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VIRUSES IN TICKS. V. VIRUSES ISOLATED FROM AFGHANISTAN TICKS DURING 1968*)

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Abstract. Six strains of Quaranfil-like virus were isolated from *Argas reflexus* group females and nymphs collected in Afghanistan. From an other 13 strains isolated from the same tick species three have been studied and found to be unrelated to any of the reference stocks.

During an expedition to Afghanistan in September, 1968, Dr. H. Hoogstraal and Mr. I. Helmy collected 846 specimens of ticks. These included the following species: *Argas* (*A.*) *reflexus* group, *A.* (*Persicargas*) *persicus*, *A.* (*Chiropterargas*) *boueti*, *Ornithodoros* (*Alectorobius*) *coniceps*, *Rhipicephalus turanicus*, *Hyalomma anatolicum excavatum*, and *Hyalomma dromedarii*. The following month Dr. Frank E. Hoopes kindly forwarded 346 specimens of *Argas* (*A.*) *reflexus* and *Ornithodoros* (*A.*) *coniceps* collected in Kabul Province, Afghanistan. All specimens were received alive at U.S. Naval Medical Research Unit No. 3, Cair, Egypt, U.A.R. for further study.

MATERIALS AND METHODS

The ticks were placed in pools according to species, development stage, and collection locality, and processed for attempts at virus isolation. There was an interval of one month between processing and inoculation of the two collections of ticks. Standard virus isolation techniques were used, and details of these are referenced. Pools were inoculated into infant mice by the intracerebral (i.c.) route (KAISER 1966). Upon indications that the inoculated mice were ill (WORK 1964), the brains of the

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mice were harvested (STROME 1953), made into a 10 per cent suspension in 2.5 per cent bovine albumin in phosphate buffered saline (BAPS), and reinoculated i.c. into infant mice. A 10 per cent suspension of brains of these mice in 0.75 per cent BAPS was passed through a Seitz EK filter pad. The filtrate was inoculated into suckling mice, and the third passage infected brains were used to prepare sucrose-acetone extracted antigens (CLARKE and CASALS 1958). These antigens were tested by the complement fixation test (CF) (modified from SEVER 1962) against reference antisera prepared in our laboratory (WORK 1964). Each isolate was tested for its ability to agglutinate goose red blood cells (CLARKE and CASALS 1958). Several passages of each isolate were made in infant mice to stabilize and adapt them to this host system. Higher passaged material was then used to determine sodium desoxycholate sensitivity of the isolates, to prepare immune sera, and to perform neutralization tests in two-day-old mice (HAMMON and WORK 1964). Neutralization indices were calculated by the method of REED and MUENCH (1938).

RESULTS

Three of the 83 pools of ticks in the September collection yielded virus isolates. The ticks comprising these three pools were of the *Argas* (*A.*) *reflexus* group and had been taken from cracks in the plaster, under seat covers, and from behind pictures tacked to the walls of pigeon shops in Kafroshy Market, Kabul Province. Arbovirus tick isolate 282 (ArT 282) was recovered from one of three pools of female ticks. ArT 284 and 285 were isolated from two pools of nymphs. ArT 282 and 284 were closely related by CFT to Quaranfil virus (Table 1). By the same test, ArT 285 was unrelated

Table 1. Complement fixation serological relationship of ArT 361 virus to ArT 282, ArT 284, and Quaranfil viruses

Antigen	Antiserum			
	ArT 282	ArT 284	ArT 361	Quaranfil
ArT 282	32/32*)	32/32	8/32	32/32**)
ArT 284	64/16	128/16	16/16	ND***)
ArT 361	64/4	128/4	32/8**)	64/32**)
Quaranfil	16/8	ND	64/32**)	ND

*) Reciprocals of: Serum titer/Optimum antigen titer.
**) Reciprocals of: Serum titer/Antigen titer.
***) ND = Not done.

to any of the following reference stocks prepared and maintained in our laboratory: Sindbis, Bunyamwera, Chenuda, Wad Medani, Nyamanini, Qalyub, Quaranfil, and sadfly fever. Qalyub virus was first isolated in Egypt by Dr. R. M. Taylor and mention of its name in this paper does not constitute initial publication (TAYLOR 1969, personal communication).

It can be seen in Table 2 that average survival times (AST) for infant mice infected with ArT 285 was longer initially than ArT 282 and 284, and has remained so on subsequent passages. ArT 285 agglutinates goose red blood cells, whereas

Table 2. Characteristics of ArT 282, ArT 284, and ArT 285 Viruses

Isolate	Average Survival Time (Days)		Agglutination of Goose Erythrocytes	Chemical Sensitivity
	Initial Passage	> Second Passage		
ArT 282	6	3—4	0	3.8 log*)
ArT 284	5—6	3	0	ND**)
ArT 285	9	5	+	5.8 log*)

*) Reduction in titer of virus treated with sodium desoxycholate as compared to non-treated control.

