ISOLATION OF WEST NILE VIRUS FROM A NATURALLY INFECTED HUMAN BEING AND FROM A BIRD, *SYLVIETTA RUFESCENS* (VIEILLOT)*

R. H. KOKERNOT, D.V.M., M.D., M.P.H., *Rockefeller Foundation*, and
B. M. MCINTOSH, D.V.SC. (PRET.), *Poliomyelitis Research Foundation, South African Institute for Medical Research*
From the Arthropod-borne Virus Research Unit†

In a previous paper in this *Journal* Weinbren[1] presented a review of the literature concerning the clinical aspects of infection with West Nile virus. The same publication reported the results of a serological survey for the presence of West Nile neutralizing antibodies in sera collected from various vertebrate hosts, including man, which indicated that the virus was active in the Union of South Africa. The present paper describes the isolation of West Nile virus from the serum of a naturally infected human being and from the tissues of a wild-caught bird, *Sylvietta rufescens* (Vieillot) commonly called a crombec warbler. Both specimens were collected in Tongaland, Union of South Africa, in April-May 1958, as a part of the regular field activities of this Unit A brief account of the field programme since its initiation in 1954 is given in the introduction of a previous paper (Kokernot *et al.*[2]).

MATERIALS AND METHODS

The methods used in the documentation and processing of huma sera for attempted virus isolation have been reported elsewhere.[2] Briefly it may be stated that the acute and convalescent sera from the case cited here were proved to have been derived from the same donor by a comparison of two sets of fingerprints‡ taken at the different times when the blood specimens were obtained.

Experience has shown that a significant number of individuals engaged in various aspects of the field programme have acquired clinical illness during the work periods. The aetiology of such cases has been confirmed by virus isolation and demonstrated by serological studies to have been due to infection with some one of the arthropod-borne viruses.[2-4] Careful surveillance of field personnel is now maintained and includes initial fingerprinting for identification, obtaining of pre- and post-employment blood specimens (the latter to detect possible inapparent infections), and taking of temperatures orally, morning and afternoon.

The crombec warbler§ was captured in a Japanese mist net and taken to the field laboratory, where it was anaesthetized. The spleen and a sample of liver were removed aseptically and stored together in a sterile glass container on CO_2 ice. At the Johannesburg laboratory the tissues were suspended in a volume of bovine plasma-albumin estimated to give a 10% suspension and then centrifuged. After treatment with penicillin and streptomycin the supernate was inoculated intracerebrally into newborn and adult mice.

The method used in identifying the virus strains isolated from the human serum and the tissues of the bird was similar to that described by Smithburn *et al.*[3] All neutralization tests, however, were done with sera previously heat-inactivated in a 60°C water bath for 30 minutes. An equal volume of fresh normal rhesus monkey serum (obtained 1-2 hours previously) was added to the inactivated sera just before the setting up of the test proper. This step was taken to assure the presence of the heat-labile accessory substance present in normal serum[5,6] which augments the activity of neutralizing antibodies to West Nile virus. Casals *et al.*[7] have described the methods utilized in doing complement-fixation (CF) tests. Haemagglutination-inhibition (HI) tests were done according to methods described by Clarke and Casals.[8]

The strain of West Nile virus used in these studies was the original isolate described by Smithburn *et al.*[9] in its 33rd mouse-brain passage.

RESULTS

Isolation and identification of virus strain from the human being

An African male (J.M.), aged 26, was employed during the period 23 April – 1 May 1958, by the field team at Ndumu to assist in the capture of wild birds with the aid of Japanese mist nets. Each morning he arrived at the nets shortly after sunrise, and remained there until sunset. His task was to remove from the nets birds that were caught, and in the intervening periods he stayed near by in the grass, obscured from birds under clumps of bush. On 28 April, after 4 days of such activity, he reported at about 18.00 hours to the doctor present at the station with vague complaints of feeling ill and having a headache. Although in no obvious distress, he appeared ill and had an oral temperature of 101·2°F. A venipuncture was made and about 15·0 ml. of blood taken. The specimen was designated H 442. The serum was separated the following morning and stored in a lusteroid tube on CO_2 ice. The day after onset the individual was afebrile and able to resume his normal duties.

