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Antigenic relationship of West Nile strains by titre ratios calculated from cross-neutralization test results

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SUMMARY

The antigenic relationship of ten South African West Nile isolates, the South African prototype virus H442, the Egyptian strain EG101 and the Indian strain G2266 were compared using titre ratios. The titre ratios or 'R' values were calculated from heterologous and homologous neutralization titres and expressed as a percentage.

Substantial antigenic differences were demonstrated between the South African prototype strain and the majority of the recently obtained South African isolates, seven of which were fairly closely related and possibly form a distinct antigenic sub-set. The recent isolates also differed from the Egyptian and Indian West Nile isolates. The heterologous results between the South African West Nile strains and the Indian strain G2266 suggest that prior infection with an Indian West Nile virus would give poor protection against the South African viruses, whereas the reverse would not be so.

INTRODUCTION

Studies on the antigenic relationship of West Nile (WN) virus strains from three geographic regions suggested the existence of two main antigenic groups, African-Middle-Eastern and Indian (Hammam, Clarke & Price, 1965). Of the three South African strains included in that study two appeared to belong to the African-Middle-Eastern group and the third was antigenically distinct from either group, suggesting the existing of at least one further group. Antigenic variation amongst isolates within these main groups has been described by Nir *et al.* (1968), Umrigar & Pavri (1977) and Odelola & Fabiyi (1976).

This paper presents the results of a study of the antigenic relationship of several South African WN virus isolates collected from one site during one season. The method of assessing the relationships of these isolates to each other, and to members of the two main WN antigenic sub-sets, was by calculating the titre ratios or 'R' values from heterologous and homologous neutralization titres. Jordan & Gaylin (1953) studied the antigenic relationship of influenza B strains using 'R' values. Rweyemamu *et al.* (1977) applied this method to evaluate the antigenic relationship between foot and mouth disease vaccine strains.

MATERIALS AND METHODS

Virus strains

Ten WN virus isolates from pools of *Culex univittatus* mosquitoes caught at the Rondebult bird sanctuary, Germiston, in 1984 (Jupp *et al.* 1986) were included in the study. The isolates, all at mouse passage 3 and tissue culture passage 3 (M3TC3) were AR20724, AR20730, AR20735, AR20739, AR20758, AR20760, AR20761, AR20763, AR20769, AR20778. The South African WN prototype strain H442, at passage level M4TC3 was also tested, together with the Indian strain G2266 (M5TC3) and the Egyptian WN strain EG101 (M13TC3), which were obtained from the Yale Arbovirus Research Unit, New Haven, Connecticut, USA.

Antisera

Hyperimmune ascitic fluids (HAF) were prepared against all the WN strains using the method described by Tikasingh, Spence & Downs (1966).

Neutralization test (NT)

The NT test was based on that used by Blackburn & Swanepoel (1980). Each virus strain was adapted to Vero cells (Yasamura & Kawakita, 1963) and stored in aliquots as third passage material at −70 °C in 10% dimethyl sulphoxide. The viruses were titrated in quadruplicate just prior to use in the test. The HAF's were inactivated at 50 °C for 30 min and dilutions were made from 1 in 8 to 1 in 8192. A volume containing a calculated 100 $TCID_{50}$ of virus was added to each well and the serum-virus mixture was incubated for 1 h at 22 °C. The microtiter wells were seeded with 8×10^4 Vero cells per well. The virus was titrated in quadruplicate and the end point calculated by the method of Kärber (1931). If the titre of the virus used in the test was outside the range of 100 $TCID_{50} \pm Log_{10}0{\cdot}25$, the test was repeated. The tests were read after 6 days incubation and endpoints recorded.

The first group of cross-NT tests consisted of the ten local WN isolates and the South African prototype WN virus H442. These tests were carried out in duplicate and the neutralization titre was the dilution at which neutralization occurred in both replicates.

The second group of cross-NT tests included selected local isolates from the first group, H442, the Egyptian strain EG101 and Indian strain G2266. The HAF's of this group were tested in quadruplicate, the neutralization titre of each replicate was recorded and the average NT titre calculated.

