VIRAL ISOLATES FROM IXODID TICKS OF WILD ANIMALS IN KENYA

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Abstract: Approximately 10,000 ixodid ticks were collected over a period of 24 months from 11 species of wild African ungulates. The ticks, sorted by species, were inoculated intracerebrally into infant mice as pools for attempted virus isolation. A total of 11 isolates were made, 8 from 62 pools of Rhipicephalus pulchellus, 2 from 9 pools of Amblyomma variegatum and 1 from 6 pools of Amblyomma cohaerens. Seven isolates were identified as Kadam virus, 1 isolate showed serological relationship with Bhanja virus and 3 isolates, of one antigenic type, remained unidentified. Virus was not isolated from 25 pools of Rhipicephalus evertsi, 11 pools of Rhipicephalus appendiculatus, 2 pools of Amblyomma lepidae, 9 pools of Haemaphysalis species and 4 pools of Hyalomma species.

Zusammenfassung: Ungefahr 10,000 Ixodid-Zecken wurden wahrend 24 Monaten von 11 Arten wildlebender Huftiere Afrikas gesammelt. Die Zecken wurden nach Arten sortiert und im Gruppen (Pools) zusammengefasst. Isolierung von Viren wurde versucht indem Babymause intracerebral mit Extrakten aus diesen Zeckenpools inokuliert wurden. Insgesamt wurden 11 Isolate gefunden, 8 aus 62 Pools von Rhipicephalus pulchellus, 2 aus 9 Pools von Amblyomma variegatum und 1 aus 6 Pools von Amblyomma cohaerens. Sieben Isolate liessen sich als Kadam-Virus identifizieren. 1 Isolat zeigte serologisch Ahnlichkeit mit Bhanja-Virus und 3 Isolate, antigenisch einheitlich, konnten nicht identifiziert werden. Eine Virusisolierung gelang nicht von 11 Pools von Rhipicephalus appendiculatus, 25 Pools von Rhipicephalus evertsi, 2 Pools von Amblyomma lepidae, 9 Pools von Haemaphysalis species und 4 Pools von Hyalomma species.

INTRODUCTION

In Kenya the most important tick-borne virus disease is Nairobi Sheep Disease (NSD). The principal virus vector is Rhipicephalus appendiculatus (6). NSD virus was registered as ungrouped in 1961, but recent unpublished work by Davies et al (2) shows that this virus is probably identical to Ganjam virus. Other tick-transmitted viruses isolated in Kenya include Thogoto virus (4) and Congo virus (9).

MATERIALS AND METHODS

Ticks: From April 1973 to April 1975 a total of approximately ten thousand ixodid ticks from various wild African herbivorous animals were processed for virus isolation. The majority of ticks were supplied by the Food & Agriculture Organization (F.A.O.) of the United Nations' Wildlife Disease Project personnel (Ken/68/013) Kabete. A number of ticks from buffaloes (Syncerus caffer) were also collected by the staff of Wellcome Institute for Research on Foot-and-Mouth Disease, Embakasi. In addition, ticks were collected from animals dying in the Nairobi National Park.

Approximately 80% of the ticks were alive on arrival at Kabete where they were sorted into species. The majority of ticks were adults, and the few immature stages were discarded.

Virus Isolation Technique: Pools of ticks varying in size from ten to one hundred and twenty individuals were ground with sterile sand and phosphate buffered saline (PBS) solution, pH 7.5 containing 1000 i.u. penicillin, 500 µg streptomycin and 250 µg mycostatin per ml. The amount of diluent varied from 2 to 10 ml. according to the number, size and state of engorgement.

The supernatants were collected after centrifugation at 2500 r.p.m. fifteen minutes and left at room temperature in the dark for thirty minutes before intrecerebral (i.c.) inoculation of 0.02 ml. into ten-twenty infant Swiss albino mice maintained as a closed LCM free colony at Kabete. If mice were not immediately available, the tick suspensions were stored in bijoux bottles at - 70 C. Inoculated mice were checked daily for sixteen days for evidence of nervous symptoms.

If Giemsa stained brain smears from sick mice were negative for bacteria, a 10% mouse brain suspension was made in the antibiotic solution and subinoculated into infant nice and BHK - 21 cells in roller tubes. The isolation of a presumptive virus was considered when deaths occurred in mice and cytopathic effect (CPE) developed in tissue culture on second, and subsequent passages

Serological Techniques: Mouse immune ascitic fluids (MAF) were prepared in adult mice by 4 weekly 0.5 ml intra-peritoneal (i.p.) inoculations of 10% suspensions of infected mouse brains in PBS emulsified in equal volumes of Freunds adjuvant. The last inoculation was followed five days later by 0.5 ml of a 1:100 suspension of a mouse sarcoma virus (Rous 180) supplied by Dr. Metzelaar, Royal Tropical Institute of Amsterdam, Nairobi.

