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ISOLATION OF HUGHES VIRUS FROM TICKS IN CUBA

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Summary. — Nine species of ticks parasitizing cattle, horses, bats and seabirds in four provinces of Cuba were examined virologically. A total of 13 strains of Hughes virus (genus *Nairovirus*) were isolated from *Ornithodoros denmarki* ticks.

Key words: ticks; virus Hughes; Nairovirus

In 1979 and 1980, during two expeditions organized by the Czechoslovak Academy of Sciences, the Cuban Academy of Sciences and the Institute of Tropical Medicine "Pedro Kouri" in Havana, ticks in some regions of Cuba were examined virologically. We are reporting the results of the first expedition organized from October till December, 1979. Main attention was directed to ticks of cattle and horses due to their potential economic importance, less to ticks parasitic on seabirds nesting in colonies and on bats which form the main part of the mammalian fauna in Cuba.

Materials and Methods

Ticks were collected directly from farm animals (cattle and horses), in 13 localities in four provinces; Havana, Matanzas, Camagüey, and Isla de la Juventud.

The following species of ticks-bat parasites were collected in caves: *Ornithodoros azteci* Matheson, 1935 in Cueva de Punta del Este — Isla de la Juventud inhabited by *Eptesicus fuscus petersoni* Silva, *Macrotus waterhousei minor* Gundlach and *Natalus micropus macer* (Miller) bats; *Ornithodors viguerasi* Cooley and Kohls, 1941, *Parantricola marginatus* (Banks, 1910) and *Antricola habanensis* Cruz, 1976 were collected in the cave Cueva del Mudo — La Habana, inhabited by *Phyllonycteris* poei Gundlach and *Pteronotus* sp. bats. The two last tick species were collected from the bat quano on the ground, the *Ornithodoros* species were collected on the cave walls.

Ticks from the nesting sites of seabirds *Sterna fuscata* L. and *Anous stolidus* (L.) were collected on the little island Cayo Mono Grande, north of the peninsula Hicacos — Matanzas, during a period when the island was not inhabited by birds. The ticks were collected under stones and logs.

Testing of three specimens of the very rare species *Ixodes capromydis* Černý, 1966 from *Capromys pilorides* G. M. Allen in Rancho Castor — Isla de la Juventud is also included.

Isolation tests were done from ticks stored at —60 °C, partly from live ticks. Suspensions from 1—100 ticks each were inoculated to 1 to 2-day-old mice (0.01 ml intraverebrally — i. c. — or 0.03 ml subcutaneously — s. c.). The mice were observed for three weeks.

The isolated viruses were tested on 2-day-old suckling mice inoculated with 0.01 ml i.c., 0.03 ml s.c. or 0.05 ml intraperitoneally (i.p.), on juvenile mice inoculated with 0.03 ml i.c., 0.05 ml s.c. or 0.1 ml i.p. and in PS Cl_4, Vero, GMK, and CV_1 cell lines. Their susceptibility to the strain K 38 was studied both based on a cytopathic effect (CPE) developing on 48-hr monolayers, and by the

Table 1. Tick species virologically examined

Species	Females	Males	Nymphs	Total
Ixodes capromydis	3	0	0	3
Boophilus microplus	3385	1144	1016	5545
Anocentor nitens	414	336	698	1448
Amblyomma cajennense	315	452	96	863
Ornithodoros denmarki	280	440	30	750
Ornithodoros viguerasi	23	82	0	105
Ornithodoros azteci	—	—	—	235
Parantricola marginatus	21	0	100	121
Antricola habanensis	250	220	550	1020
Total				10090

— = Not identified.

plaque micromethod according to de Madrid and Porterfield (1969). All other virus strains were tested only by the plaque micromethod on CV_1 cells.

Identification of isolated strains was performed by the neutralization test (NT) on suckling mice and by the plaque-reduction neutralization test (PRNT) on the CV_1 cell line (De Madrid and Porterfield, 1969). Hughes virus used for identification was kindly supplied by Dr. D. K. Lvov, Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences. Moscow.

Results and Discussion

In 196 isolation tests we examined a total of 10 090 ticks belonging to 9 species (Table 1).

A total of 7 856 ticks from cattle and horses, represented by three species of the family *Ixodidae*, namely *Boophilus microplus* (Canestr., 1887), *Anocentor nitens* (Neumann, 1897) and *Amblyomma cajennense* (Fabr., 1787), were processed in 152 pools with negative results. Testing of *Ixodes capromydis* was also negative. Ticks parasitizing bats, represented by four species of the family *Argasidae* (1481 specimens), were tested in 27 pools with negative results).

Ticks of seabirds were represented by *Ornithodoros denmarki* Kohls, Sonennshine and Clifford, 1965 of the family *Argasidae*. A total of 750 ticks was tested in 16 pools; 13 pools prepared from nymphs and adults of both sexes were positive. Virus was reisolated in 9 out of 11 reisolation tests. In cubation period on isolations varied from 5-16 days, mostly from 6-9 days. All inoculated mice died since the second passage. Sick mice lay on their side, sometimes showing paraeses of hind legs; most animals died without this symptom within 24 hr. All 13 strains behaved similarly and strain K 38 was used in subsequent experiments.

