

## VIRUSES IN TICKS. IV. VIRUSES ISOLATED IN EGYPT DURING 1968\*)

K. S. E. ABDEL WAHAB, R. E. WILIAMS, M. N. KAISER

Virus Research Center, Agouza Production Laboratories, Ministry of Health, Egypt, U. A. R., and Guest Investigator, Virology Department, United States Naval Medical Research Unit Number Three, Cairo, and Medical Zoology Department, United States Naval Medical Research Unit Number Three, Cairo.

**Abstract.** Four virus strains were isolated from *Ornithodoros erraticus* females and nymphs collected from the burrows of *Arvicanthis n. niloticus* in Upper Egypt. These strains proved to be identical with the Qalyub virus isolated from the same vector species in Lower Egypt in 1952. The identification procedure is described.

In 1952 Taylor and Dressler of the United States Naval Medical Research Unit Number Three (NAMRU-3), Cairo, isolated a virus from a pool of 43 ticks collected in Egypt. The strain was apparently lost in 1956, before adequate comparison with other arboviruses, but was revived by Dressler in 1967 from ampoules that had been sent to the Naval Medical Research Institute, Bethesda, from NAMRU-3 (TAYLOR and DRESSLER 1969). In 1968, Dr. R. M. Taylor registered the strain as a new arbovirus in the Catalogue of Arthropod-borne Viruses under the name Qalyub virus (strain Ar 370). This single isolate from *Ornithodoros erraticus* ticks collected in a burrow of the Nile grass rat, *Arvicanthis n. niloticus*, by Dr. H. Hoogstraal at Barada, Qalyub, Qalyubiya Governorate (in Lower Egypt), represents all that has been known regarding Qalyub virus in nature. We are now attempting to learn more about the natural history and range of infection of this virus.

## MATERIALS AND METHODS

During August 1968, 493 males, 546 females, and 496 nymphs of *Ornithodoros erraticus* were collected from approximately 60 burrows of the Nile grass rat, *Arvicanthis n. niloticus*, in Minya, Beni Suef, and Giza Governorates, all in Upper Egypt.

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Sera were also collected from 50 donkeys in El Manashy, Imbaba, Giza Governorate, and from 83 donkeys at Delta Barrage, Qalyub, Qalyubiya Governorate. Sera from 25 *Rattus norvegicus*, 5 *Rattus rattus*, 45 *Asomys cahirinus*, and 1 *Hemiechinus auritus aegyptius* were collected in the same village from which the primary isolation of Qalyub virus was recovered.

The 1535 ticks were brought alive to NAMRU-3, identified, and grouped into 45 pools according to sex, developmental stage, and collecting locality. They were then washed and inoculated intracerebrally into suckling mice following the procedure of KAISER (1966). Upon indications that the inoculated mice were ill (WORK 1964), their brains were manually excised by sterile technique or by the method of STROME (1953) and made into a 10 per cent suspension using 0.75 per cent bovine albumin in phosphate buffered saline. The suspensions were filtered through Seitz EK pads and reinoculated as above. Fourth through sixth passage brain material was used to determine sensitivity to sodium desoxycholate following the methods of THEILER (1957), and to prepare sucrose-acetone extracted antigens, as described by CLARKE and CASALS (1958), and immune sera (WORK 1964). Each isolate was tested for its ability to agglutinate goose erythrocytes (CLARKE and CASALS 1958). Identification of isolates by the complement fixation (CF) test was accomplished using a modification of SEVER'S (1962) technique which included 100 per cent end points in titrating complement. The methods of WORK (1964) were used in conducting neutralization tests in 2-day old mice inoculated intracerebrally. Neutralization test end points were calculated according to the REED and MUENCH (1938) formula. Animal sera were tested by CF for antibodies against Qalyub (Ar 370) antigen.

## RESULTS

Four virus strains were isolated from the 45 tick pools. From 10 pools each of males, females, and nymphs from Matai and Mallawi, Minya Governorate, three strains were isolated from female pools and one from a nymphal pool. None was isolated from 15 pools from Giza and Beni Suef Governorates.

By CF (Table 1), each isolate (ArT 237, 240, 241, and 255) was closely related to Dr. Taylor's strain Ar 370 (Qalyub virus). Antigens prepared from these isolates were unrelated to any other reference stocks maintained at NAMRU-3: Sindbis, West Nile, Bunyamwera, sandfly fever, Chenua, Wad Medani, Nyamanini, and Quarantil. In two isolates (ArT 240 and 237) tested for the effect of sodium desoxycholate, the infectivity of each was more than 2.5 logs lower following treatment. None of the four isolates agglutinated goose erythrocytes. Neutralization test

Table 1. Serological relationship of 1968 isolates to Qalyub (ArT 370) virus by complement fixation test

Antigen	Antiserum				
	Qalyub	ArT 237	ArT 240	ArT 241	ArT 255
Qalyub	32/32*)	ND**)	64/32	64/32	ND
ArT 237	256/16	256/32	128/64	128/64	256/64
ArT 240	8/4	-ve***)	128/16	32/8	-ve
ArT 241	32/8	128/32	256/64	64/16	256/32
ArT 255	256/64	128/64	128/64	128/64	128/64

\*) Reciprocal of serum titer over reciprocal of antigen titer.

\*\*) ND = Not done.

\*\*\*) -ve = negative.

results for ArT 240 and 241 are shown in Table 2. Neutralization indices were somewhat low because of use of low-titered specific immune serum, including that of Qalyub (Ar 370).

After being stored for 10 months at  $-60^{\circ}\text{C}$ , the original tick suspensions of ArT 237 and 255 were reinoculated i.c. into infant mice and these two isolates were again recovered.

In the CF tests, the mammal sera showed no antibodies against Qalyub virus.

Table 2. Relationship of 1968 isolates to Qalyub (ArT 370) virus by neutralization test

Virus	Antiserum		
	ArT 240	ArT 241	Qalyub
Qalyub (ArT 370)	1.7*)	1.9	2.0

\*) Logarithms of virus neutralized.

## DISCUSSION

Sixteen years ago Qalyub virus was first isolated from a pool of *Ornithodoros erraticus* collected from an *Arvicanthis n. niloticus* burrow in Lower Egypt. We have now isolated the same virus from pools containing females and nymphs of this tick species from burrows of the same rodent species in Upper Egypt.

*O. erraticus* is widely distributed in cultivated and semidesert areas of Egypt where its chief hosts are *Arvicanthis n. niloticus* and the Egyptian hedgehog, *Hemiechinus auritus* subsp. (HOOGSTRAAL et al., 1954). *Arvicanthis* is closely associated with human agricultural activities and occurs in all cultivated areas of Egypt (HOOGSTRAAL 1963). The hedgehog is common in gardens and in or beside buildings but its range does not extend into Upper Egypt south of the Faiyum (HOOGSTRAAL 1962). *O. erraticus* also infests *Rattus rattus* burrows in cultivated fields, buildings, and cemeteries. Other hosts are different rodent species, foxes, ground-living owls, and large lizards.

As this study progresses, we hope to learn more about the biological factors influencing the circulation of Qalyub virus in nature and whether the virus infects the human population of Egypt. Presentation of the present findings does not constitute either original publication of the name Qalyub (Ar 370) virus or of Dr. Taylor's original data on this virus.

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K. S. E. A. W., Virus Research Center, Agouza Production Laboratories, Ministry of Health, Egypt, U.A.R.