The Ecology of West Nile Virus in South Africa and the Occurrence of Outbreaks in Humans

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ABSTRACT: This paper reviews studies done on West Nile virus (WNV) in South Africa, mainly between 1962 and 1980 on the temperate inland plateau (Highveld and Karoo). The virus is maintained in an enzootic transmission cycle between feral birds and the ornithophilic mosquito *Culex univittatus*. About 30 avian species have been shown to be involved without mortality. Humans, and other mammals, although they may have antibodies, are considered blind-alleys in the transmission cycle except perhaps some dogs. *Cx. univittatus* also transfers infection to humans, almost invariably causing only a mild illness. Its usually low anthropophilism may explain why annual human infection on the Highveld is limited to sporadic cases. Besides multiple isolations from field collections of *Cx. univittatus,* this mosquito is both highly susceptible to the virus and an efficient transmitter. *Culex theileri* is a minor vector. In the summer of 1974 there was a large epidemic in the dry Karoo after unusual rains: there were many human cases, the infection rate in *Cx. univittatus* was 39.0/1000, and postepidemic immune rates in humans and birds were high. In 1984 there was an epizootic in Gauteng Province in the Highveld with an infection rate in *Cx. univittatus* reaching 9.6/1000 and more human infections than usual. The much lower immune rates in the KwaZulu-Natal coastal lowlands than on the plateau and the single isolation from *Cx. neavei,* which replaces *Cx. univittatus* in the lowlands, are explained by the low susceptibility of *Cx. neavei* to the virus. Genetic relatedness of isolates from different countries showed two lineages, with one lineage comprising only African isolates, including 25 South African strains, which had a sequence homology of 86.3–100%. This suggests that the viral enzooticity does not depend on annual importation of virus in migrant birds.

KEYWORDS: West Nile virus; South Africa; ecology; human outbreaks

INTRODUCTION

This paper reviews the studies done on West Nile virus (WNV) in South Africa, mainly between 1962 and 1980, but also some work done before and after this period. Most of the studies were carried out in the inland plateau region (FIG. 1). This is an area with a temperate climate 400–2000 meters above sea level comprising the Karoo (the western two-thirds) and the Highveld (the eastern third). The Karoo is

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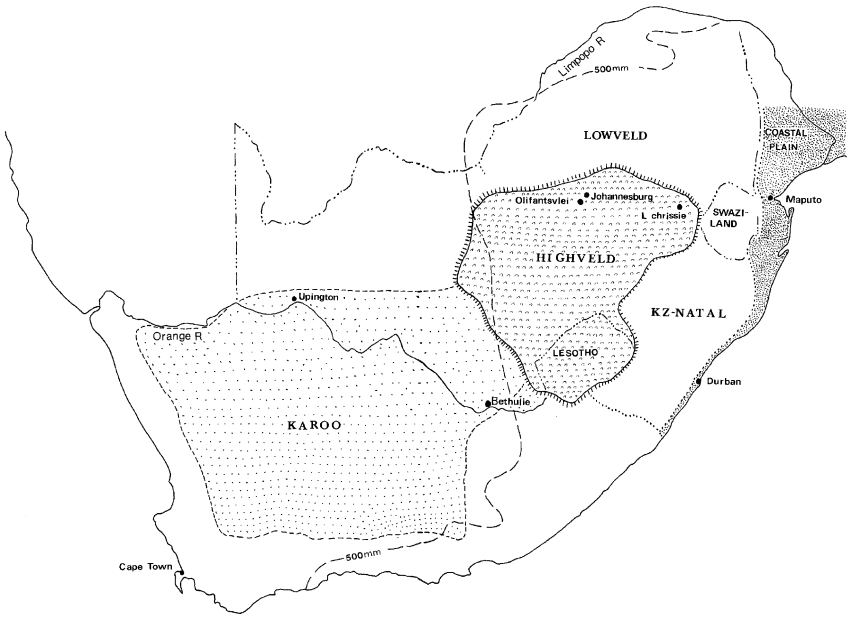

FIGURE 1. South Africa, showing the inland plateau (Karoo and Highveld) and the KwaZulu-Natal coastal plain. The 500 mm isohyet bisects the country into eastern moist and western arid parts.

arid semidesert with a mean rainfall of less than 500 mm, while the Highveld is cooler grassland with 500–700 mm of rainfall. The coastal plain of KwaZulu-Natal (KZN), the other region where studies were undertaken, is a lowland strip on the eastern seaboard with a moist subtropical climate except near the Mozambique border to the north where it becomes tropical.

