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Characterization of Palyam serogroup orbiviruses isolated in South Africa and serologic evidence for their widespread distribution in the country

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SUMMARY

The finding that there had been multiple isolations of Palyam serogroup orbiviruses from aborted cattle fetuses in neighbouring Zimbabwe, suggested that there was a need to investigate the possible occurrence of the viruses in South Africa. Unidentified viruses isolated in South Africa, which had been in storage, were examined. Four viruses which had been isolated from *Culicoides* midges collected at various sites in the years from 1969 to 1977, were identified as three strains of Gweru virus and one of the Nyabira virus (Palyam group serotypes originally described from Zimbabwe). A fifth virus, isolated in 1967 from the blood of a cow with mild fever, was found to be a distinct new member of the Vellore antigenic complex of the Palyam serogroup and was named Apies River virus. Sera from 476 cattle, 150 sheep, 24 goats and 78 humans from 10 farms were tested for neutralizing antibodies to the above three serotypes of virus plus Abadina and Marondera serotypes. Only 1 of 100 cattle sera from two farms in the southern coastal area had antibody, but elsewhere there was a high prevalence of antibody with 254 (53%) of all cattle exhibiting activity for one or more of the five serotypes of virus tested. Only 6 (4%) sheep, 3 (12·5%) goats and 11 (14%) humans had antibody.

INTRODUCTION

We recently examined 14 out of 17 Palyam serogroup orbiviruses which had been isolated in Zimbabwe and found that they belonged to two previously described serotypes plus two new serotypes (Whistler & Swanepoel, 1988). From the findings that 14 of the isolations in Zimbabwe had been obtained from aborted cattle fetuses, that neutralizing antibody to one serotype had been shown to occur widely in cattle sera and that seroconversions had been recorded in cattle which aborted, we concluded that fetotropism was possibly a common feature of Palyam serogroup viruses and that there was a need to investigate the role of the viruses in other geographic regions and in vertebrates other than cattle. There have been no reports of isolation of viruses of the serogroup in South Africa and the present paper records identification of five viruses previously isolated in the country as Palyam serogroup orbiviruses, with characterization of a further new serotype. It

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Table 1. *Strains of Palyam serogroup orbiviruses isolated in South Africa*

Strain designation	Isolated from	Year	Geographic location	
0/4518	Cow blood	1967	VRI*, Onderstepoort, Transvaal	location in Fig. 1
AR 11022	*Culicoides* sp.	1969	Kaalplaas†, Transvaal	
AR 11869	*Culicoides* sp.	1970	Bethuli, Orange Free State	
AR 17819	*Culicoides* sp.	1976	Luckhoff, Orange Free State	
AR 18422	*Culicoides* sp.	1977	Upington, Cape	

* VRI, Veterinary Research Institute.
† Adjacent to the VRI at Onderstepoort.

also records demonstration of antibodies to five serotypes of the group in man and domestic animals as a preliminary to investigation of the medical and veterinary significance of the viruses in South Africa.

MATERIALS AND METHODS

Viruses

Unidentified viruses in the collection of the Arbovirus Unit of the National Institute for Virology (NIV) were screened by complement-fixation (CF) for group-specific antigen of the Palyam serogroup and five viruses which reacted (Table 1) were subjected to fully quantitative CF tests and to neutralization tests for identification of individual serotypes. Strain 0/4518 (Table 1) had been isolated in 1967 from the blood of a cow which developed mild fever while stabled at the Veterinary Research Institute (VRI) at Onderstepoort near Pretoria. The other four viruses were isolated at NIV from pools of *Culicoides* midges collected at various sites in South Africa from 1969 to 1977.

Strains representative of the five serotypes of Palyam serogroup viruses known to occur in southern Africa and which were used in neutralization tests on human and domestic animal sera, comprised 0/4518 identified as the new serotype Apies River in the present study, plus four strains from Zimbabwe: Nyabira virus strain 792/73, Abadina strain 2041/76, Gweru strain 866/77 and Marondera strain 1070/78 (Whistler & Swanepoel, 1988). Reference strains of the remaining known members of the Palyam serogroup were obtained from Dr R. B. Tesh of the Yale Arbovirus Research Unit, New Haven, Conn., USA, as detailed previously (Whistler & Swanepoel, 1988).

