STUDIES ON THE TRANSMISSION OF WESSELSBRON VIRUS BY *AEDES (OCHLEROTATUS) CABALLUS* (THEO.)*

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During the study of an epizootic in the eastern Cape Province, Wesselsbron virus was isolated from sheep (R. A. Alexander[1]). In the course of subsequent investigations by us on the farm Crowboroughvlei undertaken principally to identify the vector or vectors of the disease, further isolations were made from sheep, from 2 species of mosquitoes (Kokernot *et al.*[2]) and from man (Heymann *et al.*[3]). *Aedes (Ochlerotatus) caballus* (Theo.) was the prevalent mosquito and one of the two species from which we isolated Wesselsbron virus. The purpose of this report is to present results which demonstrate the ability of *Aedes caballus* to transmit that agent.

METHODS AND MATERIALS

With only the few exceptions mentioned below the methods and materials employed in these experiments were the same as those previously described (Kokernot *et al.*[4]).

A lamb (AN 2348) obtained on 9 April 1957 from Grootfontein Agricultural College, Middelburg, Cape Province, was exposed to 40 *Aedes caballus* females caught in the epizootic area the afternoon of the same day. The head and neck of the lamb were placed through the sleeve of a gauze cage measuring 14 × 14 × 14 inches. The legs of the animal were secured to assure restraint for a period of approximately 1 hour between 18.00 and 19.00 hours. During this interval 6 of the mosquitoes engorged and were pooled and designated AR 731. The remaining 34 mosquitoes showed no visible evidence of having engorged and were pooled and designated AR 732. Both lots were stored on dry ice overnight and the following day processed and inoculated into litters of newborn and adult mice in Johannesburg.

Immediately after exposure the lamb and mosquitoes were transported by car in an overnight journey to Johannesburg.

Aedes caballus that had been reared in the laboratory from larvae were allowed to engorge on lamb AN 2348 on 2 occasions. The first time was 46 hours after the initial exposure to AR 731, when the lamb's temperature was 104·4°F, and 14 of the laboratory-reared *Aedes caballus* (lot 35) engorged through the gauze covers of glass vials in which they were confined. On the third day, when its temperature was 104°F or over, the lamb was placed inside a plastic-screened cage measuring 30 × 24 × 21 inches, and in a 2-hour period from 20.00 to 22.00 hours 6 laboratory-reared *Aedes caballus* (lot 36) engorged.

Other *Aedes caballus* caught biting man in the epizootic area on the afternoon of 9 April were collected in glass tubes lined with strips of saturated absorbent paper and transported alive to Johannesburg. The next day 134 of these were observed to probe or engorge on 31 newborn mice held firmly over the orifices of the tubes. Each mouse was thus bitten by more than one mosquito. After exposure, the infant mice were returned to their respective mothers. The mosquitoes were then pooled in lots of 50, 50 and 34 and designated AR 749, AR 750 and AR 751 respectively. Each lot was killed by being placed at −20°C and then processed in the usual manner.

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RESULTS

Wesselsbron virus was isolated from the 6 *Aedes caballus* (AR 731) that engorged on lamb AN 2348 on 9 April 1957, and from the 34 (AR 732) showing no evidence of engorgement.

On the 2nd, 3rd and 4th days after exposure to 40 *Aedes caballus* mosquitoes (AR 731 and AR 732), lamb AN 2348 had a temperature in excess of 104°F. The 4th day it was anorexic, dyspnoeic and weak. Death occurred on the 5th day. Lots 35 and 36 *Aedes caballus* were allowed to engorge when the temperature was in excess of 104°F and during a period when Wesselsbron virus was circulating, as confirmed by subsequent specificity test. These details are shown in Table I.

