ILLNESS CAUSED BY SINDBIS AND WEST NILE VIRUSES IN SOUTH AFRICA*

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Antibody surveys on sera from persons resident in different parts of South Africa have revealed the wide distribution of Sindbis and West Nile viruses in this country and indicated that man is fairly frequently infected. These infections are especially prevalent in the highveld region of the Transvaal and Orange Free State and along the Orange River, where high temperatures and irrigation have apparently created favourable conditions for the vector.[1-5] The presence of these viruses in South Africa has also been demonstrated by their isolation from mosquitoes and wild birds collected in northern Natal and on the highveld;[6-8] and West Nile virus has been isolated from a sick human being in northern Natal.[6]

Despite these findings, little definite evidence has hitherto been obtained to indicate the extent to which Sindbis and West Nile viruses are a cause of illness in man in South Africa. Furthermore, although Sindbis virus has been known since 1952 and has been isolated in several parts of the world from birds and mosquitoes, it was only in 1961 that it was recovered for the first time from man when its isolation from blood specimens taken from 5 ill Africans in Uganda was reported.[9] The signs and symptoms shown by these patients included fever, headache, malaise, jaundice, widespread body pains and pain in the chest and joints.[10]

It was thus of considerable interest when, in January 1963, Sindbis virus was isolated from the skin lesions of a person ill in Johannesburg.[11] This patient showed fever, malaise, pains in the joints and tendons, a maculopapular rash over the trunk and limbs, and vesicles on the fingers and toes. Subsequently, further cases with somewhat similar clinical features were brought to our notice at several localities in the Transvaal and Orange Free State from March to April 1963. Although no virus was isolated from these patients, paired sera, collected from the same individuals during the acute and convalescent stages of the illness, were available from 16 patients, thus enabling a diagnosis of Sindbis or West Nile infection to be made on serological evidence. This report describes the clinical and serological findings in these patients, as well as in an additional 5, from whom only convalescent-phase sera were available.

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METHODS AND MATERIALS

The methods used in the haemagglutination-inhibition (HI) test are those described by Clarke and Casals,[12] in which serial, two-fold dilutions of serum were tested against 8 units of viral antigen and the results expressed as the reciprocal of the highest serum dilution, causing inhibition. The lowest serum dilution tested was 1/20.

In the neutralization (N) test, undiluted serum was mixed with an estimated 100 LD_{50} of virus and, after incubation for 1 hour at 37°C, the mixture was inoculated intracerebrally into 6 mice. Mouse survival ratios of 6/6, 5/6, 4/5 or 4/4 were recorded as positive, provided the virus dose was at least 50 LD_{50}, as shown by titration at the time of the test.

In the complement-fixation (CF) test, serum dilutions of 1/4, 1/8, 1/16 and 1/32 were tested against approximately 8 units of antigen. The antigens were prepared by acetone-ether or sucrose-acetone extraction of infective infant-mouse brain.[12]

Following our usual procedure, sera were initially screened in an HI test with antigens from the following arboviruses:

Group A: Sindbis, chikungunya, Middelburg; Group B: H 336, Wesselsbron, West Nile, Spondweni (selected sera only); Bunyamwera group: Bunyamwera, Germiston; Ungrouped: Rift Valley fever.

Depending upon the outcome of this test, sera were then tested with the N and CF tests. In some instances these tests could not be done on account of lack of serum. Since Ndumu virus, a member of Group A, does not readily produce a satisfactory haemagglutinin, certain sera positive for Sindbis virus antibody were tested against Ndumu virus with the N test only.

LABORATORY RESULTS

Virus Isolation

Attempts at isolation of virus were made from 11 cases of Sindbis and from 4 cases of West Nile infection as indicated in Tables I and II. The specimens used included blood (14 cases), throat swabs (5), skin lesions (7), and stool (3). These were tested in 1 - 2 days-old white mice, and most specimens were also tested in vervet monkey kidney tissue cultures. None of the specimens has yielded virus, although it is intended to repeat some of these attempts.

It will be seen from Table I that attempts to isolate virus from Sindbis cases were made in 6 cases within 4 days of onset of the illness, and in 3 of these Sindbis virus HI antibody was absent in the blood at the time the specimens were collected. A further case in which time of onset was uncertain was also negative for antibody at the time the specimens were collected for virus isolation. An attempt to isolate the virus was made in one West Nile case within 4 days of onset and at a time when the blood was free from West Nile antibody.

Failure to isolate the virus in these cases was probably due to low concentration or the absence of virus at the time of collection of the specimens, or to loss of viability during conveyance and storage of the specimens, since the host systems used possess a high degree of susceptibility to both viruses.

