FURTHER ISOLATIONS OF WESSELSBRON VIRUS FROM MOSQUITOES*

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The purpose of this paper is to report the isolations of strains of Wesselsbron virus from wild-caught mosquitoes which have not previously been known to be host to this virus. These mosquitoes, representing 3 different genera, were collected in 1957 in 2 different regions of the Union of South Africa. In the Middelburg district of the Cape Province mosquito collections were made in the course of an intensive investigation undertaken principally to identify the vector or vectors of an epizootic occurring in sheep. The background leading to these studies and an account of

* 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 and the Veterinary Division of the Department of Agriculture.

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some of the more important results have been previously reported.[1,2]

The Ingwavuma district (Tongaland) of northern Natal was the other locality of collection. A station located near Ndumu in Tongaland has been the base for periodic activities concerned with the study of arthropod-borne viruses since January 1956. The scope of this programme has been referred to by Kokernot *et al.*[3] The selection of this site for a long-term study area was based on the results of a virus research expedition made to Tongaland from 17 April to 13 May 1955.[4] During the course of those investigations Wesselsbron virus was isolated from a naturally infected human being and from *Aedes (Neomelaniconion)*† *circumluteolus* (Theobald).[5]

METHODS AND MATERIALS

The method of collecting mosquitoes in the eastern Cape Province and their subsequent handling until arrival in Johannesburg has been previously reported.[1] Similarly the methods regularly employed for the same purpose at the Ndumu field station have been reported.[3] In the former locality all catches were made with man as bait, while in the latter region most of the catches were made while mosquitoes were resting on vegetation.[6] The technique for processing mosquitoes for possible virus isolation has previously been described.[1]

Neutralization tests were done according to the method described by Smithburn,[7] except that serum-virus mixtures were incubated for 1 hour in a water-bath at 37°C before inoculation. In tests with infant mice, litters 0 - 2 days old were inoculated either intraperitoneally or intracerebrally with 0·03 ml. of the serum-virus mixture.

The method for preparing haemagglutinin from the alkaline aqueous suspension of virus-infected infant-mouse brains and the technique of HA titrations and haemagglutination-inhibition (HI) tests were essentially the same as those described by Clarke and Casals.[8] The principal modification concerned the preparation of goose erythrocytes.[9]

Complement-fixation (CF) tests were done according to the method described by Casals *et al.*,[10] with the exception that CF antigen was obtained from a crude alkaline aqueous suspension of titrated brain material. As noted above, the haemagglutinin was prepared in the same manner.

We are indebted to Dr. R. A. Alexander, Director of Veterinary Services, Onderstepoort, for pre- and post-inoculation Wesselsbron-immune sheep sera.

† Formerly this subgenus was referred to as *Banksinella*.

RESULTS

Isolation of Virus Strains

In Table I details are summarized concerning the origin from mosquitoes of 18 strains of virus. Included in this résumé are the mosquito species from which each virus strain was isolated, the number of mosquitoes in each pool, the AR (arthropod pool) number, and the place and date of the actual field collection. The fate of mice inoculated with a suspension prepared from each pool of mosquitoes is also summarized in this table in the form of mortality

TABLE I. RÉSUMÉ OF ISOLATION OF 18 STRAINS OF VIRUS FROM MOSQUITOES

Mosquitoes			*Collection*		*Date processed*	*Fate of mice inoculated with mosquito suspension*		
						Infants		*Adults*
Species	*AR No.*	*No. in pool*	*Place*	*Date*		*Mortality ratio***	*AST*	*Mortality ratio***
Aedes (Ochlerotatus) caballus	734	50	Middelburg, C.P.*	3.4.57	10.4.57	11/12	12·3	1/6
" " "	741	50	"	5.4.57	10.4.57	6/6	11·4	0/6
" " "	742	50	"	5.4.57	10.4.57	5/6	14·3	0/6
" " "	743	50	"	5.4.57	10.4.57	6/6	8·2	0/6
" " "	746	50	"	5.4.57	10.4.57	6/6	7·2	0/6
" " "	750	50	"	9.4.57	11.4.57	12/12	6·5	1/6
" " "	755	50	"	5.4.57	11.4.57	10/12	9·5	0/6
" " "	756	50	"	5.4.57	11.4.57	12/12	6·9	0/5
" " "	758	50	"	5.4.57	10.4.57	7/7	7·4	0/5
" " "	762	50	"	7.4.57	12.4.57	5/5	8·6	0/6
" " "	763	45	"	2.4.57	12.4.57	5/6	10·2	0/6
" " "	764	44	"	3.4.57	12.4.57	6/6	6·8	0/6
" " "	778	218	"	25.4.57	26.4.57	12/12	7·2	Not inoc.
Aedes (Neomelaniconion) spp. ‡	740	19	"	9.4.57	10.4.57	6/6	7·3	0/6
" " "	748	48	"	6 & 7.4.57	11.4.57	12/12	8·8	1/6
" " "	773	4	"	9.4.57	12.4.57	6/6	8·8	0/5
Mansonia (Mansonioides) uniformis	814	41	Ndumu, Natal	8.5.57	14.5.57	1/6	19·3	0/6
Culex (Culex) univittatus	926	106	" "	19.11.57	25.11.57	9/12	13·4	Not inoc.