**) ND = Not done.

ArT 282 and 284 do not. Both ArT 282 and 285 are susceptible to the action of sodium desoxycholate. ArT 284 has not been tested for chemical sensitivity.

Of the 20 pools of ticks in the October collection, 16 yielded viral isolates. These isolates were again recovered only from the *Argas* (*A.*) *reflexus* group ticks which had been collected as before in pigeon bazaar stalls, and, in addition, from a pigeon farm that supplied many pigeons to the bazaar. Thirty *Argas* (*A.*) *reflexus* nymphs from pigeon shops were sorted into two pools. An isolate from one of these pools (ArT 361) again appeared to be related to Quaranfil and also to ArT 282 and 284 (Table 1). Three hundred nymphs from the pigeon farm were separated into 16 groups that subsequently yielded 15 viral isolates. Five of these have been studied. Of these, three are related by CFT to Quaranfil (Table 3) and two are unrelated by the same test to any of our reference stocks or to each other.

For the following tests, ArT 282 and ArT 377 were selected as the Quaranfil-like representatives of the isolates of the September and October tick collections respectively. In a reisolation attempt in infant mice the original tick suspension stored at —60 °C yielded isolate ArT 377. This reisolate was passaged 5 times, the work being done in one room used for housing infected animals and was not the same room in which the virus was originally isolated. All open tube and transfer mani-

Table 3. Complement fixation serological relationship of ArT 364, ArT 365, and ArT 377 Viruses to Quaranfil virus

Antigen	Antiserum			
	ArT 364	ArT 365	ArT 377	Quaranfil
ArT 364	32/8*)	16/8	ND	64/64**)
ArT 365	4/32	8/32	ND	64/64**)
ArT 377	ND***)	ND	128/32	64/8**)

*) Reciprocals of: Serum titer/Optimum antigen titer.

**) Reciprocals of: Serum titer/Antigen titer.

***) ND = Not done.

pulations of the test material for all passages were performed in this same room. The brains were harvested by sterile technique, macerated using mortar and pestle, and made into a 10 per cent suspension in 0.75 per cent BAPS. This suspension was placed in a centrifuge tube, sealed, and taken to the Virology Department for centrifugation. Following centrifugation, the suspension was returned to the animal room, opened, and the supernate immediately inoculated into infant mice. Sixth passage material of this isolate was used to prepare sucrose-acetone extracted antigens for CF tests and virus for neutralization tests.

Four months later, this same process of reisolation described above was repeated using isolate ArT 282 in a different animal room from that in which it was originally isolated, and from that in which isolate ArT 377 was reisolated. The results of CF tests with the reisolated material confirm our earlier findings by CFT as shown in Table 4. Results of the neutralization tests shown in Table 5 indicate that these two viruses are closely related to Quaranfil virus.

Table 4. Serological relationships of reisolated ArT 282, 377, and Quaranfil virus

Antigen	Antiserum		
	ArT 282	ArT 377	Quaranfil
ArT 282	256/64*)	256/64	512/64
ArT 377	256/64	512/64	512/64
Quaranfil	256/128	512/64	256/64

*) Reciprocals: Serum titer/Antigen titer.

Table 5. Neutralization test relationships between ArT 282, ArT 377, and Quaranfil viruses

Virus	Antiserum		
	ArT 282	ArT 377	Quaranfil
ArT 282	6.3*)	6.3	ND
ArT 377	5.8	5.8	5.8
Quaranfil	ND**)	4.6	4.6

*) Logs of virus neutralized.
**) ND = Not done.

DISCUSSION

Quaranfil virus was isolated at the United States Naval Medical Research Unit No. 3 in 1953 by Dr. R. M. TAYLOR (1966) and has been in that laboratory since that time. Inadvertent contamination during the process of initial isolation of these

viruses cannot be ruled out with absolute certainty. However, the circumstances associated with these isolations, some of which are reported here and the remainder to be published in greater detail later, are strongly suggestive that laboratory contamination did not occur. The precautions taken during the reisolation of these viruses and the subsequent results prompt us to report that we have isolated a Quaranfil-like agent on two separate occasions from two separate lots of *Argas* (*A.*) *reflexus* group ticks collected more than a month apart in Kabul Province, Afghanistan. We will in the near future supply WHO Reference Centers for Arboviruses with suspensions of these isolates to confirm the findings reported here.

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