., TIMONEY, P.J. & TENGELSEN, L.A. 1995. Clinical, virological and serologic responses of donkeys to intranasal inoculation with the KY-84 strain of equine arteritis virus. *Journal of Comparative Pathology*, 112:207–211.

MORAILLON, A. & MORAILLON, R. 1978. Results of an epidemiological investigation on viral arteritis in France and some other European and African countries. *Annales de Recherches Veterinaires*, 9:43–54.

MURPHY, T.W., TIMONEY, P.J. & McCOLLUM, W.H. 1991. Variation in genomic RNA of equine arteritis virus during long term persistance in the stallion, in *Proceedings of the Sixth International Conference on Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.F. Wade. R & W Publications (Newmarket): 327.

MURPHY, T.W., McCOLLUM, W.H., TIMONEY, P.J., KLINGEBORN, B.W., HYLLSETH, B., GOLNIK, W. & ERASMUS, B. 1992. Genomic variability among globally distributed isolates of equine arteritis virus. *Veterinary Microbiology*, 32:101–115.

NEU, S., TIMONEY, P.J. & McCOLLUM, W.H. 1988. Persistent infection of the reproductive tract in stallions experimentally infected with equine arteritis virus, in *Proceedings of the Fifth International Conference on Equine Infectious Diseases*, edited by D.G. Powell. University Press of Kentucky, Lexington: 149–154.

PAWESKA, J.T. & BARNARD, B.J.H. 1993. Serological evidence of equine arteritis virus in donkeys in South Africa. *Onderstepoort Journal of Veterinary Research*, 60:155–158.

PAWESKA, J.T. 1994. An epidemiological investigation on equine viral arteritis in donkeys in South Africa, in *Proceedings of the Seventh International Conference on Equine Infectious Diseases*, edited by H. Nakajima & W. Plowright. R & W Publications (Newmarket): 337–338.

PAWESKA, J.T., VOLKMANN, D.H. & BARNARD, B.J.H. 1994. Equine arteritis virus: Isolation from donkeys and demonstration of venereal and in-contact transmission in South Africa, in *11th Faculty Day, Faculty of Veterinary Science*, University of Pretoria: V & R Printing Works: 21.

PAWESKA, J.T., VOLKMANN, D.H., BARNARD, B.J.H. & CHIRNSIDE, E.D. 1995. Sexual and in-contact transmission of asinine strain of equine arteritis virus among donkeys. *Journal of Clinical Microbiology*, 33: 3296–3299.

PAWESKA, J.T., AITCHISON, H., CHIRNSIDE, E.D. & BARNARD, B.J.H. 1996. Transmissibility of the South African asinine strain of equine arteritis virus (EAV) among horses and between donkeys and horses. *Onderstepoort Journal of Veterinary Research*, 63:189–196.

PAWESKA, J.T., BINNS, M.M., WOODS, P.S.A. & CHIRNSIDE, E.D. 1997. A survey for antibodies to equine arteritis virus in donkeys, mules and zebra using neutralisation (VN) and enzyme linked immunosorbent assay (ELISA). *Equine Veterinary Journal*, 29:40–43.

TIMONEY, P.J. 1985. Clinical, virological and epidemiological features of the 1984 outbreak of equine viral arteritis in the Thoroughbred population in Kentucky, USA, in *Proceedings of the Grayson Foundation International Conference of Thoroughbred Breeders Organization*, Dromoland Castle, Ireland, 1984: 24–48.

TIMONEY, P.J. & McCOLLUM, W.H. 1985. The epidemiology of equine viral arteritis, in *Proceedings of the American Association of Equine Practitioners*, 31:545–551.

TIMONEY, P.J., McCOLLUM, W.H., ROBERTS, A.W. & MURPHY, T.W. 1986. Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Research in Veterinary Science*, 41:279–280.

TIMONEY, P.J., McCOLLUM, W.H., MURPHY, T.W., ROBERTS, A.W., WILLARD, J.G. & CARSWELL, G.D. 1987. The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. *Journal of Reproduction and Fertility Supplement*, 35:95–102.

TIMONEY, P.J., McCOLLUM, W.H. & ROBERTS, A.W. 1987. Detection of the carrier state in stallion persistently infected with equine arteritis virus, in *Proccedings of the American Association of Equine Practitioners*, 32:57–65.

TIMONEY, P.J., McCOLLUM, W.H. & MURPHY, T.W. 1991. A longitudinal study of equine arteritis virus infection in Standardbred stallions with special reference to occurrence of the carrier state, in *Proceedings of the Sixth International Conference of Equine Infectious Diseases*, edited by W. Plowright, P.D. Rossdale & J.F. Wade. R & W Publications (Newmarket): 231–237.