Neutralizing antibodies to WNV are widely distributed in humans in South Africa and also in neighboring Mozambique, as well as along the northern borders of Namibia and Botswana. The virus was isolated at 11 localities on the inland plateau and at 2 localities on the KZN coast. Neutralizing antibodies were found in humans more frequently on the inland plateau (17.1% in the Karoo and 8% in the Highveld) than in the KZN coastal localities (2%).[1–5] This difference has correlated positively with mosquito infection rates, observed human illness, and avian infection and is believed to be due to the presence of the efficient vector *Culex univittatus* on the plateau, which is replaced by a much less efficient vector, *Culex neavei*, on the KZN coast.[6] From the various aspects of the ecology of WNV studied on the plateau, it became clear that a maintenance or enzootic cycle existed in which the virus was transmitted between various species of wild birds by the mosquito *Cx. univittatus.*[7,8]

Sporadic human cases of WNV—invariably a mild febrile illness with myalgia, arthralgia and a maculopapular rash—occur annually each summer on the Highveld.[9] On two occasions this usual pattern changed: there was an epidemic of WNV in the Karoo in 1974[10] and an unusual epizootic accompanied by more human cases than usual in the Highveld in 1984.[11]

TABLE 1. West Nile virus isolations from mosquitoes in South Africa, 1956–1980[a]

Region	Infected species	No. tested	No.isolations
Inland Plateau	*Cx. univittatus*	70,037	128
	Cx. theileri	82,995	6
	Cx. pipiens	128,967	1
	Ae. caballus sl	1809	1
KwaZulu-Natal coastal lowlands	*Cx. neavei*	57,559	1
	Cq. microannulata	254	1
	Ae. circumluteolus	201,427	1

[a]Isolations from the 1974 Karoo outbreak are not included; see TABLE 4.

TABLE 2. Results of quantitative vector competence tests with West Nile virus

	Culex univittatus			*Culex neavei*
Titer of infective feed $\log_{10}$ LD_{50}/mL	Infection rate[a]	50% Infection threshold $\log_{10}$ LD_{50}/mL[b]	Transmission rate[c]	50% infection threshold $\log_{10}$ LD_{50}/mL
5.8 –6.3	37/37		35/36 (97%)	
4.3	24/24		5/15 (33%)	
		2.1		4.4
2.7	21/25 (84%)			
1.9	12/29 (41%)			

[a]Numerator = No. mosquitoes infected; denominator = No. mosquitoes tested.
[b]This is calculated from infection rates and is titer of virus to infect 50% of mosquitoes.
[c]Numerator = No. mosquitoes transmitting virus; denominator = No. of infected mosquitoes feeding.

The *Alphavirus* Sindbis (SIN) has the same ecology as WNV and a very similar epidemiology. Hence, the two viruses have been studied simultaneously in South Africa and what has been said above applies to both. Observations on the SIN virus will sometimes be included in this paper where the comparison with WNV is considered important.

THE MOSQUITO VECTORS

TABLE 1 lists those mosquito species collected between 1956 and 1980 from which one or more isolates of WNV were made.[8,12,13] Multiple isolations were obtained from only two species, *Cx. univittatus* and *Cx. theileri,* and nearly all came from the former. Both these mosquitoes occur on the inland plateau; laboratory vector competence tests carried out with *Cx. univittatus* (TABLE 2) have shown that this species is highly susceptible to the virus with a low 50% infection threshold of 2.1 $1\log_{10}$ LD_{50}/mL and that it has a high transmission rate (97%) after an infecting feed with

TABLE 3. Olifantsvlei birds and West Nile virus; highest viremias recorded after viral inoculation and percentage of certain species naturally infected (hemagglutination inhibition antibodies), 1962–1965

Species	Laboratory viremia $\log_{10}$ LD_{50}/mL	Natural infection: No. tested (% positive)
Bubulcus ibis (cattle egret)	6.1	39 (9)
Threskiornis aethiopicus (sacred ibis)	5.2	14 (14)
Anas undulata (yellow-bill duck)	5.4	104 (17)
Anas erythrorhyncha (red-bill teal)	4.2	56 (36)
Netta erythrophthalma (pochard)	3.9	NT
Fulica eristata (red-knobbed coot)	4.2	72 (22)
Columba livea (domestic pigeon)	4.7	NT
Streptopelia capicola (turtle dove)	5.1	NT
Streptopelia senegalensis (laughing dove)	6.7	39 (10)
Passer melanurus (Cape sparrow)[a]	4.4	NT
Ploceus velatus (masked weaver)	7.5	133 (9)
Quelea quelea (red-bill quelea)[a]	3.9	279 (11)
Euplectes orix (red bishop)	7.7	999 (11)
Acrocephalus gracilirostris (Cape reed warbler)	NT	32 (11)

