## RESEARCH COMMUNICATION

# Serological evidence of equine arteritis virus in donkeys in South Africa

J. T. PAWESKA and B. J. H. BARNARD

Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

#### **ABSTRACT**

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This paper reports the first serological evidence of exposure of donkeys to equine arteritis virus. Seven hundred and thirty-four serum samples collected between 1989 and 1992 from donkeys in different areas of South Africa were examined for the presence of antibodies against this virus by a microneutralization test. Seventeen percent of serum samples tested positive. The distribution of seropositive animals varied from none in the western Cape Province and the Transvaal Highveld to 30 % in the northern Transvaal. The country-wide distribution of serologically positive donkeys suggests a longstanding presence of the virus in South Africa.

## INTRODUCTION

Equine arteritis virus (EAV) is the sole member of the genus arterivirus within the family Togaviridae (Porterfield, Casals, Chumakov, Gaidamovich, Hannoun, Holmes, Horzinek, Mussgay, Oker-Blom, Russel & Trent 1978; Westaway, Brinton, Gaidamovich, Horzinek, Igarashi, Kaariainen, Lvov, Porterfield, Russel & Trent 1985). Recently it was proposed as a member of the coronavirus-like 'superfamily' (Den Boon, Snijder, Chirnside, De Vries, Horzinek & Spaan 1991). EAV was first isolated in 1953 from fetal lung tissue after an outbreak of respiratory disease and abortion on a Standardbred farm in Bucyrus, Ohio, in the United States of America (Doll,

Bryans, McCollum & Crowe 1957). EAV spreads by the respiratory (McCollum, Prickett & Bryans 1971; McCollum & Swerczek 1978) and venereal (Timoney, McCollum, Murphy, Roberts, Willard & Carswell 1987) routes. Clinical signs of infection are extremely variable (Mumford 1985) and in its most severe form it causes abortion (Doll, Knappenberger & Bryans 1957) and mortality among foals (Golnik, Michalska & Michalak 1981). A carrier state exists in serologically positive stallions, which excrete virus in semen (Timoney, McCollum, Roberts & Murphy 1986; Timoney, McCollum & Murphy 1991).

Although serological surveys suggest that EAV is widespread throughout the world, relatively few outbreaks of clinical disease have been reported (Huntington, Ellis, Forman & Timoney 1990) and it would appear that infection continues to be subclinical (Timoney 1992).

EAV infection has been diagnosed mostly in Standardbred horses (McCollum & Swerczek 1978), but it was an outbreak in Kentucky Thoroughbreds in 1984 (Timoney & McCollum 1988) which generated widespread interest and publicity and which led to the imposition of some of the severest restrictions ever on the international movement of horses.

The virus was first isolated in South Africa in 1987 from the semen of a serologically positive Lippizaner stallion and early in 1988 the first seropositive Thoroughbred horses were identified (Erasmus 1988). Other shedder stallions and cases of clinical disease have not as yet been recorded.

This report presents serological evidence of EAV infection in donkeys in South Africa.

## **MATERIALS AND METHODS**

## Serum samples

Serum samples collected from 734 donkeys in 49 districts in the Transvaal, the Orange Free State, Natal and the Cape Province between 1989 and 1992 were kindly provided by Mr G. Venter, Division of Entomology, Onderstepoort Veterinary Institute. Age and sex of the animals were unknown.

#### Virus serum neutralization test

A microneutralization test (Moraillon & Moraillon 1978) with minor modifications was used to test the samples for the presence of antibody. Sera were inactivated at 56 °C for 30 min and then mixed with  $100 \text{ TCID}_{50}$  of the Bucyrus strain of EAV. Ten percent guinea pig serum (complement) was added to the diluent used to prepare the virus suspension. The serum-virus mixtures were incubated at 37 °C for 1 h. Finally, RK-13 cells were added. Results were read on the 7th d of incubation. The titre was expressed as the reciprocal of the highest serum dilution completely inhibiting the appearance of a cytopathic effect. A serum was considered positive when it had a titre  $\geq$  4.