Serum samples

Sera utilized in the antibody survey comprised samples collected from humans and domestic animals on 10 farms in South Africa, as indicated in the results section, by members of staff of the Department of Health and Division of Veterinary Services from 1983 to 1987 in the course of unrelated studies. The sera were stored at −70 °C and in order to obtain broad geographic cover of South Africa, four farms were selected for testing in the large Cape province and two farms each in the remaining three provinces.

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Table 2. *The results of fluorescent focus reduction tests with reference mouse immune ascitic fluids and Palyam serogroup orbiviruses of the Vellore antigenic complex plus virus 0/4518 isolated in South Africa*

	Virus			
Mouse immune ascitic fluid	Bunyip Creek	Vellore	Marondera	0/4518
Abadina	0	0	0	0
Kasba	0	0	0	0
Marrakai	0	0	0	0
Nyabira	0	0	0	0
D'Aguilar	0	0	0	0
Bunyip Creek	2·7	2·1	1·8	0
Vellore	3·2	3·4	1·7	0
Marondera	2·1	1·8	2·2	1·0
0/4518	0	0	1·8	4·6
Palyam	0	0	0	0
CSIRO Village	0	0	0	0
Petevo	0	0	0	0
Gweru	0	0	0	0

Results are expressed as $\log_{10}$ neutralizing indices and values <1 are reported as 0.

Serological tests

Preparation of antigens and reference mouse immune ascitic fluids (MIAFs) and the performance of CF, indirect-immunofluorescence (IF) and fluorescent-focus reduction (FFR) neutralization tests were achieved as described previously (Clarke & Casals, 1958; Sartorelli, Fisher & Downs, 1966; Blackburn & Swanepoel, 1980; Johnson, Elliot & Heymann, 1981; Whistler & Swanepoel, 1988). Cytopathic effect neutralization (CPEN) tests were performed in microculture plates as described previously for Rift Valley fever (Swanepoel *et al.* 1986).

RESULTS

The five South African isolates were confirmed as being members of the Palyam serogroup in the quantitative CF test (data not shown). The viruses were separated into serotypes on the basis of results obtained in FFR tests with reference MIAFs. Isolates AR 11022, AR 11869 and AR 18422 proved to be indistinguishable from Gweru virus with heterologous and homologous $\log_{10}$ neutralizing indices ranging from 3·2 to 3·6. Isolate AR 17819 reacted as a strain of Nyabira virus in the D'Aguilar complex with heterologous and homologous $\log_{10}$ neutralizing indices ranging from 3·2 to 4·3, while the remaining virus. 0/4518, appeared to be a distinct new member of the Vellore antigenic complex (Table 2).

The results of screening 476 cattle sera from 10 farms for CPEN antibody to five serotypes of virus are shown in Table 3 in relation to results obtained with sera from other species. Two herds from the southern coastal area of the Cape province had little or no antibody but elsewhere there was a high prevalence of antibody in cattle to one or more of the serotypes of virus tested (Table 3; Figure 1). The sera

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Table 3. *Summary of results obtained in cytopathic effect neutralization (CPEN) tests for antibodies to five Palyam serogroup orbiviruses performed on sera of cattle, sheep, goats and humans from 10 farms in South Africa*

						Number with CPEN antibody to				
Province	District	Year	Species	Number tested	Total CPEN positive (%)	Nyabira	Gweru	Abadina	Marondera	Apies River
Cape	Vredenburg	1984	Cattle	50	0	0	0	0	0	0
	Knysna	1984	Cattle	50	1 (2)	0	0	0	0	1
	Steynsburg	1984	Cattle	50	44 (88)	1	36	14	36	21
	Prieska	1986	Cattle	26	12 (46)	6	9	6	7	8
			Sheep	50	0	0	0	0	0	0
			Goat	24	3 (13)	0	1	2	0	0
			Human	10		0	0	0	0	0
Orange Free State	Jacobsdal	1986	Cattle	50	24 (48)	2	15	18	12	11
			Sheep	50	4 (8)	2	2	3	1	1
			Human	68	11 (16)	9	0	0	1	1
	Theunissen	1987	Cattle	50	42 (84)	38	28	13	16	0
			Sheep	50	2 (4)	0	0	2	0	0
Natal	Umvoti	1984	Cattle	50	33 (66)	0	33	14	18	7
	Hlabisa	1984	Cattle	50	28 (56)	16	23	17	2	0
Transvaal	Potgietersrus	1983	Cattle	50	46 (92)	6	46	0	12	12
	Christiana	1984	Cattle	50	24 (48)	0	15	18	18	12

