WESSELSBRON VIRUS INFECTIONS IN MAN*

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Wesselsbron virus was first described by Weiss *et al.*[1] as the causative agent of an outbreak of illness in sheep, often associated with abortion, which occurred on a farm in the Wesselsbron area of the Orange Free State during the late summer of 1954–55. The virus was isolated from infected tissues of a lamb that had died 2 days before.

Wesselsbron virus was first isolated from a naturally infected human being, and from mosquitoes (Smithburn *et al.*[2]), about a month after the isolation of the above-mentioned prototype strain. The agent is an arthropod-borne virus (Muspratt *et al.*[3]) which attacks both sheep and man in nature and causes an infection with clinical manifestations.

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The aim of this paper is to record 2 further cases of natural infection by Wesselsbron virus in human beings.

Materials and Methods

The procedures and techniques that were employed in the laboratory for the diagnosis of Wesselsbron virus infection in the two human cases were essentially the same as those described by Smithburn *et al.*[2]

Clinical observations were limited to a history and physical examination without clinical pathological investigations.

RESULTS

The two cases of Wesselsbron-virus infection to be described in this report were in personnel who undertook field investigations during an outbreak of illness among sheep in the Middelburg district of the Cape Province in the late summer of 1957. The Director of Veterinary Services had stated that Wesselsbron virus had been isolated from the tissues of a sheep which died in the area and these two individuals, one from the South African Institute for Medical Research and the other from the Medical Ecology Centre of the Union Health Department, undertook to study the arthropod vector or vectors active in the epizootic. An account of the disease outbreak and a description of the topography of the Middelburg region has been published in an article reporting the isolation of an agent other than Wesselsbron virus which was made following these field investigations. This agent has been designated Middelburg virus (Kokernot *et al.*[4]).

After their arrival in the area these two individuals (de M. and P.) proceeded to catch mosquitoes, from which Wesselsbron and Middelburg viruses were subsequently isolated (Kokernot *et al.*[4]). During these operations both men were frequently bitten by mosquitoes. Two days after his arrival de M. performed a post-mortem on a lamb that had died after a short illness and a second autopsy on a ewe that had died in convulsions.

On the morning of the 5th day after his arrival in the Middelburg area de M. awoke feeling stiff and sore, especially at sites of old traumata, which included knee, ankle, left shoulder and back. He had a severe frontal headache with a feeling of pressure behind the eyes. The headache was not relieved by taking 2 aspirin tablets. His temperature at the time was 99°F; there was a further rise to 101°F by mid-afternoon. In the late afternoon, when his temperature was 100·5°F, a blood specimen was taken. During the day he was anorexic and only took fluids. By evening the muscular and joint pains had mostly gone and though he still had a headache it was less intense. The feeling of pressure behind the eyes continued. The next day the patient felt much better and although febrile in the early morning his temperature returned to normal except for a slight rise at midday. He was examined by a doctor that evening, who was unable to elicit any significant physical findings.

The blood specimen was kept on water ice for 3 days before the serum was separated and inoculated intracerebrally into newborn and adult mice for the purpose of attempted virus isolation. Of the 6 adult mice inoculated with the patient's serum, one was dead from a non-specific cause the day after inoculation and another on the 12th day. An attempt at brain passage of this second mouse was unsuccessful. The other 4 mice remained well. Inoculated newborn mice sickened on the 8th and 9th day and brain passages were made from them which led to the establishment of a transmissible agent in both newborn and adult mice. This agent was designated H 112, corresponding to the accession number given to the field specimen.

Sixteen days after initial inoculation of the patient's serum into mice a re-inoculation of the serum (which had been stored in a mechanical deep-freeze) into 6 newborn mice was done. All 6 mice either died or sickened between the 8th and 13th days. Re-isolation of the agent was accomplished.

In an adult-mouse intracerebral neutralization test with adapted H 112 virus against pre- and post-inoculation Wesselsbron-virus sheep sera, the latter neutralized approximately 5·9 logs of the virus. These results indicated that H 112 virus strain is identical with or closely related to Wesselsbron virus.

An intracerebral neutralization test in adult mice with H 112 virus was done against heat-inactivated acute- and convalescent-phase sera obtained from de M. The test showed a sharp rise in antibodies during convalescence of the donor and confirmed the origin of the virus from him. The results of this test are shown in Table I.

TABLE I. RESULTS OF INTRACEREBRAL ADULT-MOUSE NEUTRALIZATION TEST WITH H 112 VIRUS AGAINST THE ACUTE AND CONVALESCENT SERA OBTAINED FROM THE PERSON FROM WHOM THE VIRUS WAS ISOLATED

Serum with date of bleeding*	*Dilution of virus*	*Number of mice*		*Calculated titre of virus 1 to :*
		Died	*Survived*	
Acute-phase 7.4.57	10^{-4}	6	0	500,000
	10^{-5}	6	0	
	10^{-6}	1	5	
	10^{-7}	1	5	
	10^{-8}	0	6	
Convalescent-phase 24.4.57	10^{-2}	1	5	63 or less
	10^{-3}	2	4	
	10^{-4}	0	6	
	10^{-5}	0	6	

* Sera heat-inactivated for 30 minutes at 60°C before testing.

