Sexual and In-Contact Transmission of Asinine Strain of Equine Arteritis Virus among Donkeys

J. T. PAWESKA,^{1*} D. H. VOLKMANN,² B. J. H. BARNARD,¹ AND E. D. CHIRNSIDE³

Department of Virology, Onderstepoort Veterinary Institute,¹ and Department of Theriogenology, Faculty of Veterinary Science, University of Pretoria,² Onderstepoort, 0110 Republic of South Africa, and Centre for Preventive Medicine, Animal Health Trust, Newmarket, Suffolk, CB8 7DW, England³

Received 17 April 1995/Returned for modification 9 August 1995/Accepted 19 September 1995

Two in a group of five naturally seropositive donkey stallions were found to shed equine arteritis virus (EAV) in their semen as demonstrated by virus isolation. Direct intramuscular inoculation of sonicated semen from one virus-shedding stallion (S3) caused clinical disease in two donkeys from which virus was recovered and in which seroconversion was detected. Sexual transmission was confirmed in two mares mated to S3 when after a febrile response during which EAV was isolated from buffy coats and nasal and ocular exudates, both mares were found to have seroconverted. In-contact transmission in a susceptible stallion was demonstrated after its exposure to a sexually infected mare. The 3' end of the asinine virus was amplified directly from donkey semen with EAV-specific primers, and its nucleotide sequence was found to be homologous to that of the prototype Bucyrus virus isolated from horses. These results indicate that EAV and its disease transmission are analogous in donkeys and horses.

Equine arteritis virus (EAV) has recently been designated the prototype of the genus *Arterivirus* (2), which includes lactate dehydrogenase-elevating virus of mice, the virus causing porcine respiratory and reproductive syndrome (or Lelystad virus), and simian hemorrhagic fever virus. The *Arterivirus* classification is based on similarities in viral genome size, organization, and replication strategy.

Equine viral arteritis has assumed increased significance for the international horse breeding and racing industry since the 1984 epidemic in Kentucky (25). The recent outbreak of equine viral arteritis in 1993 in the United Kingdom (32) confirmed earlier concerns that increased international movement of horses, and less onerous restrictions on testing, would increase the risk of importation of EAV into susceptible populations (3).

EAV was first isolated in 1953 from fetal lung tissue during an outbreak of respiratory disease and abortion in Standardbred horses in the United States (8). Only one serotype of EAV has been demonstrated (12), although strains of EAV have been shown to differ in their pathogenicities (1, 14). RNase T₁ mapping (17, 18) and sequence analysis (6) have identified genetic variation among virus isolates. Serological surveys indicate that EAV infection is widespread among horses, and there is an increasing incidence of disease outbreaks reported throughout the world (26, 27). The vast majority of EAV infections in the field are subclinical in nature, and previous infection is often detected only retrospectively within a population during serosurveys. Clinical signs of EAV vary widely, but in its most severe form, infection causes abortion (8) and mortality among foals (11, 12). EAV is spread by the respiratory (13) and venereal (19, 29) routes. A high proportion of seropositive stallions (28–30) which excrete virus in semen are carriers, and these stallions are thought to play the major epidemiological role in the dissemination, persistence, and perpetuation of the virus.

The only reported host for EAV is the horse, although serological surveys indicate that the host range may include other equids (16, 21). However, there are no published records of clinical disease in equids other than horses and no studies of the effect of an alternate equid host on the causal agent or clinical presentations of the disease. The objectives of this study were to determine if naturally infected seropositive donkey stallions persistently shed virus in semen, to investigate the routes of viral transmission between donkeys, and to compare an asinine EAV isolate with equine isolates by cDNA sequence analysis of the matrix (M) and nucleocapsid (N) genes (6).

MATERIALS AND METHODS

Animals and disease transmission experiments. Following confirmation of their seropositivity in a serosurvey, five clinically healthy donkey stallions (S1 to S5) were purchased from their owners. One seronegative stallion (S6) and four seronegative mares (M1 to M4) were supplied by the Onderstepoort Veterinary Institute. Donkeys were maintained in separate stalls, except during test breeding and in-contact periods, in the following experiments. (i) Four stallions (S1 to S4) were test bred to seronegative mares twice a day for 4 days during the same estrus period; S1 was bred to M1, S2 to M2, S3 to M3, and S4 to M4. (ii) Having failed to seroconvert to EAV positivity, M4 was reused and test mated to S3. Following four daily coverings, M4 was introduced to S6 and the two donkeys were maintained in the same stall for a period of 3 weeks. (iii) In the absence of a virus-neutralizing antibody response following test mating, M1 and M2 were inoculated intramuscularly with 5 ml of sonicated S3 semen containing 10^{3.5} 50% tissue culture infective doses of virus per ml.