were tested at dilutions from 1 in 8 to 1 in 128 only and there was a clear tendency for the occurrence of high antibody titres in cattle, $\geqslant 128$, to coincide with high prevalence (data not shown). In the few instances where it was possible to establish the ages of individual cattle from which the sera were obtained, the prevalence of antibody increased directly with age and at the Jacobsdal farm, for instance, 22 of 30 $\geqslant$ 1-year-old cattle had antibody to one or more serotypes of virus, whereas 18 of 20 $\geqslant$ 1-year-old cattle lacked antibody to any of the five serotypes of virus.

CPEN tests were performed on sera from livestock other than cattle on three of the farms which had a high prevalence of antibody in cattle. The prevalence of antibody in sheep and goat sera was found to be markedly lower than that in cattle sera on the same properties (Table 3). Human sera from two of the farms were subjected to group-specific IF tests for antibody to Palyam viruses as well as to CPEN tests for antibody to the five individual serotypes of virus. No antibodies were detected in 10 human sera from the farm in the Prieska district while 14 of 28 human sera from the Jacobsdal farm had group-specific IF antibody activity. CPEN antibody to individual serotypes of virus were demonstrated in 11 of 14 of the sera with group-specific IF antibody. The CPEN titres ranged from 32 to $\geqslant 128$ and it is notable that in most instances the antibodies in human sera were directed against Nyabira virus, while in cattle sera from the same property the antibodies were mainly directed against the other four serotypes of virus (Table 3).

DISCUSSION

The occurrence of Palyam serogroup orbiviruses in South Africa was demonstrated in the present study by characterization of viruses isolated previously from the blood of a cow and from *Culiocoides* midges collected at various sites in the country (Figure 1). Two of the three serotypes isolated in South Africa, Nyabira and Gweru, have been described previously (Swanepoel & Blackburn, 1976; Whistler & Swanepoel, 1988), but the third virus, strain 0/4518, represents a new serotype. In keeping with the practice of using geographic names for viruses in the serogroup, the new virus is designated Apies River for the stream which flows past the VRI at Onderstepoort where the prototype strain was isolated. This brings to five the number of serotypes known to occur in southern Africa and antibodies to all five were demonstrated at widely separated localities in South Africa.

It appears that the viruses of the Palyam serogroup have a particular association with cattle. Prior to the present identification of a new serotype from cattle and demonstration of antibodies in cattle sera in South Africa, there had been 15 isolations of four serotypes from cattle in Zimbabwe (Swanepoel & Blackburn, 1976; Whistler & Swanepoel, 1988), 112 isolations of three serotypes from cattle in Australia (Cybinski & St George, 1982) and antibodies had been found in cattle in India, Australia, Nigeria and Zimbabwe (Myers *et al.* 1971; Cybinski & St George, 1982; Moore & Kemp, 1974; Blackburn, Searle & Phelps, 1985). Antibody to one serotype of virus was more common in buffalo sera than in cattle sera in Australia (Cybinski & St George, 1982), but in all other instances the prevalence of antibody to Palyam serogroup viruses was found to be higher in

