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OBSERVATIONS ON SOME VIRUSES ISOLATED FROM TICKS IN SENEGAL*)

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Abstract. Following the isolation of Jos virus from cattle ticks (*Amblyomma variegatum*), a serological survey was carried out on bovine material collected in three different zones of Senegal. One hundred and forty-four sera were investigated: thirty-nine (27.1 %) were protective. The highest incidence was found in Casamance where 37.6% of cattle had antibodies to this virus.

In 1965, a new type of virus designated "Bandia" (IPD/A611) was isolated from *Ornithodoros erraticus sonrai* collected in rodent burrows in the Bandia forest (N 14°35′—W 17° 01′) (BRÈS, CORNET and ROBIN 1967). This paper deals with the characteristics of two other viruses isolated from *Amblyomma variegatum* in the year 1967 and with the incidence of antibodies in cattle in three different areas of Senegal. These isolates appear to be related to (if not identical with) Ib Ar 18 735 previously isolated in Nigeria and named Jos (CAUSEY 1967).

MATERIALS AND METHODS

Collection of ticks. Ticks were collected on cattle at the slaughter-house (Table 1). A total of 1071 pools including 17,111 specimens were handled. They were kept two days in glass containers at 25—30 °C. Then they were classified and pooled alive. The pools were stored at —50 °C.

Method of virus isolation. Each pool was triturated by mortar and pestle and suspended in phosphate buffer containing 10 % normal rabbit serum and antibiotics. The suspension was clarified by centrifugation at 2000 rpm for 15 minutes. The supernatant was stored at —50 °C for one month and then used for inoculum. Infant mice, 1—2 days old, were inoculated intracerebrally (i.c.) with 0.02 ml of the inoculum. Brains harvested from moribund mice were used for further passaging in suckling mice.

Identification of virus strains. Virus strains were identified by complement fixation (CF) and neutralization (N) tests. CF tests were done according to the LBCF method (HAMMON and WORK 1964). Neutralization tests were performed using serial virus dilutions with undiluted serum.

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Serological survey. Virus strains were confined to assays for neutralizing antibodies in cattle sera. Equal amount of a suspension of previously titrated virus and the serum under test were mixed so that the inoculum contained at least 100 LD_{50} of virus. The mixture after incubation for 1 hour at 37 °C, was inoculated i.c. into 6 suckling mice, each mouse receiving 0.02 ml. The mice were observed 2 weeks and the deaths were recorded daily. Survival of all or 5 of the 6 infant mice was interpreted as positive (+), survival of 2 to 4 as inconclusive (±) and the survival of none or 1 of the mice as negative (—).

Table 1. Ticks collected for virus isolation (1967—1968)

Ticks collected	No.	% of total
Amblyomma variegatum		
Adults	3290	
Nymphs	1240	23.1
Larvae	5	
Hyalomma truncatum	8995	45.9
Hyalomma impeltatum	1510	7.7
Hyalomma rufipes	360	1.8
Hyalomma impressum	445	2.3
Others Ixodidae	2630	13.4
Argasidae	1140	5.8
Total	19,615*)	100

*) 17,111 specimens inoculated.

RESULTS

Isolation. During the investigations 2 strains were isolated from *A. variegatum*. The first positive pool (strain No. PA 3287) contained 20 ♂♂ collected July 17, 1967 at the Dakar slaughter-house. On the 5th day after inoculation, 2 mice were found dead and 2 looking ill were harvested. After two brain-to-brain passages, the virus killed 1-day-old mice in 4—5 days. It kills adult mice by i.c. but not by i.p. route. Attempts to reisolate virus 2 months after the original isolation failed. The second strain No. PA 3491 from 20 ♀♀ *Amblyomma variegatum* collected August 21, 1967 behaved similarly.