Observation and monitoring of clinical signs. Following test mating, in-contact exposure, or experimental infection, rectal temperatures and clinical signs were monitored twice daily at approximately 0730 and 1600 h for 4 weeks.

Collection and processing of specimens. (i) **Semen.** Samples were collected during test breeding with an artificial vagina. Mares were synchronized in estrus by using dinoprost tromethamine (Lutalyse; Upjohn [Pty.] Ltd., Isando).

(ii) **Preejaculatory fluid.** Prostaglandin F_{2α} was used to induce spontaneous ex copula ejaculation in seropositive stallions (15), and a large volume (40 to 150 ml) of preejaculatory fluid was collected into sterile plastic bags tied to the stallions' penises.

(iii) **Blood.** Blood samples were taken daily for 2 weeks from test-bred mares and in-contact exposed and intramuscularly infected donkeys and then every 2 to 3 days for a minimum of 2 more weeks. Blood samples from seropositive stallions were collected 1 week before and 2 and 3 weeks after test breeding. Heparinized blood for buffy coats was taken from test-bred mares and the in-contact-exposed stallion during the febrile response and 2 postfebrile days and from inoculated mares every second day for a period of 3 weeks postinfection. Heparinized blood was collected from seropositive stallions at the same time as were blood samples for serum.

* Corresponding author. Mailing address: Department of Virology, Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 Republic of South Africa. Phone: 27 12 5299123. Fax: 27 12 5299418.

(iv) **Nasal and ocular exudates.** Volumes of approximately 2 ml of nasal exudate and 1 ml of ocular exudate were collected directly into sterile tubes from infected animals during and for 2 days after the febrile response. Inoculated mares were sampled daily for 1 week and then every second day for another week postinfection.

(v) **Urine.** Volumes of 50 to 100 ml of urine were collected into sterile plastic beakers 1 week before test mating and then once a week for 2 weeks thereafter.

Virus isolation. All samples for virus isolation were chilled on freezer packs and transported with a minimum of delay to the laboratory, where they were either processed immediately or frozen at -20°C prior to virus isolation on monolayers of RK-13 cells (ATCC CCL 37), passage level 174 to 195, in plastic 25-cm² culture flasks. Cells were inoculated with 0.5 ml of sample treated as described below and examined daily for 9 days for cytopathic effects. In the absence of cytopathic effects, cultures were frozen at -20°C , thawed, and repassaged for two blind passages, after which the samples were regarded as EAV negative. The identity of cultured virus was confirmed in a virus neutralizing test using EAV-specific horse serum and by genome amplification (5).

Preejaculatory fluids were diluted 1:50 and used for cell culture inoculation. Procedures for isolation from semen followed that used by Timoney et al. (28, 30), with minor modifications; complete ejaculates were frozen at -20°C , subsequently thawed, sonicated, and centrifuged at $2,500 \times g$ for 15 min at 4°C , and the supernatant was diluted 1:40 for inoculation. Buffy coats were processed as described by Fukunaga et al. (9). Nasal and ocular discharge samples were centrifuged at $3,000 \times g$ for 20 min at 4°C . The supernatant was filtered through a sterile Millipore filter (0.45- μm pore size), and the resulting solution was used to inoculate cell sheets. Urine was precentrifuged at $3,000 \times g$ for 30 min at 4°C . The resulting supernatant was ultracentrifuged at $100,000 \times g$ for 2 h at 4°C , and the pellet was diluted 1:10 and used to inoculate cell sheets.

VN test. EAV-neutralizing antibodies were detected in a complement-dependent virus neutralization (VN) test in microtiter plates with RK-13 cells and the Bucyrus isolate of EAV (21).

EAV ELISA. Antibodies to EAV were detected in an enzyme-linked immunosorbent assay (ELISA) which was based on a recombinant EAV antigen as described for horse sera (4).