The second individual (P.) participated in the same activities as de M. with the exception that at no stage did he handle living or dead infected animals. He was, however, frequently bitten by mosquitoes while engaged in mosquito catching. The evening of the same day that de M. awoke feeling ill, i.e., the 5th day after their arrival in the Middelburg area, P. felt a sudden headache and pain in his back and left shoulder while working at a catching site. Shortly afterward his temperature was found to be 101·6°F. During the night he suffered rigors and perspired profusely. The following day his febrile reaction continued and he too complained of pain behind the eyes as well as generalized body pains. The patient experienced great discomfort in flexing his limbs owing to muscular pains. He also complained of a pain high up in his abdomen. His temperature at this stage was 98·8° and it was then that a blood specimen was taken for possible virus isolation*. The next day the patient's temperature was normal but he still complained of upper abdominal pain. He was examined by a doctor that morning and showed both hepatomegaly and splenomegaly, the liver being firm and

* The authors acknowledge the cooperation of Dr. I. H. Mathieson, deputizing for the District Surgeon, Middelburg, C.P., for obtaining this specimen.

tender. The convalescence of this individual was protracted in that he continued feeling discomfort in his abdomen although the liver and spleen were no longer palpable.

The blood specimen from P. was stored on water ice for 2 days. After this the serum was separated and inoculated intracerebrally into 2 litters of newborn and a group of 6 adult mice. There were no deaths or signs of illness in the inoculated mice during the 21-day period they were observed. A 1-in-10 dilution of the serum in bovine plasma-albumin was re-inoculated intracerebrally into a litter of mice with no evidence of pathogenic effects.

This acute-phase specimen of serum and one obtained from P. 42 days later were tested against H 112 virus in an intracerebral adult-mouse neutralization test. Each serum was heat-inactivated for 30 minutes at 60°C before the testing. The convalescent specimen neutralized 3·3 logs of H 112 virus in the test controlled by the acute-phase serum. Such an increase in neutralizing antibodies was taken as firm evidence that the aetiologic agent of illness in the second case (P.) was the same virus that was isolated from de M.

DISCUSSION

In the two cases of Wesselsbron-virus infection cited in this paper and those reported elsewhere,[1, 2] there have been no pathognomonic signs or symptoms elicited during the course of the illness. The total number of cases with the diagnosis confirmed by virus isolation or a significant immune response in convalescence has been small. The clinical spectrum may thus be broader than is indicated by these reports.

There are certain epidemiological factors that might assist the clinician in his attempt to determine the aetiology of such cases. Illness due to Wesselsbron-virus infection acquired under natural conditions would be expected to have a seasonal incidence. This period would be expected during the warmer months and especially if rainfall had been above normal. Both factors favour higher mosquito populations.

The mode of transmission might be direct contact with infected tissues or the bite of a vector mosquito. Wesselsbron virus has been isolated from several species of wild-caught mosquitoes collected in the Union of South Africa.[3, 5] In the laboratory two of these species have been shown to be capable of transmitting the virus by bite after a significant intrinsic incubation period (Muspratt *et al.*[3] and Kokernot *et al.*[6]).

There appears to be a rather widespread geographical distribution of Wesselsbron virus infection in man and domestic animals in southern Africa. The virus has been isolated in the Free State, in northern Natal and in the eastern part of the Cape Province. Serological surveys for the presence of neutralizing antibodies to this virus in the sera of indigenous human beings and domestic animals of northern Natal indicate a high incidence of immunity.[7] Weiss *et al.*[1] report the finding of antibodies in bovine sera collected at Knysna and also in the Rhodesias.

A third factor of epidemiological significance is the patient's occupation. This virus has been responsible for epizootics among the sheep population in South Africa. Therefore individuals engaged in sheep husbandry have a greater degree of exposure to infection with the agent, both in handling infected animals and in their exposure at work in the infected zone.

The aetiology of Wesselsbron virus infection in man can be determined with certainty only if the virus is isolated or if there is a significant rise in titre of antibody in a convalescent serum when compared with a specimen taken during the acute phase of the illness. To attempt virus isolation the blood specimen should be obtained in the early stage of illness and during the period of pyrexia, as the period of viraemia in arthropod-borne virus diseases is short. In the second case (P.) reported in this paper the blood was taken soon after the termination of pyrexia and virus isolation was unsuccessful. If the diagnosis is to be made by serological study it is necessary to have paired sera. The first should be 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. Two clinical cases of Wesselsbron-virus infection in man are described.

2. The diagnosis was confirmed by virus isolation in one case and by significant increase in titre of neutralizing antibodies in both cases.

3. Epidemiological factors are discussed which might assist the practitioner in making a clinical diagnosis.

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