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TABLE I. RESULTS OF ANTIBODY TESTS ON SERA FROM PERSONS SUSPECTED OF HAVING BEEN INFECTED WITH SINDBIS VIRUS

Case No.	Locality	Patient	Days after onset blood collected	Group A viruses							Group B virus	
				Sindbis			Chikungunya		Middelburg	Ndumu	West Nile	
				HI	N	CF	HI	N	HI	N	HI	N
1	Johannesburg	M.L.*	5	N	N	—	N	—	N	N	N	—
		,,	27	1,280	P	—	N	—	N	—	N	—
2	Vereeniging	J.D.*	3	80	N	N	N	—	N	—	>1,280	P
		,,	12	>1,280	P	16	N	—	N	N	>1,280	P
3	,,	L.P.*	6	80	N	N	N	—	N	—	N	—
		,,	15	>1,280	P	N	N	—	N	N	N	—
4	,,	M.W.*	7	1,280	P	N	N	—	N	—	N	—
		,,	16	2,560	P	8	—	—	N	N	N	—
5	,,	M.B.*	2	N	—	—	N	—	N	—	80	P
		,,	16	320	P	8	20	—	N	—	20	P
6	,,	L.B.*	2	N	—	—	N	—	N	—	N	—
		,,	15	640	—	—	N	—	N	—	N	—
7	,,	M.E.*	2	80	N	N	N	—	N	—	N	—
		,,	14	2,560	P	N	N	—	N	N	N	—
8	Vanderbijlpark	J.H.*	4	20	N	N	N	—	N	—	1,280	P
		,,	31	640	P	4	N	—	N	—	1,280	P
9	,,	A.W.*	?	N	—	—	N	—	N	—	N	—
		,,	>18	1,280	P	N	N	—	N	N	N	—
10	,,	M.K.	10	80	N	N	N	—	N	—	N	—
		,,	39	640	P	32	N	—	N	N	N	—
11	,,	R.B.*	3	N	N	N	N	—	N	—	N	N
		,,	20	320	P	8	N	—	N	—	40	N
12	Carletonville	V.L.	6	320	P	N	N	—	N	N	N	N
		,,	20	1,280	P	8	80	N	20	—	40	N
13	Welkom	C.B.*	5	N	—	—	N	—	N	—	—	—
		,,	43	640	P	4	N	—	N	N	N	N
14	,,	S.M.	2	N	—	—	N	—	N	—	40	—
		,,	20	640	P	N	N	—	N	N	N	—

*Cases on which virus isolation was attempted. N = negative. — = not tested.

Serological Findings

(*a*) *Sindbis infections*. Paired sera from 14 patients showed a diagnostic rise in titre against Sindbis virus. The results of the antibody tests on these sera with West Nile and 4 Group A viruses are shown in Table I. The results against other viruses tested are excluded, since these were either negative or due to obvious immunological overlap with West Nile virus.

In all cases HI antibody against Sindbis virus either appeared or rose during convalescence. The results showed a high degree of specificity in that only cases 5 and 12 showed HI antibody against the other A viruses included in this test, and this antibody was of much lower titre than that against Sindbis.

The 13 convalescent sera tested with the N test against Sindbis virus were all positive, and there was conversion from negative to positive antibody status in 7 of the 9 cases from which paired sera were subjected to this test.

The CF test was carried out on 12 convalescent sera, and 8 of these were positive. In 6 cases there was a conversion from negative to positive CF antibody status during convalescence.

HI antibody against West Nile virus was observed in 6 cases. In 3 of these N antibody was present, and HI antibody was present at approximately the same levels in the acute and convalescent phases. It is therefore concluded that in these 3 cases the presence of antibody to West Nile virus was the result of a previous infection. In the remaining 3 cases the HI antibody was at a low level and 2 of them were negative to West Nile virus with the N tests.

(*b*) *West Nile infections*. Sera were obtained from 7 cases which were negative for HI antibody against Sindbis and the other Group A viruses and in which HI antibodies against West Nile virus were higher than against the other Group B viruses tested. The results on the sera are shown in Table II. Results with Spondweni virus are omitted since these were either negative or of low titre and obviously due to immunological overlap. Significantly, sera with HI antibody against H 336 and Wesselsbron viruses were negative for N antibody against these viruses, and with one exception (No. 16) were positive for N antibody against West Nile virus. Although the convalescent serum from case 16 was recorded as negative for N antibody against West Nile virus, 2 of the 6 mice inoculated in this test survived, indicating the presence of low titre N antibody. Paired sera were available from only 2 patients, but both showed a rise in West Nile HI antibody and one also showed a rise in CF antibody.

