

## ESTERO REAL VIRUS: A NEW VIRUS ISOLATED FROM ARGASID TICKS *ORNITHODOROS TADARIDAE* IN CUBA

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*Summary.* — Isolation and serologic identification of a virus isolated from argasid *Ornithodoros tadaridae* ticks collected in the province Sancti Spiritus in Cuba are described. The virus was named according to the isolation locality El Estero Real. The virus circulation in nature is discussed.

*Key words:* Estero Real virus; argasid tick *Ornithodoros tadaridae*; Cuba

During virologic studies on ticks in Cuba (Danielová *et al.*, 1982; Málková *et al.*, in press) a single collection of argasid ticks (*Ornithodoros tadaridae* Černý and Dusbábek, 1967) was made on April 26, 1980 from the leaves of a palm tree (*Copernicia vespertilionum* Léon, 1931), the crown of which was colonized by bats (*Mormopterus minutus* Miller, 1899). The tree was located near a settlement, El Estero Real, in the northern part of Sancti Spiritus province (latitude 22°22' North, longitude 79°04' West). These argasids are known to be parasites of two species of bats, *Mormopterus minutus* Miller, 1899) and *Tadarida laticaudata yucatanica* (Miller, 1902), which occur in the Caribbean region and in Central America. These two species take shelter in palm trees during the day, forming colonies which often number several hundred specimens (Silva Taboada, 1979).

One hundred ninety nymphs and 340 adult argasid ticks *O. tadaridae* were tested for virus presence. Specimens used in isolation tests were alive and engorged. Prior to testing, ticks were kept in refrigerator at 4°–8 °C. Suspensions from 40–60 nymphs or 40–60 adults were macerated in PBS supplemented with 10% inactivated bovine serum and antibiotics. After centrifugation at 2500 rev/min for 10 min, the supernatant was inoculated into each of a litter of 1- to 2-day-old mice, in volumes of 0.01 ml intracerebrally (i.c.) and 0.03 ml subcutaneously per mouse. The animals were observed daily for three weeks. The brains of sick and dead mice were passaged to fresh suckling mice in the form of 10% clarified suspensions.

Identification tests were carried out by neutralization test (NT) (Lennette and Schmidt, 1969; Lindsey *et al.*, 1976), indirect immunofluorescence (IFA) (Holubová, 1980) and complement-fixation (CF) test (Casey, 1965). A list of immune ascitic fluids (IAF) used in the CFT is given in Table 1.

**Table 1. Group-specific-polyvalent-monospecific immune ascitic fluids (IAF) used in CF-test for K 329 virus identification**

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Group-specific:

BUN, KEM, QRF, Rabies, TRC

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Polyvalent:

No. 1 : BAH, Tete, MTR, MTY, EgAn 1398-61, BEA  
 No. 2 : JUR, MNT, MARU 11079, GAM, BeAn 141106  
 No. 3 : KOO, WON, BAK, KET, MAP, TRU, MPK  
 No. 4 : NYM, UUK, GA, THO  
 No. 5 : HUG, SOL, SAW, MAT, LS  
 No. 6 : MCO, CHO, TIM, PAC  
 No. 7 : HP, FLA, KC, KLA, MEB  
 No. 8 : EHD, CGL, IRI, CFT, BLU, IbAr 22619  
 No. 9 : NAV, TNT, ARU, PCA  
 No. 10 : UPO, DGK, WAN, DHO  
 No. 12 : OKO, OLI, WIT, BIA, TAT

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Monospecific :

AVA, AB, CTF, BAU, GI, HUG, HUA, MAT, ML, LS, NM, POW, PS, SAK, SAW, SIL, SC, SOL, SCA, TYU, UUK, WM and YH.

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Twelve pools of ticks (4 of nymphs and 8 of adults, respectively) were tested for the presence of virus. Eight virus strains were isolated, of which 7 were confirmed in re-isolation tests. The incubation period in the first passage ranged from 9 to 18 days, in the second passage it was reduced to 7—10 days, and in the third and subsequent passages it became stabilized at 6—8 days. By the third passage in mice, i.e. titres of individual strains ranged between 4.4 and 6.8 log LD<sub>50</sub>/0.01 ml.