TABLE I. COURSE OF WESSELSBRON INFECTION IN LAMB AN 2348 WHICH WAS SOURCE OF INFECTIVE FEED FOR LABORATORY-REARED *Aedes caballus*, LOTS 35 AND 36

Date	*Hour*	*Temperature*	*Remarks*
9.4.57	18.00	Not recorded	Exposed 1 hour to 40 wild-caught *Aedes caballus* collected 9.4.57 biting man.
11.4.57	08.30	100·2°F	
	12.00	101·6	
	15.50	104·4	14 *Aedes caballus*, lot 35, engorged; lamb then bled and Wesselsbron viraemia confirmed.
	22.15	103·6	
12.4.57	08.25	102·8	
	12.00	103·8	
	16.00	105·3	Confirmed Wesselsbron viraemia.
	20.00	104·4	During this 2-hour interval 6 *A. caballus* lot 36, engorged.
	22.00	104·0	
13.4.57	08.50	102·7	
	11.50	102·8	
	16.15	104·3	Anorexic, dyspnoeic and weak.
14.4.57	08.15	101·3	Critically ill.
	12.00		Died—Wesselsbron virus isolated from liver, kidney and spleen.

On the 19th post-feeding day one of the 14 *Aedes caballus* in lot 35 died. A suspension was made of this mosquito and inoculated into infant mice. An identification test confirmed the presence of Wesselsbron virus in this mosquito (Table III, experiment 2). After the lapse of 21 and 22 days, selected

TABLE II. FATE OF NEWBORN MICE BITTEN BY *Aedes caballus* WHICH HAD PREVIOUSLY ENGORGED ON A LAMB (AN 2348) CIRCULATING WESSELSBRON VIRUS

Mosquito lot no.	*Interval between feeding on lamb and mice*	*Number of mosquitoes biting mice*	*Apparent transmission rate*	*Fate of mice*
35	21 days	6	1/7	1 moribund 9th day—confirmed transmission (Table III, experiment 3). 6 remained well.
36	22 days	3	7/7	1 sick 7th day. 6 sick 8th day Confirmed transmission by means of pooled brains of 2 mice.

mosquitoes of lots 35 and 36 respectively were allowed to bite newborn mice. The results are summarized in Table II. Although 6 mosquitoes of lot 35 were utilized in biting newborn mice many times, only one mouse developed illness during the 21-day observation period. The illness in this mouse was confirmed as being due to Wesselsbron virus (Table III, experiment 3). The 3 mosquitoes of lot 36 bit another group of 7 newborn mice with 100% apparent transmission rate. Two of these mice, sick on the 8th post-biting day, were sacrificed and their brains pooled and then set up in a neutralization test to confirm Wesselsbron virus transmission. Over 4 logs of the agent in the pooled mouse brains was neutralized by a post-inoculation Wesselsbron guinea-pig serum when compared to the pre-inoculation serum.

Thus, laboratory-reared *Aedes caballus* from 2 lots differentiated by the time and method of exposure for engorgement on a lamb circulating Wesselsbron virus were able to transmit the virus to infant mice. The details confirming Wesselsbron virus as the agent involved in a successful lamb—*Aedes caballus*—mouse transmission are shown in Table III.

In addition to the transmission of Wesselsbron virus by the technique described above with laboratory-reared *Aedes caballus*, it was also transmitted by wild-caught mosquitoes (AR 749, AR 750 and AR 751). However, only one of the 31 mice bitten 2 to 4 times by a group of 134 such mosquitoes sickened. In a neutralization test the aetiology of the illness as having been due to Wesselsbron-virus infection was confirmed.

The three lots into which the 134 wild-caught *Aedes caballus* were divided for processing after having bitten mice (lots nos. 749, 750 and 751) yielded 4 strains of virus. From AR 749 a mixture of Middelburg (Kokernot *et al.*[4]) and Wesselsbron viruses was isolated. Wesselsbron virus was isolated from AR 750 and Middelburg virus from AR 751.

DISCUSSION

Wesselsbron virus was isolated from the wild-caught *Aedes caballus* (AR 731) that fed on lamb AN 2348 and this animal was subsequently proved to be infected with that virus. Nevertheless, this cannot be regarded with certainty as an experimental transmission because the lamb was obtained from a flock of sheep in which morbidity and mortality due to Wesselsbron virus were occurring.* When obtained, the lamb appeared healthy, but it is possible that Wesselsbron virus infection, acquired from a different source than mosquitoes of lot AR 731 or AR 732, was in an incubation phase. Regardless of the source of the lamb's infection, the Wesselsbron virus which was present in its blood served as the infecting feed for 2 lots of laboratory-reared *Aedes caballus* (lots 35 and 36).