## **RESULTS**

Evidence of EAV infection in donkeys in South Africa was demonstrated by the detection of virus neutralizing antibodies in their serum.

The antibody titres varied from 4–128 (Table 1). One hundred and twenty-three (17 %) of 734 donkeys tested and 24 of 49 districts yielded positive results (Table 2).

The geographic location of serologically positive donkeys in the Transvaal, the Orange Free State, Natal and the Cape Province is depicted in Fig. 1. The highest prevalence was in the northern Transvaal where 30 % tested positive. The other positive areas identified are western Transvaal (13 %), the

Orange Free State (19 %), Natal (18 %) and the eastern Cape Province (8 %). The 2 areas which tested negative are the Transvaal Highveld and the western Cape Province. However, a limited number of specimens were available from these two areas, 17 and 79 respectively. No positive cases were detected in 55 % of districts, while a high prevalence was encountered in others — Bochum (39 %), Nebo (48 %), Seshego (33 %) and Sekhukhuneland (50 %).

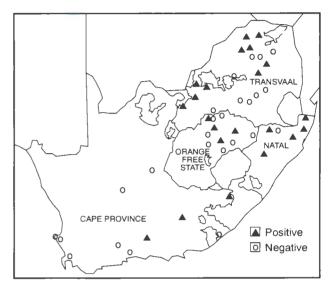


FIG. 1 Distribution of donkeys in South Africa with antibodies against equine arteritis virus. 1987–1992

#### DISCUSSION

Horses are the only known host of EAV (Chirnside 1992). The presence of antibodies in 17 % of 734 serum samples of donkeys, however, indicates that they can become infected by and harbour the virus. The widespread distribution of serologically positive donkeys suggests a longstanding presence of this virus in South African donkeys. The absence of positive donkeys in 24 of 49 districts is not an indication of the absence of EAV, but can probably be attributed to the low number of specimens tested.

In recent years it has become evident that venereal transmission by the carrier stallion represents the major route of infection, except in circumstances where clinical respiratory disease or abortion provides a substantial source of virus in aerosols and also where prolonged close contact occurs (Huntington, Forman, Ellis 1990). The incidence of positive cases among donkeys may be linked to the system of management practised. For example, castration is infrequently performed and animals of a number of owners usually share communal grazing which promotes close contact. The movement of donkeys over long distances is rare and dissemination of virus by this means is probably limited.

TABLE 1 Distribution of antibody against EAV in 734 donkey sera collected from several areas in South Africa, 1989–1991

	Neutralizing antibody titre								
Area	Negative	4	8	16	32	64	128		
Northern Transvaal Western Transvaal Transvaal Highveld Orange Free State Natal Eastern Cape Province Western Cape Province	121 122 17 121 86 65 79	7 5 0 10 8 3	12 5 0 13 4 2	15 4 0 3 4 1	10 3 0 1 2 0	3 1 0 1 0 0	5 0 0 1 0		
Total	611	33	36	27	16	5	6		

TABLE 2 Prevalence of virus neutralizing antibodies against equine arteritis virus in South African donkeys, 1989–1992

Area	Number of districts		Number specimer	% positive	
	Tested	Positive	Tested	Positive	podiavo
Northern Transvaal Western Transvaal Transvaal Highveld Orange Free State Natal Eastern Cape Province Western Cape Province	9 7 5 11 6 5 6	6 6 0 4 5 3 0	173 140 17 149 105 71	52 18 0 28 19 6 0	30 13 0 19 18 8 0
Total	49	24	734	123	17

Current information on the occurrence of EAV in South Africa (Erasmus 1988) indicates a restricted distribution of the virus among South African horses and suggests that the high prevalence and wide distribution of the virus among South African donkeys could possibly be explained by the introduction of infected donkeys in previous decades.

Further investigation should be undertaken to evaluate the significance of the present data in the epidemiology of the disease and to review strategies for the prevention and control of EAV in South Africa.

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