Hyperimmune serum was prepared in mice against strain K329 which was selected at random according to virus titre and course of infection in mice; the homologous neutralization index (NI) of this serum was 3.5—4.0 log units. In mouse NT and IFA tests the serum reacted significantly with all strains isolated (Table 2), indicating their close antigenic relationships. To verify the identity of strains, a cross IFA test with K329, K331 and K341 strains was carried out, since in the 3rd passage in mice these strains showed slight differences in titres, incubation period, and NI when using K329 antiserum. Nevertheless, the IFA results revealed that the strains were antigenically identical (Table 3).

To establish the antigenic relationships of isolates, strain K329 was used as the prototype for identification tests. At the first stage of these tests, using NT in mice and IFA, we were able to rule out recognized viruses transmitted by ticks in the Caribbean region (Hughes, Soldado, Wad Medani, Sawgrass and African Swine Fever) and certain viruses from outside this region [Powassan, Eyach (Colorado Tick Fever serogroup), Tribeč (Kemerovo), tick-borne encephalitis]. Thereafter, arboviruses of Group A were ruled out using polyvalent IAF for WEE, EEE, VEE, Chikungunya, Sindbis, for Group B using

Table 2. Virus titres and antigenic relationship of isolated strains

Virus strain	Virus titre <sup>1</sup>	Antibody tests with mouse anti-K 329 serum	
		NI	IFA
K 327	4.9*	> 3.48	128
K 328	5.9	2.12	256
K 329	6.6	3.7	256
K 330	6.8	> 4.35	256
K 331	5.8	2.95	256
K 334	4.4*	2.77	256
K 341	5.8	> 2.85	512
K 342	6.3	1.69	256

<sup>1</sup> Mouse i.c. log LD<sub>50</sub>/0.01 ml (3rd mouse passage).

NI - neutralization index.

\* In the 6th mouse passage the virus titres were similar.

polyvalent IAF for dengue 1-4 and yellow fever and individual hyperimmune serum to St. Louis encephalitis, WN and California Group anti-Tahyňa hyperimmune serum. Strain K329 was also tested with antibody to rabies and Tett-nang (related to murine hepatitis virus) viruses. At the second stage of identification, K329 antigen was examined extensively in CF test with polyvalent IAF (Table 1). These tests were also negative. The physico-chemical and biological properties of the virus will be described elsewhere (Málková *et al.*, in press).

The argasid ticks *O. tadaridae* are parasites of bats with a strict total specificity for their hosts (Hoogstraal and Aeschlimann, 1982). Their known hosts are two species of bats, namely *Mormopterus minutus* (Miller, 1899) and *Tadarida laticaudata yucatanica* (Miller, 1902) (Silva Taboada, 1979). In this connection it can be assumed that the above-mentioned bats are also hosts of the isolated virus. Considering that bats and their bloodsucking parasites can harbour different arboviruses as well as other viruses — a significant role being played by *Tadarida* bat species — (Sulkin and Allen, 1974), the isolated virus was investigated serologically for a wide range of arboviruses and other viruses using 5 grouping, 13 polyvalent and 31 individual IAF and sera, all selected according to geographical occurrence, vectors and possible range of hosts.

Table 3. Cross IFA test with 3 virus strains<sup>1</sup> isolated from *O. tadaridae*

Antigen	Serum							
	K 327	K 328	K 329	K 330	K 331	K 334	K 341	K 342
K 329	256	512	<b>1024</b>	512	128	256	128	512
K 331	256	256	1024	512	<b>128</b>	512	128	512
K 341	256	256	512	256	128	256	<b>256</b>	1024

Because all results were negative, we conclude that the Estero Real virus is a new, at present unclassified virus. The high infection rates in tested ticks (8 of 12 pools positive) were apparently due to the fact that all these ticks came from a single site, i.e. from the crown leaves of the same palm tree and even possibly from the same host species.

As for the circulation of virus in nature, the following may be deduced: the fact that *O. tadaridae* from which the virus was isolated feeds on bats and has a strict host specificity suggests that the circulation of Estero Real virus in nature takes place primarily among the argasid ticks and bats of the above-mentioned species. Any relationship to other host species, e.g. birds nesting in palm trees, is improbable. Geographic distribution and circulation of the virus in nature are probably limited, because the two species of bats are rare; one of them is found only in Cuba and the other is known only in the Caribbean and adjacent (pericaribbean) areas (Silva Taboada, 1979). Both bat species may take shelter in human dwellings, but it is at present unknown, whether man is included in the transmission of Estero Real virus.

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