Genome amplification, cloning, and sequencing. Viral RNA was extracted directly from S3 semen and subjected to reverse transcription PCR amplification (5). The sequences of the primers employed were as follows: reverse transcription primer (negative sense) 5'-TGGTTCTCTGGGTGGC-3' (nucleotides 12692 to 12706), negative-sense PCR primer 5'-TTGGATCCTGGGTGGCTAATACTACTT-3' (nucleotides 12670 to 12707), and positive-sense PCR primer 5'-GGCTCAACGATGTTATCTATGA-3' (nucleotides 11138 to 11159). The negative-sense PCR primer was modified from the published EAV genome sequence (7) to include a *Bam*HI site (underlined in primer sequence) to aid cloning. Following reverse transcription PCR, the 1,569-bp DNA product was purified by standard techniques and digested with *Bam*HI-*Eco*RI. The resulting 1,219-bp band was recovered from a 1% agarose gel, ligated into M13 mp19 digested with *Bam*HI-*Eco*RI, and transformed into *Escherichia coli* TG1. M13 single-stranded DNA was prepared and sequencing was performed with ³⁵S-dATP by using modified T7 DNA polymerase (Sequenase; U.S. Biochemicals) and EAV-specific primers.

RESULTS

(i) **Sexual transmission by shedding stallions.** Of five seropositive donkey stallions two (S3 and S5) were identified as EAV semen shedders by virus isolation on one or more occasions over a 6-month period. Virus was recovered from all the S3 semen samples but was not isolated from preejaculatory fluids, buffy coats, or urine. The presence of EAV RNA was detected by genome amplification in one semen sample from S3, and infection was confirmed experimentally by direct intramuscular inoculation of sonicated semen (see item iii below).

Test-bred mares M1, M2, and M4 remained completely seronegative 6 to 10 weeks following repeated test matings with seropositive stallions S1, S2, and S4, respectively (Table 1). Two mares (M3 and M4) seroconverted on days 9 and 8, respectively, following the first of 4 successive days of mating to S3 (Table 1 and Fig. 1a and b). Increased rectal temperatures from 38.8 to 39.1 $^{\circ}\text{C}$ and 38.8 to 38.9 $^{\circ}\text{C}$ were recorded for M3 and M4 on days 7 to 11 and 8 to 9, respectively, with maximum temperatures on days 9 and 10 following the first day of mating. Clinical signs were most prominent at the time of the febrile response 1 week after mating; mild conjunctivitis, lacrimation, and a serious nasal discharge were observed for a period of 6 to 8 days. EAV was isolated from all buffy coat

TABLE 1. Serological, virological, and clinical responses in experimental donkeys

Expt	Sero-conversion	Virus isolation ^a	Fever		Clinical signs
			Maximum temp ($^{\circ}\text{C}$)	Duration (days)	
Test mating					
S1 to M1	—	—	None	None	None
S2 to M2	—	—	None	None	None
S3 to M3	+	+	39.1	5	Mild
S4 to M4	—	—	None	None	None
S3 to M4	+	+	38.9	2	Mild
In contact, S6 ^b	+	+	40.0	8	Moderate
Direct inoculation					
M1	+	+	39.9	7	Mild
M2	+	+	39.4	7	Mild

^a Virus isolated on RK-13 cells from buffy coats and nasal and ocular exudates.

^b S6 was exposed to M4 after M4 was test bred to S3.

preparations and from 5 of 11 nasal and 3 of 11 ocular samples collected from M3 and M4 during the febrile response and 2 postfebrile days.

(ii) **In-contact transmission between donkeys.** Stallion S6 seroconverted on day 12 after in-contact exposure to M4 (Table 1 and Fig. 1c), which in turn had seroconverted on day 8 following test mating to S3. This indicated that S6 was infected sometime between the third and seventh day of its introduction to M4, which coincided with the viremic phase in M4. A rise in rectal temperature from 38.8 to 40.0 $^{\circ}\text{C}$ was recorded for S6 on days 8 to 15, with the maximum temperature observed on day 13. The clinical signs of disease were most prominent at the time of febrile response and more severe in S6 than in the test-mated mares. In addition to the clinical signs observed in sexually infected mares, anorexia, depression, general weakness, and lameness were noted in S6. Virus was isolated from all the buffy coat samples collected, from nasal discharges on days 9, 11, and 14, and from ocular discharges on days 9 to 11.