The susceptibility of mice and cell lines was tested with second passage virus. In suckling mice, the titre of the strain K 38 reached 5.71 log LD_{50}/ /0.01 ml and 4.62 log LD_{50}/0.05 ml after i. c. and i. p. inoculation respectively. Single mice inoculated s.c. died after a prolonged incubation period. Juvenile mice died only after i.c. inoculation (3.37 log LD_{50}/0.03 ml). Monolayers of $PSCl_4$ cells showed no specific changes during 14 days p.i. In Vero and

Table 2. Cross-neutralization test with strain K 38 and Hughes virus

Antigen	Serum			
	K 38 log LD_{50}	Hughes log LD_{50}	K 38 log PFU	Hughes log PFU
K 38	2.47	1.38	2.47	2.17
Hughes	3.07	$\leqq$ 2.10	2.22	2.20

GMK cells, mild alteration of cells appeared after 48 hr of incubation; a mild CPE appeared 5 days p.i. up to a dilution of 10^{-4}. In CV_1 cells a clear-cut CPE was observed 96 hr p.i. and was stabilized till the 7th day p. i.; the titre reached 6.3 log $TCID_{50}$/0.2 ml. Plaques were formed only in CV_1 cells. They were very small, clear-cut, reaching the largest size and number 7 days p. i. The plaques of the various strains isolated were homogeneous, mostly 1 mm in diameter. Hughes virus, tested for comparison, produced larger plaques (2 mm).

The susceptibility of suckling mice and the CV_1 cell line to all isolated strains was compared by titration of the strains in their 2nd passage. In repeated tests, the titres reached values from 3.83 log LD_{50}/0.01 ml (K 49) to 5.71 log LD_{50}/0.01 ml (K 38) in mice and from 0.53×10^6 (K 49) to 0.33×10^8 (K 52) PFU/0.2 ml in CV_1 cells.

The isolated virus proved to be a weak antigen and induced a very poor neutralizing antibody formation. A neutralization index (NI) of > 2 log LD_{50} was obtained after application of 10 immunizing doses of strains K 38, K 42 or K 52. All isolated strains were tested in NT and PRNT with anti-K 38 serum. The NI of the various strains differed more in the NT than in the PRNT, but most of them were > 2 log LD_{50} (up to 2.97 log LD_{50}). Strain K 52 showed the lowest NI (1.1 log LD_{50}). In a cross-neutralization test between the strains K 38 and K 52, the NI of homologous and heterologous antigen and antiserum differed by more than 1 log LD_{50}. Values of NI in PRNT were more uniform, reaching 2.11—2.70 log PFU in repeated tests. We assume, therefore, that all isolated strains are related but not antigenically identical.

A cross-neutralization test with strain K 38 and virus Hughes suggests (Table 2) that the isolated virus is identical or closely related to virus Hughes (Hughes serogroup of the *Nairovirus* genus).

During an arbovirus study in Cuba we placed special emphasis on ticks. Their highest number was from cattle and horses because of their possible economic importance. All three species found do not belong to the indigenous fauna of Cuba; they were introduced there together with cattle and horses in the historical period. Therefore it could not be excluded that some arbovirus not autochthonous in this region had been introduced together with them. Although we were unable to study ticks in the whole territory of Cuba, the largest area of cattle rearing, Camagüey, was included and ticks were collected in several farms there. The number of cattle ticks tested seem

to offer sufficient evidence that these ticks are not vectors of any economically important viruses.

The identification of the isolated virus was directed by the fact that *Ornithodoros* ticks of seabirds were the source of Hughes and Soldado virus isolations in Central America (Hugnes *et al.*, 1964; Aitken *et al.*, 1968; Jonkers *et al.*, 1973). The isolated strains clearly differed from Soldado virus in incubation period and course of infection. According to one of us (A. Fernández) some years ago Hughes virus had been isolated from ticks of seabirds on a little island south of Cuba. Isolation of 13 virus strains from *O. denmarki* on a rather small island gives evidence of a high prevalence of Hughes virus in ticks there.

References

Aitken, T. H. G., Jonkers, A. H., Tikasingh, E. S., and Worth, C. B. (1968): Hughes virus from Trinidadian ticks and terns. *J. med. Entomol.* **5**, 501–503.

Hughes, L. E., Clifford, C. M., Thomas, L. A., Denmark, H. A., and Philip, C. B. (1964): Isolation and characterisation of a virus from soft ticks (*Orgnithodoros capensis* group) collected on Bush Key, Dry Tortugas, Florida. Amr. *J. trop. Med. Hyg.* **13**, 118–122.

Jonkers, A. H., Casals, J., Aitken, T. H. G., and Spence, L. (1973): Soldado virus, a new agent from Trinidadian Ornithodoros ticks. *J. med. Entomol.* **10**, 517–519.

Madrid, A. T. de, and Porterfield, J. S. (1969): A simple micro-culture mehod for the study of group B arboviruses. *Bull. Wld. Hlth. Org.* **40**, 113–121.