The indirect fluorescent antibody technique (FAT) was employed as described by Davies et al. (3) using fluorescein isothiocyanate conjugated swine anti-mouse gamma-globulin purchased from Nordic Immunological Laboratories, Tilburg, Netherlands.

Any positive reaction by FAT was subsequently checked by complement fixation tests (CFT) in microplates. Antigens were prepared from infected suckling mouse brains, which were purified by treatment with fluorocarbon (Arcton 113). As a final test for identity between related strains the serum neutralisation test (SNT) was employed using constant immune fluid (MAF) and virus in decimal dilutions.

Prototype strains of the virus isolates were compared by use of FAT and CFT with the most common vector borne viruses causing disease in Kenya livestock available at Kabete, namely: ephemeral fever virus (EF), bluetongue virus (BT), Rift Valley fever virus (RVF) and Nairobi sheep disease virus. In addition the following mouse immune fluids were supplied by Yale Arbovirus Research Unit (YARU), U.S.A.: Polyvalent 1 (Bahig, Tete, Matruh, Matanya, Burg el Arab), polyvalent 4 (Nyamanini, Uukunemi, Grand Arbaud, Thogoto), Polyvalent 10 (Upolu, DGK, Wanowlie, Dhori), Polyvalent group California, Polyvalent group Kemerovo, polyvalent group B (Flavivirus), monovalent Congo, Dugbe, Ganjam, Bhanja, Hazara. Kadam virus immune fluid was supplied by the East African Virus Research Institute, Entebbe, Uganda.

RESULTS

A total of 128 pools of ixodid ticks were processed for virus isolation. Sixty-two pools consisted of Rhipicephalus pulchellus, 25 of Rhipicephalus evertsi, 11 of Rhipicephalus appendiculatus, 9 of Amblyomma variegatum, 6 of Amblyomma cohaerens, 2 of Amblyomma lepidae, 9 of Haemaphysalis species and 4 pools of Hyalomma species.

Ticks were collected from the following animals: Buffalo (Syncerus caffer), 39 pools; bushbuck (Tragelaphus scriptus), 11 pools; eland (Taurotragus oryx), 4 pools; Grant's gazelle (Gazella granti), 4 pools; impala (Aepyceros melampus), 2 pools; kongoni (Alcelaphus buselaphus cokei), 11 pools; rhinoceros, black (Diceros bicornis), 12 pools; topi (Damaliscus korrigum korrigum), 16 pools; wildebeest, blue (Connochaetes taurinus), 24 pools; zebra common (Equus burchelli), 3 pools; and giraffe (Giraffa camelopardus), 2 pools.

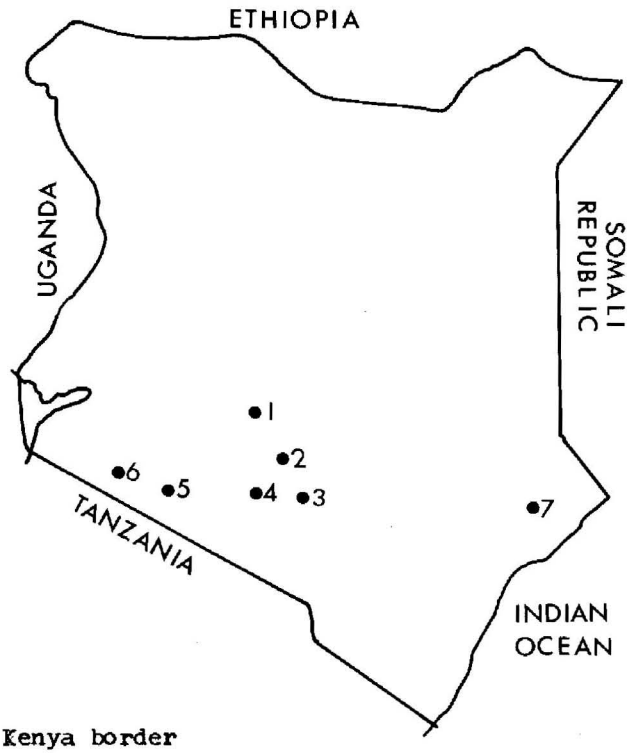
Most of the ticks were collected in Central and Rift Valley Provinces from animals inhabiting dry forms of woodland, wooded grassland and bushed grassland. Figure 1 shows the main tick collection sites.