(iii) **Experimental infection.** M1 and M2 became febrile between the 4th and 10th days after intramuscular inoculation, and maximal rectal temperatures ranged from 39.4 to 39.9 $^{\circ}\text{C}$. In both mares, mild depression, weakness, conjunctivitis, and serious rhinitis were observed during and 2 to 3 days following the febrile response. Seroconversion was recorded 8 to 9 days after inoculation (Fig. 1d and e), and EAV was recovered from all specimens collected. Virus shedding from the respiratory tract and lacrimal secretions was detected between days 4 to 11, and viremia was detected from days 3 to 14 postinoculation in both mares.

(iv) **Antibody responses following infection.** All the donkey mares, despite the different routes or modes of infection, became seropositive 8 to 9 days postinfection (Fig. 1). Peak antibody titers of log₁₀ 2.7 to 3.0 were detected on days 12 to 15 postinfection, and 4 to 6 weeks after test breeding or intramuscular infection, mares were seropositive at log₁₀ 2.1 to 2.4. Seroconversion of S6 was detected on day 12 after exposure to M4, and a peak titer of log₁₀ 2.4 to 2.7 was reached on days 16 to 19. The appearance and levels of immunoglobulin G (IgG) antibodies detected by ELISA corresponded to the dynamics of the immunological response measured by the VN test (Fig. 1). An increase in IgM antibody levels was detected after 6 to 8 days; the levels peaked at about 2 weeks and started to decline 2 to 3 weeks postinfection (Fig. 1). The level of IgM in M4 returned to preinfection levels after 9 weeks (Fig. 1b).

FIG. 1. Immune response of donkeys following exposure to EAV. (a) Test-bred mare M3; (b) test-bred mare M4; (c) in-contact stallion S6; (d) intramuscularly inoculated mare M1; (e) intramuscularly inoculated M2. Sera were tested by the VN test (▲) and by ELISA for EAV-specific IgG (■) and IgM (□) antibodies.

(v) **Genome sequence of the asinine isolate of EAV.** The region of the asinine EAV genome sequenced corresponds to nucleotides 11615 to 12705 of the Bucyrus sequence (7). The initiation codons of open reading frames 5 and 7, the termination codons of open reading frames 5 to 7 and the nucleotide sequence lengths of the M and N genes of the donkey isolate corresponded precisely to equine isolate sequences (6). Only two differences between the asinine sequence and the prototype Bucyrus sequence were found. Base substitutions of A to G at position 11695 and G to A at position 11934 were silent, i.e., neither encoded amino acid changes in either M or the carboxy terminus of G_L.

DISCUSSION

This study demonstrates the existence of naturally infected, seropositive donkeys in southern Africa that persistently shed EAV in their semen. This study also provides virological evidence that the natural host range of EAV includes donkeys, illustrates the existence of sexual and in-contact transmission routes of infection among donkeys, and confirms that a high degree of similarity exists between the asinine and equine virus isolates at the genetic and antigenic levels.

Irrespective of the route or mode of infection, febrile responses of variable severities and durations between 2 and 8 days were observed some 4 to 15 days following exposure. EAV was isolated from buffy coat samples and from nasal and ocular secretions taken during the febrile response, and all

donkeys seroconverted to EAV positivity. Clinical signs of infection were most prominent following the onset of the febrile response and included conjunctivitis, lacrimation, nasal discharge, depression, anorexia, general weakness, and lameness. The temporal development of clinical signs, fever, virus excretion, and seroconversion is in accordance with that described for infectious outbreaks and experimental infections of horses with EAV (3, 24, 27, 32).

Seroconversion to EAV positivity was confirmed by ELISA with no temporal advantage in sensitivity. However, the EAV-specific rise in IgM preceded the IgG response and began falling within 20 days of infection. Consequently, isotype-specific ELISAs may be useful in differentiating recently infected animals from animals with long-standing seropositivity, which is similar to the current use of the complement fixation (10) and VN tests (23). Both the VN test and ELISA were based on the Bucyrus equine isolate, and serological test results indicate that a high degree of antigenic cross-reactivity between the equine and asinine viruses exists. In addition, sequence analysis of the M and N genes of the asinine virus showed it to be closely related to the prototype Bucyrus isolate. This close homology suggests that the asinine virus could infect horses and that the Bucyrus isolate could infect donkeys, although no such cross-infections have been reported.