A successful transmission by means of mosquitoes collected from nature within such a short period as 24 hours might include specimens capable only of mechanical transmission. However, the results presented include successful transmission by laboratory-reared *Aedes caballus* after a 21- and 22-days post-infective feeding period. There is a significant difference between the transmission rate to mice by bite of mosquitoes in lots 35 and 36. Lot 35 mosquitoes were exposed to AN 2348 a day earlier in the course of the illness, and it is probable that at that time the viraemia level was at a low infective threshold. The two lots of mosquitoes were exposed to the lamb by different methods. Lot 35 mosquitoes were maintained individually in glass vials. They were allowed to probe and feed upon the lamb through the gauze-covered orifice. Lot 36 mosquitoes became engorged with blood by feeding upon the lamb restrained in the cage with the mosquitoes.

The significance of *Aedes caballus* as a vector of Rift Valley fever virus (Gear *et al.*[5]) and Middelburg virus (Kokernot *et al.*[4]) has previously been reported. It is apparent from the current results that it is also a vector of Wesselsbron virus. The bionomics of this little-studied species merits intensive investigation with the hope that some practical method of control will be revealed.

Aedes caballus is the second member belonging to this

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TABLE III. RESULTS OF 3 NEUTRALIZATION EXPERIMENTS THAT CONFIRM WESSELSBRON VIRUS AS BEING THE AGENT IN A SUCCESSFUL LAMB-*Aedes Caballus* (LOT 35)-INFANT MOUSE TRANSMISSION

Experiment No.	*Source of infected infant mouse brain*	*Serum pre- or post-Wesselsbron virus**	*Dilution of infected brain suspension added to equal volume of serum*	*Results (Fate of mice)* Died	Lived	*Titre*
1. Identity of agent circulating in blood of lamb AN 2348 which was the source of infective feed for lot 35 *Aedes caballus*	Mice inoculated with serum taken from AN 2348 immediately after feeding 14 *Aedes caballus*, lot 35	Pre-	10^{-3}	3	0	3·86
			10^{-4}	5	7	
			10^{-5}	0	12	
			10^{-6}	0	12	
		Post-	10^{-1}	0	6	0·5†
			10^{-2}	0	6	
2. Identity of agent present in 1 *Aedes caballus* on the 19th post-feeding day	Mouse inoculated with the mosquito suspension	Pre-	10^{-3}	12	0	4·6†
			10^{-4}	12	0	
			10^{-5}	1	5	
			10^{-6}‡			
			10^{-7}	0	6	
		Post-	10^{-1}	0	12	0·5†
			10^{-2}	0	10	
3. Identity of agent causing illness in 1 of 7 infant mice bitten by *Aedes caballus* on their 21st post-feeding day	Mouse moribund on the 9th post-mosquito biting day	Pre-	10^{-3}	6	0	5·17
			10^{-4}	12	0	
			10^{-5}	6	6	
			10^{-6}	2	4	
			10^{-7}	0	6	
		Post-	10^{-1}	0	12	0·5†
			10^{-2}	0	10	

* Sera in experiments 1 and 2 were from guinea-pigs and serum in experimen 3 was from a sheep.
† Approximate titre since no clear end-point could be calculated.
‡ Unsatisfactory litter

genus shown in this laboratory to be capable of transmitting Wesselsbron virus. Muspratt *et al.*[6] demonstrated the ability of *Aedes* (*Banksinella*) *circumluteolus* to transmit the virus. Furthermore, the virus has been isolated in nature from the latter species (Smithburn *et al.*[7]).

SUMMARY

1. Laboratory-reared *Aedes caballus* were allowed to engorge on a lamb circulating Wesselsbron virus.

2. On the 21st and 22nd post-engorgement days these mosquitoes transmitted the virus by bite to new-born mice.

3. 134 wild-caught *Aedes caballus* were allowed to bite 31 new-born mice. From this a successful transmission resulted and Wesselsbron virus was confirmed in the single mouse showing illness.

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