Of the 128 pools eleven were positive for virus (8.6%). In Table 1 the isolates are listed by pool number, month of isolation, species of ticks, animal host and place collected. As Table 1 shows, viruses were recovered from three species of ticks, R. Pulchellus (8 isolates), A. variegatum (2 isolates) and A.cohaerens (1 isolate).

All 11 isolates were found to be sensitive to ether and chloroform, and the relationship between them determined by FAT, CFT and SNT showed that they fall into 3 different immunological groups, as follows:

a. T 39.
b. T 97, T 100, T101, T 103, T 129, T 163 and T 176.
c. T 45, T 83 and T 178.

T 39, T 83 and T 100 were chosen as prototype strains for further characterisation and identification. Their biological characteristics in tissue culture systems and their pathogenicity for mice are shown in Table II.



---- Kenya border

1 Naivasha

2 Nairobi N. Park

3 Machakos

4 Kajiado

5 Keekerok

6 Lolgorien

7 Ijara (Coast Province)

Figure 1. Tick Collection Sites April 1973 - April 1975

Table I. Virus Isolations from Ixodid Ticks in Kenya 1973/75.

Pool No.	Month of Isolation	Tick Species	Animal Host	Place of Collection
T 39	Jan. '74	R.pulchellus	Topi	Ijara
T 45	" '74	R.pulchellus	Wildebeest	Kajiado
T 83	Mar. '74	R.pulchellus	Eland	Machakos
T 97	Apr. '74	R.pulchellus	Rhinoceros*	Nairobi
T 100	" '74	R.pulchellus	"	National
T 101	" '74	R.pulchellus	"	Park
T 103	" '74	R.pulchellus	"	
T 129	Jun. '74	A.variegatum	Buffalo	Lolgorien
T 163	Mar. '75	A.variegatum	"	Keekorok
T 176	" '75	A.cohaerens	"	"
T 178	Apr. '75	R.pulchellus	Giraffe	Kajiado

* The animal was found in Nairobi National Park unable to stand. It had an extreme tick-burden. Twelve pools of R.pulchellus were processed for virus isolation of which 4 yielded a virus with identical characteristics.

Table II. Some Biological Characteristics of Virus Isolates From Ticks.

Virus	Growth in tissue culture					Pathogenicity for Mice			
	BHK	L-cells	Vero	CK	BFM	IM ic	IM ip	AM ic	AM ip
T 39	+	-	+	-	-	+	-	-	-
T 83	+	+	+	+	+	+	+	+	+/-
T 100	+	-	-	-	-	+	+	+	+/-

+/- irregular mortality in mice
+ CPE or plaques, death in mice
BFM Bovine foetal muscle
CK Calf Kidney
IM Infant mice, 2-4 days old
- No CPE or plaques, no deaths in mice
AM Adult mice, 6 weeks or more

Identification of prototype strains: T 100 showed specific immune fluorescence with polyvalent Group B immune fluid and was subsequently identified as Kadam virus by both FAT and CFT.

T 39 reacted specifically with Bhanja virus immune fluid in FAT, but we were unable to demonstrate a significant reaction in CFT. We have therefore tentatively identified T 39 as related to Bhanja virus while awaiting the results from a reference laboratory.

T 83 showed no serological cross reaction with any of the immune reagents listed above.

DISCUSSION

Kadam virus, a member of Flaviviruses (Gr. B Arboviruses) was first isolated in Uganda from Rhipicephalus pravus (5). Our findings are the first report of Kadam virus isolation in Kenya and incriminate other species of ticks as carrier of this agent.

The immunological relationship between T 39 and Bhanja virus could be demonstrated on repeated occasions in the FAT. Shah & Work (7) first reported the isolation of Bhanja virus from Haemaphysalis intermedia collected from a paralysed goat in India. Subsequently this virus has been isolated from other Haemaphysalis ticks in Italy (8), in Nigeria from Amblyomma variegatum, Boophilus decoloratus, Hyalomma truncatum and from cattle and sheep blood (1).

Tests in our laboratory indicate that T 83 has some of the characteristics of an arbovirus, such as pathogenicity for mice, and sensitivity to ether and chloroform. It differs, however, in some respects. For example it multiplies in primary calf kidney, and foetal bovine muscle cells with production of CPE and on repeated examinations infected monolayers have been found to contain multiple intranuclear eosinophilic inclusion bodies, stained by Hematoxyline Eosin.

Work is in progress in cooperation with the F.A.O. Wildlife Disease Project, Kabete to determine the pathogenicity of T 39, T 100 and T 83 for sheep, cattle and captive wild African ungulates. Preliminary results suggest that these viruses are of little consequence as animal pathogens.

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