On 28 May, after it had been stored for 3 days on CO_2 ice and 27 days in a frozen state in a mechanical deep-freeze (−20°C), the specimen H 442 was inoculated intracerebrally into 2 litters of one-day-old mice. After a 4-day incubation period, 3 infants from one litter were missing (presumably eaten by their mother) and all others of both litters were sick. Brains of the sick mice were harvested for passage, pathological examination and storage. Passage was successful and a serially transmissible agent was established. The 2nd-passage infected mouse-brain material was pathogenic for adult mice inoculated intracerebrally. The infective agent was easily filterable through a Seitz pad.

In the attempt to re-isolate the agent from H 442 serum, made on 2 June 1958, 3 different dilutions were made and each was inoculated intracerebrally into 2 litters of newborn

* The studies and observations on which this paper is based were financed jointly by the South African Institute for Medical Research, the Poliomyelitis Research Foundation, the South African Council for Scientific and Industrial Research and the Rockefeller Foundation, and were conducted with the collaboration of the Union Health Department.

† P.O. Box 1038, Johannesburg.

‡ We acknowledge with thanks the examination of and opinion on these prints by a member of the South African Police force, Lt. G. Retief, Officer-in-Charge, Local Finger Print Office, Johannesburg.

§ The authors acknowledge with thanks the identification of this bird by Mr. R. Liversidge, Ornithologist on the staff of the Port Elizabeth Museum.

mice. Eleven of the 12 infants inoculated with a $10^{-0.3}$ serum dilution were either missing, dead or sick between the 5th and 7th days following inoculation. In 2 litters inoculated with a $10^{-1.3}$ serum dilution, 2 mice were missing on days 4 and 7, while the rest remained well for 21 days. In the litters inoculated with a $10^{-2.3}$ dilution, only one mouse died, on day 9.

An intracerebral adult-mouse neutralization test with H 442 virus was done against a serum obtained from J.M. 4 days before onset of illness as well as his acute- and convalescent-phase sera. The test showed a significant rise in neutralizing antibodies during convalescence of the donor and thus confirmed the fact that the virus had originated with him. The results of this test are shown in Table I.

TABLE I. RESULTS OF AN INTRACEREBRAL ADULT-MOUSE NEUTRALIZATION TEST WITH H 442 VIRUS AGAINST 3 SERA OBTAINED FROM THE PERSON FROM WHOM THE VIRUS WAS ISOLATED

Serum with date of bleeding	*Dilution of virus*	*Fate of mice*		*Calculated titre of virus 1 to:*
		Died	*Survived*	
	10^{-6}	6	0	
Routine pre-employment 24.4.58	10^{-7}	5	0	
	10^{-8}	3	3	130,000,000
	10^{-9}	1	5	
	10^{-10}	0	6	
	10^{-6}	6	0	
Acute-phase 28.4.58	10^{-7}	6	0	
	10^{-8}	4	2	178,000,000
	10^{-9}	0	6	
	10^{-10}	0	6	
Convalescent-phase 4.6.58	10^{-4}	5	1	
	10^{-5}	1	5	31,000
	10^{-6}	0	6	

A rise in HI antibodies to the homologous virus as well as to West Nile virus and AN 2842 (designation given virus strain isolated from the crombec) was also demonstrated in a test with the donor's one acute- and two convalescent-phase sera. In this test, one of the later serum specimens was taken over 7 months after onset of illness and a significant drop in HI antibodies was noted. These results are shown in Table II.

TABLE II. RESULTS OF HI TEST WITH H 442 ANTIGEN SHOWING ORIGIN FROM A HUMAN BEING AND RELATIONSHIP TO WEST NILE AND AN 2842 VIRUSES

Serum†	*Antigen**		
	West Nile	*H 442*	*AN 2842*
Acute			
28.4.58	10	10	10
Convalescent			
4.6.58	640	640	640
9.12.58	80	40	80

* Eight units antigen used.

† Results expressed as reciprocal of serum dilution giving complete HI.

An intracerebral adult-mouse cross-neutralization test with West Nile and H 442 viruses against their respective pre- and post-inoculation guinea-pig sera indicated a significant reciprocal cross-relationship between the two viral strains. These results, incorporated in Table III, thus identify H 442 virus as a strain identical with or closely related to West Nile virus.