AR = arthropod

AST = average survival time in days

* In the Middelburg District of Cape Province mosquitoes were collected on Crowboroughvlei Farm, Conway.

** Mortality ratio: denominator represents no. of mice inoculated and numerator no. of mice dying.

‡ These pools contained both *Aedes (Neomelaniconion) lineatopennis* (Ludlow) and a second species which is closely related to or identical with *Aedes (Neomelaniconion) albothorax* Theobald.

TABLE II. RESULTS OF SEROLOGICAL STUDIES WITH 18 VIRUS STRAINS WHICH INDICATE THAT THEY ARE CLOSELY RELATED TO OR IDENTICAL WITH WESSELSBRON VIRUS

Virus strain	*Type serological test*								
	Haemagglutination inhibition		*Complement fixation*				*Neutralization*		
	Antigen — method of preparation	*Results*	*Antigen — method of preparation*	*Results*			*Age of mice*	*Route of inoc.*	*Logs virus neutralized*
				Wesselsbron	*H336*	*Spondweni*			
AR 734	crude	Group B	crude	128/64	O	O	Adults	IC*	3·23
AR 741	,,	,,	,,	8/8	O	O	,,	,,	2·72
AR 742	,,	,,	,,	32/32	O	O	,,	,,	3·67
AR 743	,,	,,	,,	16/32	O	O	,,	,,	4·50
AR 746	,,	,,	,,	128/32	O	O	,,	,,	2·37
AR 750	ND	ND	ND	ND	ND	ND	,,	,,	2·80
AR 755	crude	Group B	crude	16/8	O	O	,,	,,	4·60
AR 756	ND	ND		ND	ND	ND	Newborn	IP	4·0
AR 758	crude	Group B	crude	32/16	O	O	Adults	IC	4·1
AR 762	,,	,,	,,	8/16	O	O	,,	,,	3·94
AR 763	,,	,,	ND	ND	ND	ND	,,	,,	5·0
AR 764	,,	,,	ND	ND	ND	ND	,,	,,	3·33
AR 778	ND	ND	ND	ND	ND	ND	Newborn	IC	4·25
AR 740	ND	ND	ND	ND	ND	ND	,,	IP	3·2
AR 748	ND	ND	ND	ND	ND	ND	Adults	IC	3·4
AR 773	crude	Group B	ND	ND	ND	ND	Adults	IC	5·0
AR 814	crude	Group B	crude	128/32	O	O	Adults	IC	3·3
AR 926	ND	ND	ND	ND	ND	ND	Newborn	IC	4·7

* Code for this and other abbreviations as follows: IC=intracerebral; IP=intraperitoneal; ND=not done.

ratios for newborn and adult mice. In the first group the average survival time has been calculated. This value is only an approximation since, in all cases, mice were sacrificed for further passage. However, only mice showing definite signs of illness were taken for this purpose.

The majority of strains were lethal for adult mice by intracerebral inoculation only after 2 passages in infant-mouse-brain. Following establishment, the average survival time in adult mice usually raged between 6 and 8 days, while in infant mice this period was usually 2 - 3 days less.

Four of the virus strains (each derived from a different one of the 4 mosquito species) were found to be filterable through asbestos (Ford's sterimat SB) pads. The suspensions of infected infant-mouse brain used for this purpose were at either the 1st (AR 750), 2nd (AR 814) or 3rd (AR 748 and AR 926) passage levels. The remaining 14 strains were not filtered. Their viral nature was assumed, because in each case the transmissible agent was readily established in newborn mice and the mouse brain suspensions used for this purpose were found to be bacteriologically sterile. Furthermore, histopathological examination of mice after intracerebral inoculation revealed lesions only in the brain and these were typical of viral encephalitis.

Identification of Virus Strains

Table II summarizes the results of immunological studies with the 18 viral strains. Three serological techniques were used in the identification of 9 strains, and 2 techniques for a further 3 strains. The mouse protection test was used for the identification of all of the strains and was the only method used for 6 of them. In 14 protection tests adult mice were inoculated intracerebrally and in 4 tests infant mice were inoculated either intraperitoneally (2 tests) or intracerebrally (2 tests). Neutralization indices ranged from 2·8 to 5 logs and the larger values were associated with high-titre virus preparation. This index for virus strains AR 748, AR 750 and AR 814 was determined with pre- and post-Wesselsbron-inoculated sheep sera. The same value for virus strains AR 740 and AR 756 was determined with pre- and post-Wesselsbron-inoculated guinea-pig sera. For the other virus strains normal monkey serum was used as a quantitative control and a Wesselsbron-immune monkey serum was used as a qualitative control.