Titre ratios

The 'R' values were calculated as described by Jordan & Gaylin (1953) where two virus strains were compared using homologous and heterologous antibody titres.

$$R = 100 \sqrt{r_1 \times r_2},$$

where

$$r_1 = \frac{\text{Heterologous titre (Virus 2)}}{\text{Homologous titre (Virus 1)}},$$

and

$$r_2 = \frac{\text{Heterologous titre (Virus 1)}}{\text{Homologous titre (Virus 2)}}.$$

Using this formula 'R' is expressed as a percentage. A value of 50% or greater represents an up to fourfold total difference in heterologous and homologous cross NT titres, 24–49% represents a four to eightfold difference and 0–24% a greater than eightfold difference.

RESULTS

The cross neutralization test results for the first group of WN strains are shown in Table 1, and the 'R' values are in Table 2. The South African prototype strain H442 had a less than 25% relationship to six of the local strains and a 50% relationship to only one strain, AR20735.

Five of the local strains were selected to be tested against the strains G2266 and EG101, these were the prototype H442, AR20735 because of its fairly close relationship to H442, and representatives of the remaining local strains AR20730, AR20739 and AR20778. The cross neutralization results of the second group are shown in Table 3, the r_1 and r_2 results in Table 4 and the 'R' results in Table 5. The relationship of the local isolates to H442 was confirmed and with the exception of AR20735, they showed a closer relationship to the strain EG101 than to H442 or G2266.

DISCUSSION

The use of the 'R' value to assess the relationship between virus strains is discussed by Jordan & Gaylin (1953). This calculation reduces the four figures taken from heterologous and homologous titres to one figure expressed as a percentage, taking into account variation in r_1 and r_2 titre ratios.

The titre of virus used in the NT test is probably the most important factor for accurate reproducible results. In cross-neutralization studies wide variation in virus titre used will result in incorrect NT titres, therefore the 'R' value calculations will be invalid. Rweyemamu *et al.* (1977) took great care to ensure that 100 $TCID_{50}$ virus was used in all the tests. In this study the test results were only accepted if the virus dosage was within $\log_{10}0{\cdot}25$ of 100 $TCID_{50}$. The first group of viruses studied consisted of the South African WN prototype virus and ten local isolates. The majority of the isolates were significantly different to the prototype strain, only one strain, AR20735, having an 'R' value of over 35% against H442. Seven of the local isolates were fairly closely related, with the majority having 'R' values of 50% or greater to each other, these isolates were AR20724, AR20730, AR20758, AR20761, AR20763, AR20769 and AR20778. These results would suggest that these strains form one group distinct from AR20735 which is antigenically closer to H442 and AR20760 and AR20739 which give a range of 'R' values from 18% to 100 per cent against the seven isolates above.

None of the local isolates included in the second group of tests were significantly

Table 1. *Titres of cross-neutralizing antibody among 11 South African strains of West Nile virus*

	Hyperimmune ascitic fluid											
West Nile virus strains	H 442	AR 20735	AR 20761	AR 20763	AR 20769	AR 20724	AR 20730	AR 20739	AR 20758	AR 20760	AR 20778	$TCID_{50}$ used in test
H 442	*1024*	64	64	64	16	16	16	32	32	8	64	178
AR 20735	256	*64*	64	32	64	16	256	128	32	16	128	100
20761	128	8	*128*	32	32	64	128	64	32	32	128	56
20763	512	32	256	*256*	128	256	512	128	64	64	512	100
20769	512	128	128	128	*128*	256	512	128	128	32	128	178
20724	256	8	128	64	64	*256*	256	32	32	32	128	178
20730	128	16	256	256	128	64	*512*	64	128	32	128	178
20739	256	16	256	64	64	64	256	*256*	256	16	128	100
20758	128	32	256	128	64	256	512	256	*128*	32	256	178
20750	128	8	128	128	32	128	256	64	128	*128*	128	100
20778	128	16	256	64	32	128	512	256	256	64	*256*	56

Table 2. *Titre ratio or 'R' value between the 11 West Nile strains expressed as a percentage using the 'R' value formula (see Materials and Methods)*

	West Nile virus strains										
West Nile virus strains	H 442	AR 20735	AR 20761	AR 20763	AR 20769	AR 20724	AR 20730	AR 20739	AR 20758	AR 20760	AR 20778
H 442	100										
AR 20735	50	100									
20761	25	25	100								
20763	35	25	50	100							
20769	25	100	50	71	100						
20724	12	9	50	50	71	100					
20730	6	35	71	100	100	35	100				
20739	18	35	71	35	50	18	35	100			
20758	18	35	71	50	71	50	100	100	100		
20760	9	12	50	50	25	35	35	18	50	100	
20778	18	35	100	71	35	50	71	71	100	50	100