TABLE III. RESULTS OF CROSS-NEUTRALIZATION TESTS WITH WEST NILE, H 442 AND AN 2842 VIRUS STRAINS AGAINST THEIR RESPECTIVE PRE- AND POST-INOCULATION GUINEA-PIG SERA

Serum	*Virus strain, logs neutralized*		
	West Nile	*H 442*	*AN 2842*
West Nile	3·3	5·0	2·6
H 442	4·0	4·7	3·0
AN 2842	4·2	4·0	2·5

Isolation and identification of virus strain from tissues of a crombec warbler—Sylvietta rufescens (*Vieillot*)

This virus strain was isolated from a suspension of pooled spleen and liver tissue taken from a crombec caught in one of the Japanese mist nets on 7 May 1958. It is significant that one week before the capture of the crombec the donor of H 442 virus had been assigned to the net detail for an 8-day period and that in the interval the position of the nets remained unchanged.

The pooled tissues from the crombec were kept on CO_2 ice until processed in Johannesburg on 12 May. In the litter of 6 newborn mice inoculated intracerebrally with 0·03 ml. of the suspension, one was moribund on the 7th post-inoculation day and the remaining 5 remained well for 21 days. The group of 6 adult mice similarly inoculated remained well for the same period. A brain suspension prepared from the moribund infant mouse and inoculated intracerebrally into newborn and adult mice caused illness in both after a 4-day incubation period. The infective agent in 2nd-passage brain material was readily filterable through a Seitz pad. The agent was designated AN 2842 virus strain to correspond with the accession number given to the tissues obtained from the crombec.

A crude HA antigen was prepared from AN 2842 virus-infected infant-mouse brains. The results of a screening test with this antigen indicated that AN 2842 was a group-B agent related more closely to West Nile virus than to any of the other agents of the group represented in the test.

In a screening CF test with AN 2842 antigen and 5 group-B-virus mouse hyperimmune sera, complement was fixed only in the presence of West Nile immune serum.

The results of cross-neutralization test with the human isolate (H 442), the bird isolate (AN 2842), and the prototype strain of West Nile virus, against their respective pre- and post-inoculation guinea-pig sera are presented in Table III. The results of these tests indicate the relationship of the virus strain isolated from the human being to the strain isolated from the crombec. Furthermore, they show that both strains are identical with or closely related to West Nile Virus.

DISCUSSION

The isolation of West Nile virus from a naturally infected human being in the Union of South Africa should serve to alert the medical profession to a consideration of this agent in the differential diagnosis of cases of pyrexia. The fact that it has been isolated from a bird as well tends to confirm the results of the South African serological survey presented by Weinbren,[1] which indicated that neutralizing antibodies due to West Nile virus occur in these two vertebrate hosts.

The aetiology of West Nile virus infection in man can be determined with certainty only if the virus is isolated or if the convalescent serum shows a significant rise in antibody titre over the acute-phase serum. In attempts at virus

isolation, the blood specimen should be obtained in the early stage of illness and during the period of pyrexia, since the period of viraemia in arthropod-borne virus diseases is short. If the diagnosis is to be made by serological study, it is necessary to have paired sera, the first taken as early as possible in the course of the illness and the second during the 2nd or 3rd week of convalescence. Both specimens should be refrigerated without actual freezing and handled in such manner as to prevent bacterial contamination and minimize haemolysis.

SUMMARY

1. The isolation of a strain of West Nile virus from the blood of a naturally infected human being is described.

2. Following infection there was a significant increase in titre of both neutralizing and haemagglutination-inhibiting antibodies in tests controlled by the inactivated acute serum from which the virus was isolated.

3. The isolation of a strain of West Nile virus from pooled tissues (liver and spleen) of a wild-caught crombec warbler is also described.

REFERENCES

1. Weinbren, M. P. (1955): S. Afr. Med. J., **29**, 1092.
2. Kokernot, R. H., Smithburn, K. C., de Meillon, B. and Paterson, H. E. (1958): Amer. J. Trop. Med. Hyg., **7**, 579.
3. Smithburn, K. C., Kokernot, R. H., Weinbren, M. P. and de Meillon, B. (1957): S. Afr. J. Med. Sci., **22**, 113.
4. Heymann, C. S., Kokernot, R. H. and de Meillon, B. (1958): S. Afr. Med. J., **32**, 543.
5. Morgan, I. M. (1945): J. Immunol., **50**, 359.
6. Whitman, L. (1947): *Ibid.*, **56**, 97.
7. Casals, J., Olitsky, P. K. and Anslow, R. O. (1951): J. Exp. Med., **94**, 123.
8. Clarke, D. H. and Casals, J. (1958): Amer. J. Trop. Med. Hyg., **7**, 561.
9. Smithburn, K. C., Hughes, T. P., Burke, A. W. and Paul, J. H. (1940): Amer. J. Trop. Med., **20**, 471.