Although relatively few occurrences of clinical infection have been reported, equine viral arteritis is widely distributed in horse populations around the world (26). Serological evidence of EAV infection in donkeys has been reported in Mo-

rocco (16), and more recently in South Africa (20, 21), although no clinical disease or abortion in donkeys has been attributed to field infection. The reasons for clinical EAV infection in donkeys remaining unreported, and for the failure to observe sexual transmission, at least in South Africa, are likely to be economic and social; in many countries donkeys are not kept on the same premises, and do not come into close contact with competition and thoroughbred horses, whose health is generally screened more assiduously. Such population compartmentalization may inhibit cross-infection between donkeys and horses.

The number of seropositive virus-shedding stallions and their effect within the donkey and other equid populations, and the abortogenic potential of equine viral arteritis in donkeys, remain to be investigated. In view of the similarities in clinical signs, modes of transmission, and the antigenic and genetic compositions of the equine and asinine arteritis viruses, there may be a need for horse, donkey, and possibly zoo and wildlife park owners to review their blood screening and management practices for Equidae and to include all the members of this family.

ACKNOWLEDGMENTS

We thank Pamela Francis, Hannelize Fourie, and G. C. Retief for technical assistance, M. Binns for oligonucleotide synthesis, and A. van Dijk and J. A. Mumford for their support of the sequencing work.

This study was supported by the Onderstepoort Veterinary Institute (Republic of South Africa), The Animal Health Trust (United Kingdom), and the Ministry for Agriculture Fisheries and Food (United Kingdom). J. T. Paweska was additionally supported by the award of a Wellcome Trust fellowship.