CLINICAL FEATURES

(*a*) *Sindbis*

A full description of the case encountered in January, has already been given.[11] While this case was unusually

TABLE II. RESULTS OF ANTIBODY TESTS ON SERA FROM PERSONS SUSPECTED OF HAVING BEEN INFECTED WITH WEST NILE VIRUS

Case No.	Locality	Patient	Days after onset blood collected	Group A virus Sindbis	Group B viruses								
					West Nile			H336			Wesselsbron		
				HI	HI	N	CF	HI	N	CF	HI	N	CF
15	Vereeniging	D.C.*	13	N	80	P	—	N	—	—	80	N	—
		,,	22	N	1,280	P	—	N	—	—	320	N	8
16	Middelburg	J.B.*	4	N	N	N	N	N	N	—	N	—	—
		,,	21	N	320	N	32	40	N	—	N	—	—
17	Welkom	T.H.*	10	N	320	P	32	80	N	—	160	N	N
18	,,	S.H.*	21	N	40	P	4	40	N	N	20	N	—
19	,,	S.P.	10	N	320	P	32	40	N	N	160	N	N
20	,,	V.K.	28	N	640	P	32	80	N	16	320	N	8
21	Vanderbijlpark	R.A.	14	N	320	P	32	40	N	N	160	N	—

*Cases on which virus isolation was attempted. N = negative — = not tested.

severe, essentially the same clinical features were observed as in the others. The patients were all adults with ages ranging from 18 to 56 years.

Incubation period. Since most of the patients had been exposed to mosquitoes over a prolonged period, it was not possible to determine the incubation time of the infection.

Prodromal symptoms. The onset of illness was usually marked by the sudden appearance of the rash; but 4 patients experienced, for 1 or 2 days before the rash, symptoms which included lassitude, headache, joint pains and generalized body aches.

Exanthem. The rash involved chiefly the trunk and limbs, being most profuse over the buttocks and legs. It affected the palms and soles; but the head was usually, though not invariably, spared. Lesions occurred in crops for the first few days and lasted up to 10 days, leaving brown stains.

The spots remained discrete, appearing first as macules but soon becoming papular. The papules were usually about 3 mm. in diameter, but much variation in size was noted. Vesicles could be discerned in nearly half the cases, occurring chiefly on the hands and feet; but removal of the skin over papules elsewhere on the body frequently revealed a small amount of clear fluid. The lesions tended to be pruritic, and were occasionally very irritating, especially on the extremities.

Enanthem. Evidence of lesions in the oro-pharynx was usually lacking, although small ulcers irregularly distributed in the mouth or pharynx were seen in 4 cases. Some patients complained of sore throat, and mild inflammation of the pharynx was occasionally observed.

Other clinical features. Low fever not much exceeding 100°F and lasting only for the first few days was noted in a number of cases. Lassitude and fatigue on slight exertion were early symptoms which tended to persist through convalescence. Rigors were not observed, but alternating feelings of heat and cold were experienced by a number of patients.

Muscle tenderness and deep aches in the limbs were occasionally encountered. Pains in the small joints of the hands and feet were almost invariably present, and larger joints were frequently affected. There was a tendency for the extremities to become swollen. Pain in the extensor tendons on the dorsum of the hand and in the tendo calcaneus (Achillis) was occasionally present.

Several patients had periocular pain, but photophobia was not common and conjunctivitis was not seen. Headache was frequently present, but it was usually mild and there was no clinical evidence of involvement of the central nervous system. A few patients complained of paraesthesiae such as pricking or tingling sensations, particularly in the hands; and shooting pains in the limbs were noted in 2 cases.

Lymphadenopathy was usually absent, although enlargement of inguinal, occipital and posterior cervical nodes was encountered. In one case the spleen could be felt. Right subcostal tenderness was experienced by several patients, but the liver was not palpable. Anorexia was usual, but nausea and vomiting uncommon. Bowel function was not markedly disturbed.

The respiratory system did not appear to be involved. In one case extrasystoles were a notable feature of the acute illness, and during convalescence this patient experienced an anginal attack with pain radiating from the chest down the left arm, but there was no electrocardiographic evidence of myocardial damage.

In most cases the symptoms disappeared within 10 days, but a few patients experienced fatigue and tendon pains for several weeks.

Laboratory investigations. Blood counts performed during the acute phase on 10 patients were within normal limits, but the erythrocyte sedimentation rate, as measured by the Wintrobe method, was markedly raised in 5 cases (13 - 31 mm. in one hour). Liver-function tests were carried out in 2 cases, but were normal, except for positive colloidal red reactions.

Pathological changes. The histological changes occurring in suckling mice inoculated with virus recovered from the first case seen have already been described.[11] With due reservations concerning extrapolation from mouse to man, it may be postulated that the joint pains and swelling of extremities are probably due to necrobiosis and oedema in subcutaneous, periarticular and tendinous tissues, rather than to pathology within the joint cavities.

(*b*) *West Nile*

Features common to both Sindbis and West Nile infections were lassitude, generalized body pains, headache, joint pains, and a maculo-papular rash; and it was not possible to distinguish between the two infections on clinical grounds.