NT = not tested.
[a]Bloods titrated after storage, hence actual titers possibly higher than values shown.

a titer of about 6.0 logs/mL.[14–16] This rate was still quite high (33%) following an infective feed on a viremic chick circulating 4.3 logs of virus.[16] As the maximum titers of virus reached in feral birds varies from about 4.0–7.7 logs/mL (TABLE 3) it is clear that *Cx. univitattus* would be able to infect a range of birds or become infected by feeding on such avian species when they were viremic. Although *Cx. theileri* has a low 50% infection threshold of 3.2 logs/mL, it has low transmission rates viz. 0% and 25% following an infective meal of 6.2 and 7.1 logs, respectively.[17] If *Cx. theileri* is almost as easily infected as *Cx. univittatus* in experiments, why are so few viral isolates made from wild populations? It is probably because of differences in feeding habits. *Cx. univittatus* is highly ornithophilic while *Cx. theileri* is only moderately so.[18,19] Furthermore there are differences between their host preferences among various avian species, *Cx. theileri* feeding largely at ground level while *Cx. univitattus* feeds both on the ground and in the canopy of trees.[19] *Cx. theileri* is thus regarded as only a minor vector. It is notewothy that no other mosquito species, particularly *Aedes* species, have been incriminated as vectors in South Africa.

As mentioned earlier, WNV activity is low on the KZN coastal plain as compared to the inland plateau. An explanation for this is the poorer susceptibility of *Cx. neavei* to the virus as compared to *Cx. univittatus* (TABLE 2).[6] *Cx. univittatus* is replaced by *Cx. neavei* in the coastal plain,[20] and only one virus isolation has been made from this species. Apparently, therefore, *Cx. neavei* is less readily infected after feeding on birds.

THE AVIAN HOST

Thirteen common avian species collected at our study site Olifantsvlei (near Johannesburg) have circulated the virus at significant titers after inoculation in the laboratory (TABLE 3).[21] Viremia lasted 3–4 days and no illness or mortality was attributed to the virus.

Fifty-seven of 62 birds (92%) had hemagglutination inhibition (HI) antibodies and 42 of 60 birds (70%) had neutralizing (N) antibodies on the 30th day. From 1962–1965 wild birds were trapped at Olifansvlei and tested for antibodies and infection by WNV.[22] Two hundred and fifty-two of 2022 birds (12.5%) were found to have HI antibodies to WNV in their plasmas. Twenty-seven species were among the positively reacting birds. TABLE 3 shows the percentage of reacting birds from among 10 of these species which are common on the plateau. No isolation of WNV was made from 1015 plasmas tested for virus by inoculation into infant mice.

Both fowls and pigeons have been used for surveillance of virus transmission in the field. Fowls were exposed in fowl-pen mosquito traps at Olifantsvlei and at another site on the plateau—Lake Chrissie—over three consecutive summers from 1962–1965.[7] They were bled periodically for HI antibody tests. Infections (antibody conversions) were recorded at both localities each summer, with a total of 38 infections at Olifantsvlei (53% of the fowls) and 13 at Lake Chrissie (54% of the fowls). Later, fowls were replaced by pigeons as sentinels. Pigeons were preferred since they were as susceptible to the virus as fowls but hardier, easier to handle, and less expensive to maintain. These were exposed at Olifantsvlei from 1967–1971 and at Bethulie in the Karoo from 1968–1971.[23] At Olifansvlei a total of 20 infections were recorded during four of the five years and at Bethulie a total of 41 during three of the four years. Infections mainly occurred in the summer and but sometimes in the autumn. The higher infection rate in Bethulie compared to Olifantsvlei was significantly different ($P < 0.025$). Insects collected in suction traps, which were sometimes suspended at the bottom of the sentinel cages, revealed the numerical prevalence of *Cx. univitattus*.[23] This type of trap proved to be the most efficient of the various methods used for sampling ornithophilic mosquitoes.