Table 3. *Titres of cross-neutralizing antibody among South African Indian and Egyptian West Nile strains*

West Nile virus strains	Hyperimmune ascitic fluid against: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778	$TCID_{50}$ used in test
EG101	*596*	178	106	45	354	64	150	100
G2266	256	*1024*	708	16	224	64	128	100
H442	150	89	*1024*	64	16	32	64	100
AR20735	89	16	256	*64*	208	64	89	178
AR20730	149	16	256	16	*355*	89	178	100
AR20739	89	106	128	8	64	*89*	64	178
AR20778	53	53	128	16	128	64	*150*	178

Table 4. *Cross-neutralization activity ('r' values) between the South African, Indian and Egyptian West Nile strains*

West Nile virus strains	Hyperimmune ascitic fluid against: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778
EG101	1	0, 17	0, 1	0, 7	1	0, 72	1
G2266	0, 43	1	0, 7	0, 25	0, 63	0, 72	0, 85
H442	0, 25	0, 09	1	1	0, 05	0, 36	0, 43
AR20735	0, 15	0, 02	0, 25	1	0, 84	0, 72	0, 59
AR20730	0, 25	0, 02	0, 25	0, 25	1	1	1, 19
AR20739	0, 15	0, 1	0, 125	0, 125	0, 18	1	0, 43
AR20778	0, 09	0, 05	0, 125	0, 125	0, 36	0, 72	1

Table 5. *Titre ratio or 'R' value between the South African, Indian and Egyptian West Nile strains expressed as a percentage using the 'R' value formula (see Materials and Methods)*

West Nile virus strains	West Nile virus strains: EG 101	G 2266	H 442	AR 20735	AR 20730	AR 20739	AR 20778
EG101	100						
G2266	27	100					
H442	16	25	100				
AR20735	32	7	50	100			
AR20730	50	10	11	46	100		
AR20739	33	27	21	30	42	100	
AR20778	30	21	23	38	65	56	100

closely related to either EG101 or G2266, although AR20735 and AR20730 were much closer to the Egyptian strain than the Indian. Contrary to the results of Hammam, Clarke & Price (1965) the South African prototype H442 was shown to be antigenically distinct from the Egyptian strain with an 'R' value of 16%. This is possibly due to the NT test being more specific than the modified haemagglutination inhibition (HI) test used in that study. Rweyemamu *et al.* (1977) found that 'R' values from NT results showed much wider differences between strains

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than those calculated from the less specific complement fixation test. The differences between the specificity of the HI, CF and NT in flavivirus serology is clearly demonstrated by Blackburn & Swanepoel (1978).

Strain H442 had a 25% relationship to the Indian strain G2266, this was interesting in that the r_1 and r_2 values were so different. The r_1 value representing the ratio of H442 antibody titre against H442 virus and the heterologous G2266 virus was 0·7, whereas the r_2 value was 0·09. These results indicate that H442 antibodies have a significant neutralizing effect on G2266 virus, whereas the reverse would not be so. This also applied to three of the four local isolates included in the study group.

G2266 is antigenically fairly representative of the Indian strains, according to the results of Umrigar & Pavri (1977), although there must be some reservations about these results as they were based on the erroneous assumption that the calculation of the 'R' values eliminated discrepancies of different virus dosage. The possibility is that a prior infection with an Indian strain of WN virus will give poor protection against the introduction of South African strains.

The minor antigenic differences between the isolates could be due to minimal changes in the glycoprotein during the bird-mosquito transmission cycle. Mah Lee & Westaway (1983) observed that Kunjin virus, closely related to West Nile, underwent alteration in the properties of the envelope protein on passage in *Aedes albopictus* cells. The major antigenic differences demonstrated between the H442 strain and the local isolates, and these viruses against EG101 and G2266 would appear to place these viruses into separate groups. It needs to be determined if the more specific cross NT test is detecting subgroups of the two main antigenic groups, or the number of main groups is larger than previously suspected.

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