Virus strain AR 778 is of special interest because a lyophilized stock was made from the suspension of wild-caught *Aedes caballus* from which it was derived. As noted in Table I, 218 mosquitoes were collected during a second visit to Crowboroughvlei Farm about 2 - 3 weeks after the first visit, when the majority of mosquitoes yielding virus had been collected. The collection on 24 April was thus made after it became apparent that the rate of virus infection in *Aedes caballus* was unusually high at the time. One of the objects of the second visit was to prepare stock virus from wild-caught mosquitoes without previous mouse passage. It was thought that a preparation of such an unadapted virus might be useful in subsequent studies concerned with virus transmission, host susceptibility and immunological response.

Observation and Results arising from the Study of other Virus Strains

All but 2 of the virus strains listed in Table I were from mosquitoes collected at Crowboroughvlei, Conway, a farm in the Middelburg district of the Cape Province. During the period that these collections were made

another type of agent, previously unknown, referred to as Middelburg virus,[1] was isolated from *Aedes caballus* and *Aedes* (*Neomelaniconion*) spp. In fact, virus was so prevalent in the mosquitoes taken during the primary phase of the investigations at Crowboroughvlei that 29 of 30 lots of *Aedes caballus* and 4 of 4 lots of *Aedes* (*Neomelaniconion*) spp. yielded either Middelburg or Wesselsbron virus. Each type of virus was recovered from lots of both types of mosquitoes. Middelburg virus has a shorter incubation period in infant mice than Wesselsbron and only the latter adapts to adult mice; hence it was early expected that some of the isolates might be mixtures of the 2 agents. The recovery of Wesselsbron virus from an adult mouse inoculated with Middelburg-infected first-passage infant-mouse brain was, indeed, reported in a previous paper.[1]

About 16 months after the original isolations, passages were made from 15 lots of frozen brains of infant mice infected with different strains of early-passage Middelburg virus. Middelburg virus was recovered from 9 of these, both agents from 1, and Wesselsbron alone from 3; 2 lots were found to have lost their virulence completely. These results not only gave further indication that some of the original short-incubation isolates were mixtures, but showed also that Middelburg is the more labile of the two under refrigeration at −20°C.

DISCUSSION

Since the original description of the isolation of Wesselsbron virus by Weiss *et al.*[11] other studies have established the importance of its role as an aetiological agent of disease among human beings and domestic animals. Reports[5,2] have been published concerning naturally acquired infection in man. In surveys for Wesselsbron-neutralizing antibodies in man a high incidence has been detected in the sera of residents in northern Natal,[12] and throughout Portuguese East Africa.[13] Several epizootics in sheep have been reported,[11,14] and Weiss *et al.*[11] report the finding of antibodies in sera from domestic quadrupeds collected at Knysna, Cape Province, and also in the Rhodesias. A high incidence of neutralizing antibodies has been detected in sera collected in Tongaland from cattle, sheep, goats and donkeys.[15]

The evident widespread distribution of Wesselsbron virus might be explained in part by the relatively large number of mosquito species found infected in nature. In addition to the species incriminated in this report, another has been found infected by Smithburn *et al.*[5] Among these species are representatives known to have a wide distribution in Africa. Wesselsbron virus has already been shown to be transmitted by the bite of 2 of these species, namely, *Aedes circumluteolus*[16] and *Aedes caballus*.[17]

It is, therefore, not presumptuous to anticipate that activity of Wesselsbron virus will be detected in regions other than southern Africa. However, this does not imply that the mosquito species incriminated in one region will necessarily be the important host of this virus in other regions.

The two most important potential vectors (*Aedes caballus* and *Aedes circumluteolus*) are both pool breeders and could probably be controlled by the same techniques. Eradication of a mosquito has so far proved well-nigh impossible except under highly specialized conditions. To get good control, however, eradication is not always essential. Mere reduction in numbers and in the length of life of vectors is proving effective in other vector-borne diseases.

Had only adult mice been inoculated with mosquito suspensions in the primary isolation attempts few, if any, of the strains of this virus would have been detected. This relative absence of pathogenicity for adult mice was one of the determining reasons for discontinuing their use in this laboratory for primary isolation. Since this observation, the routine has been to inoculate 2 litters of newborn mice intracerebrally with all specimens that are processed for attempted virus isolation.

SUMMARY

1. Strains of Wesselsbron virus were isolated from wild-caught *Aedes caballus*, *Aedes* (*Neomelaniconion*) spp., *Mansonia uniformis* and *Culex univittatus* mosquitoes collected in northern Natal and the eastern Cape Province, Union of South Africa.

2. Multiple strains of the virus were isolated from mosquitoes collected during an epizootic in sheep in the Middelburg district of the Cape Province.

3. Reports are cited which indicate that Wesselsbron virus is an important pathogen for man and domestic animals, with widespread distribution in southern Africa, and a few observations are discussed which suggest that the agent is probably important in other regions.

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