HUMAN INFECTION AND EPIDEMICS

Host preference studies in the Highveld region of the plateau showed that while *Cx. univitattus* is strongly ornithophilic it does feed on humans to a limited extent and even enters houses.[19] This limited anthropophily must be an important factor limiting the number of human cases of WNV and preventing the regular occurrence of epidemics. Human infections occur annually on the plateau, but in small numbers, and humans are incidental hosts to the virus. Because of low viremia in man, human infection is totally dependent spatially and temporally on avian infection and humans are "blind alleys" in the transmission cycle.

Since work started on WNV in South Africa, there has been only one large epidemic and one more localized epizootic accompanied by an increase in human infections. The epidemic occurred in the summer of 1973–1974 in the Karoo following exceptional summer rains. The mosquitoes collected at Upington in April 1974 are shown in TABLE 4. Thirty-three isolations were obtained from only 1325 *Cx. univit-*

TABLE 4. Number of mosquitoes collected in April 1974 at Upington during the Karoo epidemic and number of isolations of West Nile and Sindbis viruses made from them

Species	No. of mosquitoes tested	No. isolations		MIR[a] (IR[b])	
		WN	SIN	WN	SIN
Cx. univittatus	1325	33	8	24.9	6.0
Cx. theileri	4889	4	4	(39.0)	(6.5)
Cx. pipiens	1200			0.8 (0.8)	0.8
5 other species	100				(0.8)

[a]Minimum infection rate = No. infected mosquitoes/1000.
[b]Statistical infection rate/1000 calculated by method of Chiang and Reeves (1962).

tatus and four isolations from 4889 *Cx. theileri*. This epidemic was accompanied by one of SIN virus, although the mosquito infection rates were much higher for WNV (TABLE 4). This event and the related studies have been fully reported by McIntosh *et al*.[10] Studies of the mosquitoes, birds and humans in the Upington area revealed that WNV was much more active than SIN virus. Further mosquito collections made at Upington during the first wet summer after the outbreak (1976), which also had unusually heavy rain, showed that *Cx. univittatus* was an important anthropophilic species there. This might have been due to a very high population density of *Cx. univittatus* or it could indicate that the local population of the mosquito is more anthropophilic than the Highveld population. All eight species of birds trapped and bled in the Karoo at Upington after the epidemic revealed a high positivity in antibody tests for WNV, and there was also a high percentage positive in the sera of some bird species for SIN virus (TABLE 5). A mean of 55% human sera were positive for WNV after the epidemic and 16% were positive to SIN virus. The outbreak covered 2500 km^2, from the Orange river in the north, Laingsburg to the south, Beaufort West to the east and the Atlantic seaboard to the west. It is thought that two key factors favoring the occurrence of this epidemic were the unusual rains, which led to a greatly increased *Cx. univittatus* population density, and the higher summer temperatures in the Karoo, which enhanced viral replication in the vector.

TABLE 5. Percentage of bird sera, collected from various species in Upington after the Karoo epidemic in 1974, reacting in hemagglutination inhibition and/or neutralization tests with West Nile virus

Species	No. sera tested	Percent sera positive
Turdus olivaceus (olive thrush)	24	92
Streptopelia senegalensis (laughing dove)	72	86
Streptopelia capicola (turtle dove)	2	100
Pycnostictus nigricans (red-eyed bulbul)	9	22
Passer domesticus (house sparrow)	48	50
Euplectes orix (red bishop)	153	40
Quela quela (red-billed quelea)	5	60
Ploceus velatus (masked weaver)	9	33
Total/Mean	322	53

TABLE 6. Isolations of Sindbis and West Nile viruses from *Culex univittatus* collected at four Witwatersrand (Gauteng) localities in February–March 1984

Locality	No. *Cx. univittatus* tested	No. pools	Sindbis		West Nile	
			No. isolations	MIR (IR)	No. isolations	MIR (IR)
Germiston	1980	79	5	2.5 (2.5)	19	9.6 (10.9)
Rietfontein	817	33	2	2.4 (2.5)	3	3.7 (3.8)
Modderfontein	558	23	3	5.4 (5.6)	1	1.8 (1.6)
Florida/Rietfontein	22	1	—		1	—

The 1983–1984 epizootic of WN and SIN viruses occurred in the Witwatersrand-Pretoria (Gauteng) area of the Highveld.[11] The spring and early summer experienced unusually good rains (October to December). This was followed by higher than average temperatures in January to March, which again are thought to have increased the transmission of the virus by *Cx. univittatus*. The number of *Cx. univitattus* collected at four localities in the Witwatersrand, the number of isolations of both WN and SIN viruses, and the field mosquito infection rates are shown in TABLE 6. The infection rates of both WN and SIN viruses were higher than usual in the *Cx. univittatus* populations, indicating that an epizootic of both viruses had occurred. However, the number of human infections was much higher in the case of SIN virus, reaching epidemic level, while the increase in WNV cases over the previous season was only slight. It is unclear why the transfer from the feral cycle to humans occurred to such a limited extent with WNV.