Identification. The agent was filtered through a millipore pad (0.220 μ) and was shown to be chloroform-sensitive. An antigen prepared by sucrose-acetone extraction of infected suckling mouse brain was tested by CF with antisera for 63 viruses (Table 2). No reaction occurred except with Ib Ar 18 735 mouse ascitic fluid. Cross complement fixation is shown in Table 3. In N tests, the virus was neutralized by mouse ascitic fluid prepared from Ar 18 735 (Table 4). Attempts to prepare a hemagglutinin from brain of suckling mice infected with both strains (PA 3287 and PA 3491) were unsuccessful.

The following pathological changes were observed in infant mice: neuronal

degeneration in the spinal anterior horns, the hippocampus and even the cortex, and very important focal necrosis in the liver.

Serum survey: 144 bovine sera were collected in different parts of Senegal: Casamance, between Gambia and Guinea, 93; Kedougou, Eastern Senegal, 26;

Table 2. Sera with which the isolates were compared in CF test

Group A

Semliki
Chikungunya
O'nyong-nyong
Sindbis
Middelburg
N'Dumu

Group B

Group antiserum
Ntaya
Wesselsbron
Usutu
West-Nile
Dakar-Bat
Uganda S
Yellow Fever
Zika
Spondweni
Bukalasa Bat
DakAr B 209
DakAr Y 310
Entebbe bat sg.
DakAr B 490
M. M. L.

Bunyamwera group

Bunyamwera
Germiston
Ilesha
Shokwe
Olifantsvlei

Bwamba group

Bwamba
Pongola

California group

Group antiserum
Lumbo

Simbu group

Simbu
Ingwavuma
Yaba 7
Sango
Shanonda
Sabo
Shuni

Turlock group

BA 365 (M'Poko)

Nyando group

Nyando
Eretmapodites 124 (YM 176)

Kamerovo group

Chenuda

Uukuniemi group

Gd Arbaud
Ponteves

Quaranfil group

Quaranfil

Qalyub group

Bandia

Phlebotomus group

Group antiserum

Ungrouped

Vect: mosquito: Witwatersrand, Tataguine
tick: Nyamanini, Wad-Medani, Thogoto Bhanja, Congo, Ib Ar 18 735
unknown: Mossuril, Lagos bat, Tanga, Fika (3150), Lebombo

Not classified

DakAn D 318,
DakAn D 401
DaKan D 763.

Table 3. Virus identification. CF test.

MAF		ANTIGENS		
		PA 3287	PA 3491	Ar 18 735**)
PA 3287	68—1727	**32/4***)	32/8	16/8
PA 3491	68—1725	16/8	**16/8**	
Ib Ar 18 735		16/4	16/16	**16/8**

*) Serum titer/antigen titer.

**) Test performed by Dr. D. R. Causey, Ibadan, Nigeria.

and Saint-Louis, Senegal River Delta, 25. They were examined for neutralizing antibodies, using one of the strains isolated in Senegal (PA 3491). 39 sera were protective (27.1%), 4 inconclusive (2.8%) and 101 negative (70.1%). As a rather large dose of virus (LD_{50} = 400) was used in these tests, the inconclusive reactions are probably of some significance.

According to the place of collection, the percentage of protective sera are as follows: Casamance 37.6, Saint-Louis 20, Kedougou 7.6.

Table 4. Virus identification. Neutralization test

MAF	VIRUS		
	PA 3287	PA 3491	Ar 18735
Normal (log virus titer)	4.6	4.6	—
PA 3287 68—1727	2.8*)	3.2	—
PA 3491 68—1725	2.9	3.1	—
Ib Ar 18 735	—	3.2	—

*) Log neutralization index.

DISCUSSION

Two strains of a virus, previously isolated in Nigeria, have been obtained from pools of *Amblyomma variegatum* ticks collected on bovine hosts. A limited serological survey of bovine sera from different parts of Senegal produced evidence of natural infection with this virus. As to geographical distribution, it should be noted that Casamance has a very high incidence of virus infection. The fact that the cattle is rather scattered in this area suggests that the virus is very active. This requires further investigation.

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