REFERENCES

- Bürki, F. 1970. The virology of equine arteritis virus, p. 125–129. *In* (ed.), *Proceedings of the Second International Conference on Equine Infectious Diseases*, Paris 1969. Karger, Basel.
- Cavanagh, D., D. A. Brien, M. Brinton, L. Enjuanes, K. V. Holmes, M. C. Horzinek, M. M. C. Lai, H. Laude, P. G. W. Plagemann, and S. Siddell. 1994. Revision of the taxonomy of the Coronavirus, Torovirus and Arterivirus genera. *Arch. Virol.* **135**:227–237.
- Chirnside, E. D. 1992. Equine arteritis virus: an overview. *Br. Vet. J.* **148**: 181–197.
- Chirnside, E. D., P. M. Francis, A. A. F. de Vries, R. Sinclair, and J. A. Mumford. 1995. Development and evaluation of an ELISA using recombinant fusion protein to detect the presence of host antibody to equine arteritis virus (EAV). *J. Virol. Methods* **54**:1–13.
- Chirnside, E. D., and W. J. M. Spaan. 1990. Reverse transcription and cDNA amplification by the polymerase chain reaction of equine arteritis virus (EAV). *J. Virol. Methods* **30**:133–140.
- Chirnside, E. D., C. M. Wearing, M. M. Binns, and J. A. Mumford. 1994. Comparison of M and N gene sequences distinguishes variation amongst equine arteritis virus isolates. *J. Gen. Virol.* **75**:1491–1497.
- den Boon, J. A., E. J. Snijder, E. D. Chirnside, A. A. F. de Vries, M. C. Horzinek, and W. J. M. Spaan. 1991. Equine arteritis virus is not a togavirus but belongs to the coronaviruslike superfamily. *J. Virol.* **65**:2910–2920.
- Doll, E. R., R. E. Knappenberger, and J. T. Bryans. 1957. An outbreak of abortion caused by the equine arteritis virus. *Cornell Vet.* **47**:69–75.
- Fukunaga, Y., H. Imagawa, E. Tabuchi, and Y. Akiyama. 1981. Clinical and virological findings on experimental equine viral arteritis in horses. *Bull. Equine Res. Inst.* **18**:110–118.
- Fukunaga, Y., and W. H. McCollum. 1977. Complement fixation reactions in equine viral arteritis. *Am. J. Vet. Res.* **38**:2043–2046.
- Golnik, W., Z. Michalska, and T. Michalak. 1981. Natural equine viral arteritis in foals. *Schweiz. Arch. Tierheilkd.* **123**:523–533.
- McCollum, W. H. 1969. Development of a modified virus strain and vaccine for equine viral arteritis. *J. Am. Vet. Med. Assoc.* **155**:318–322.
- McCollum, W. H., M. E. Prickett, and J. T. Bryans. 1971. Temporal distribution of equine arteritis virus in respiratory mucosa, tissues and body fluids of horses infected by inhalation. *Res. Vet. Sci.* **2**:459–464.
- McCollum, W. H., and T. W. Swerczek. 1978. Studies of an epizootic of equine viral arteritis in racehorses. *Equine Vet. J.* **2**:293–299.
- McDonnell, S. M. 1992. Pharmacologically induced copula ejaculation in stallions, p. 1894–1896. *In* *Proceedings of the 12th International Conference of Animal Reproduction*, The Hague, 1992.
- Moraillon, R., and A. Moraillon. 1978. Results of a serological survey for viral arteritis in France and some other European and African countries. *Ann. Rech. Vet.* **9**:43–54.
- Murphy, T. W., W. H. McCollum, P. J. Timoney, B. W. Klingeborn, B. Hyllseth, W. Golnik, and B. Erasmus. 1992. Genomic variability among globally distributed isolates of equine arteritis virus. *Vet. Microbiol.* **32**:101–115.
- Murphy, T. W., P. J. Timoney, and W. H. McCollum. 1988. Analysis of genetic variation among strains of equine arteritis virus, p. 3–12. *In* D. G. Powell (ed.), *Proceedings of the Fifth International Conference on Equine Infectious Diseases*, Lexington, 1987. The University Press of Kentucky, Lexington.
- Neu, S. M., P. J. Timoney, and W. H. McCollum. 1988. Persistent infection of the reproductive tract of stallions persistently infected with equine arteritis virus, p. 149–154. *In* D. G. Powell (ed.), *Proceedings of the Fifth International Conference on Equine Infectious Diseases*, Lexington, 1987. The University Press of Kentucky, Lexington.
- Paweska, J. T. 1995. An epidemiological investigation of equine viral arteritis in donkeys in South Africa, p. 337–338. *In* H. Nakajima and W. Plowright (ed.), *Proceedings of the Seventh International Conference on Equine Infectious Diseases*, Tokyo, 1994. R&W Publications (Newmarket) Ltd.
- Paweska, J. T., and B. J. H. Barnard. 1993. Serological evidence of equine arteritis virus in donkeys in South Africa. *Onderstepoort J. Vet. Res.* **60**: 155–158.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Senne, D. A., J. E. Pearson, and E. A. Carbrey. 1985. Equine viral arteritis: a standard procedure for the virus neutralization test and comparison of results of a proficiency test performed at five laboratories, p. 29–34. *In* *Proceedings of the 89th Annual Meeting of the United States Animal Health Association*, Milwaukee, Wisconsin.
- Timoney, P. J. 1985. Clinical, virological and epidemiological features of the 1984 outbreak of equine viral arteritis in the Thoroughbred population in Kentucky, USA, p. 24–48. *In* *Proceedings of the Grayson Foundation International Conference of Thoroughbred Breeders Organizations*, Dromoland Castle, Ireland, 1984.
- Timoney, P. J., and W. H. McCollum. 1988. Equine viral arteritis—epidemiology and control. *J. Equine Vet. Sci.* **5**:54–59.
- Timoney, P. J., and W. H. McCollum. 1991. Equine viral arteritis: current clinical and economic significance. *Proc. Am. Assoc. Equine Pract.* **36**:403–409.
- Timoney, P. J., and W. H. McCollum. 1993. Equine viral arteritis 1993: international perspective, p. 11–12. *In* *Proceedings Equine Infectious Disease: Developing an Action Plan*, 1993. The University of Kentucky, Lexington.
- Timoney, P. J., W. H. McCollum, T. W. Murphy, A. W. Roberts, A. W. Willard, and J. G. Carswell. 1987. The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of transmission. *J. Reprod. Fertil. Suppl.* **35**:95–102.
- Timoney, P. J., W. H. McCollum, and A. W. Roberts. 1987. Detection of the carrier state in stallions persistently infected with equine arteritis virus. *Proc. Am. Assoc. Equine Pract.* **32**:57–65.
- Timoney, P. J., W. H. McCollum, A. W. Roberts, and T. W. Murphy. 1986. Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Res. Vet. Sci.* **41**:279–280.
- Vaala, W. E., A. N. Hamir, E. Dubovi, P. J. Timoney, and B. Ruiz. 1992. Fatal, congenitally acquired infection with equine arteritis virus in a neonatal Thoroughbred. *Equine Vet. J.* **24**:155–158.
- Wood, J. L. N., E. D. Chirnside, J. A. Mumford, and A. J. Higgins. 1995. The first recorded outbreak of equine viral arteritis in the United Kingdom. *Vet. Rec.* **136**:381–385.