There have only been four human cases of WNV in South Africa in which the usual mild illness has developed into a more serious condition. In two cases there has been renal failure associated with the virus. The first of these was a 55-year-old man who tested with an HI titer of 1:1280 and a positive IgM by ELISA.[24] The second was a 33-year-old man from whose serum the virus was isolated, but this patient had a three-year history of brucellosis.[25] The remaining two cases, also males, were a 60 year old who had meningoencephalitis, an HI titer of 1:1280 and positive IgM but who recovered[24] and a 28 year old who died from liver failure. WNV was isolated from a liver biopsy from the latter patient, who has been the only fatality in South Africa associated with the virus.[25]

INFECTION IN MAMMALS

Serological surveys undertaken on the inland plateau have shown the presence of neutralizing antibodies in cattle and to a lesser degree in sheep and horses.[3,4] These hosts are all considered to be incidental ones and the mammals concerned blind alleys in the transmission cycle. Experimental viremia studies have concurred with this view, since after inoculation with virus calves and goats had no detectable viremia.[5] However, if pregnant ewes are inoculated their lambs may have developmental abnormalities including hydranencephaly.[26] *Otomys* rodents showed no detectable viremia,[5] and of six other rodent species inoculated with the virus, only *Aethomys*

circulated virus with a maximum titer of 3.2 $\log_{10}LD_{50}$/mL.[27] Blackburn *et al.* inoculated three dogs with WNV, and one developed a slight viremia with a maximum of 2.8 logs/mL.[28] At this level of viremia some of the *Cx. univittatus* mosquitoes could become infected, but the efficiency of their subsequent transmission to feral birds would be limited after such a low infecting dose. Hence dogs might be able to feed back a small amount of virus into the enzootic mosquito-avian cycle.

GENETIC RELATEDNESS OF WNV ISOLATES

The genetic relatedness of South African WNV isolates with isolates from other countries has been investigated by the comparison of nucleotide sequences determined for each isolate. This study showed two lineages as follows. The first comprises 17 isolates which originate from Europe (3), Israel (2), New York (1), India (1), North Africa (3), The Central African Republic (CAR) (2), Kenya (1), Senegal (1), Ivory Coast (1), and Australia (Kunjin virus). The second lineage comprises 35 isolates from South Africa (25), Mozambique (1), Botswana (1), Namibia (1), Madagascar (3), Uganda (1), Senegal (1), Kenya (1), and CAR (1). The sequence homology of the South African isolates was 86.3%–100%, which indicates that the virus is remarkably constant in this country. This in turn may indicate the infrequency with which new strains of the virus are being imported into the country in infected migrant birds. Furthermore this may have a bearing on the mechanism which is responsible for survival of the virus through the relatively mild winter period on the inland plateau. This mechanism is probably the continuation of transmission between endemic birds and *Cx. univittatus* at a very low level, perhaps at only certain foci on the plateau. A minimum of genetic variability would be expected in South Africa if the virus overwinters here and if the enzootic cycle does not depend upon annual reintroduction of the virus by migrant birds.

CONCLUSION

The studies indicated that WNV is widespread in South Africa, where it is the cause of one of the most common arbovirus infections in humans. Although most active on the inland plateau, where it uses a highly efficient mosquito vector, it has adapted to diverse climates and is active on the KZN coast as well as in other southern African countries. The virus uses a variety of avian species as vertebrate hosts, which presumably provide it with the means to move from locality to locality, perhaps even from region to region, although there has been no evidence to suggest that birds reintroduce the virus into South Africa each year onto the South African plateau. The remarkable genetic constancy of South African isolates of the virus supports this view. Humans, livestock, and other mammals are all blind alleys in the transmission cycle except perhaps dogs. There is no evidence of a real amplifying nonavian host that would promote human infection, nor is there a likelihood of an alternative more anthropophilic and equally efficient vector superseding *Cx. univittatus* on the plateau. Owing to these factors, the pattern of endemic human infection with intermittent epidemics is expected to continue.

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