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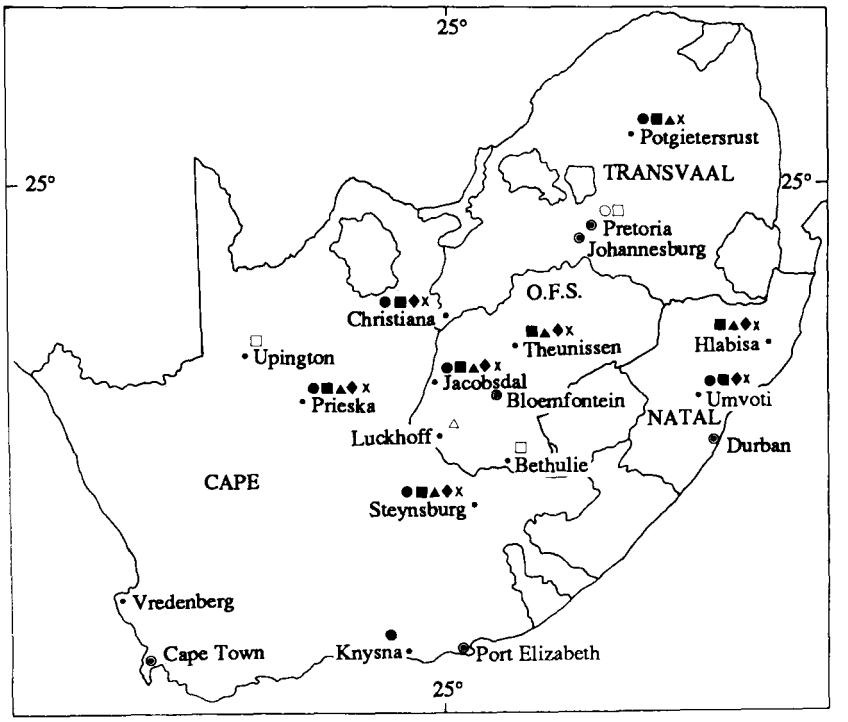

Fig. 1. Locations in South Africa where Palyam serogroup orbiviruses were isolated and where neutralizing antibodies to the viruses were demonstrated in the sera of livestock and/or humans. Virus isolations: Gweru (□), Nyabira (△) and Apies River (○) serotypes. Antibodies: Gweru (■), Nyabira (▲), Apies River (●), Abadina (◆) and Marondera (×) serotypes. O.F.S., Orange Free State. The Veterinary Research Institute at Onderstepoort is near Pretoria.

cattle sera than in other species. Inadequate information exists to indicate whether the higher prevalence of antibodies in cattle reflects the relative susceptibility of the various species of livestock to infection, or the host preferences of haematophagous vectors of the viruses, or merely farming practices which affect exposure of the different species of infection.

Prior to the present study, reported isolations of Palyam serogroup viruses from invertebrates included 4 from mosquitoes in India and Nigeria (Dandawate, 1974; Lee, Causey & Moore 1974), 99 from *Culicoides* midges in Nigeria, Australia and Zimbabwe (Lee, Causay & Moore, 1974; Cybinski & St George, 1982; Blackburn, Searle & Phelps 1985) and 2 from ticks in the Central African Republic and Guinea (Saluzzo *et al.* 1982; Boiro *et al.* 1986). The isolation made from midges in Zimbabwe (Blackburn, Searle & Phelps, 1985) and those reported in the present study, suggest that *Culicoides* midges serve as vectors for the Palyam serogroup viruses of southern Africa, just as they do for the African horsesickness (AHS) and bluetongue (BT) serogroups of orbiviruses. Isolations of AHS serotypes made from midges in South Africa in a study extending over a period of years (B. J. Erasmus, unpublished laboratory records), indicate that the viruses occur relatively infrequently in the south-western Cape province and that elsewhere the prevalence

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of individual serotypes varies with location and year, and this accords well with the pattern of distribution of antibodies to Palyam serogroup viruses observed in the present study. The relative paucity of orbiviruses in the south-west of the country possibly derives from the colder climate of that region while marked increases in orbivirus activity in particular years seem to be occasioned by the occurrence of heavy rainfall.

The 112 isolations of Palyam serogroup viruses made from cattle in Australia, were obtained from blood samples of sentinel animals in the absence of evidence of disease (Cybinski & St George, 1982). In contrast, 14 isolations in Zimbabwe were obtained from aborted cattle fetuses and one from the visceral organs of a cow which died during a Rift Valley fever epizootic (Swanepoel & Blackburn, 1976; Whistler & Swanepoel, 1988), while one isolation reported in the present study was made from a cow with mild fever in South Africa. The apparent differences in findings on pathogenicity, stem from differences in emphasis and in the nature of specimens tested in the various studies. The implication is that the viruses produce relatively mild infection in adult cattle, but that they may be abortigenic in pregnant cattle. Clearly, there is a need for pathogenicity trials in cattle and other species of livestock with African and other Palyam group viruses, and for appropriate monitoring of diagnostic specimens from a variety of species, including man.

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