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ISOLATION OF THOGOTO VIRUS FROM TICKS IN PORTUGAL

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Summary. — An agent pathogenic for mice was isolated from *Rhipicephalus sanguineus* ticks collected from goats from Vila Vicosa, Portugal. The virus was shown serologically to be closely related to or identical with Thogoto virus, which had previously been isolated from ticks over a wide geographic area including Sicily, the African continent, and Iran.

Key words: *Thogoto virus*; *tick-borne virus*; *arbovirus isolation*; *Portugal*; *Europe*; *orthomyxovirus*

More than ten years ago, a research program was initiated to investigate the presence, prevalence, and public health importance of tick-borne viruses in Portugal. In 1971, Dhori virus was isolated from *Hyalomma m. marginatum* ticks collected from bovines recently arrived in Lisbon from Vidigueira, 140 km southeast of Lisbon (Filipe and Casals, 1979). In 1978, two strains of another virus were isolated from pooled *Rhipicephalus sanguineus* ticks taken from goats arriving in Lisbon from the Vila Vicosa region. This virus was shown serologically to be closely related to or identical with Thogoto virus, which has been isolated from ticks over a wide geographic area including Africa, Iran, and Sicily.

In June and July 1978, a total of 79 ticks were collected at a slaughterhouse in Lisbon, from goats recently arrived from the Vila Vicosa region of Portugal. Vila Vicosa (Lat. 38°47′ N; Long. 7°25′ W) is approximately 150 km east of Lisbon. The ticks were identified as *Rhipicephalus sanguineus* (Latreille, 1806) and were stored at —70 °C until processed for virus isolation. Four mixed pools of male and female *R. sanguineus* ticks were homogenized in 3 ml of phosphate-buffered saline containing 0.75% bovine albumin and antibiotics. The suspension was centrifuged at 3000 rev/min for 30 min at 4 °C. After filtration (450 nm), the supernatant fluids were inoculated intracranially (i.c.) into 2-day-old Swiss albino mice, Charles River strain. Mice were observed daily for 14 days, sick animals were killed, and brains from these infected mice were serially propagated by i.c. passage in suckling mice until the average survival time of the mice stabilized.

Alkaline extracts of infected mouse brains were centrifuged at 10,000 rev/min for 30 min and used as crude antigens for complement-fixation (CF) tests (Casey, 1965). This and control CF antigens (Clarke and Casals, 1958) were tested with the following antibody preparations: (Grouping fluids) NIH-1 (Bahig, Tete, Matruh, Matariya, EgAn 1398-61, Burg el Arab), NIH-2 (Ju-

rona, Minatitlan, Belem, Alajuela, Gamboa), NIH-3 (Koongol, Wongal, Bakau, Ketapang, Maputta, Maprik, Trubanaman), NIH-4 (Nyamanini, Uukuniemi, Grand Arbaud, Thogoto), NIH-5 (Hughes, Soldado, Sawgrass, Matucare, Lone Star), NIH-6 (Marco, Chaco, Timbo, Pacui), NIH-7 (Hart Park, Flanders, Kern Canyon, Klamath, Mount Elgon Bat), NIH-8 (Epizootic Hemorrhagic Disease of Deer, Changuinola, Colorado Tick Fever, Irituia, Bluetongue, IbAr 22619), NIH-9 (Navarro, Triniti, Aruac, Pacora), NIH-10 (Upolu, Dera Gazi Khan, Wanowrie, Dhori), NIH-12 (Okola, Olifantsvlei, Wirwatersrand, Bobia, Tataguine), A, B, C, Anopheles A (Anopheles A, Lukuni, Tacaiuma, Anopheles B, Boraceia), Bwamba (Bwamba, Pongola, Nyando, Eretmapodites 147, Mossuril, Kamese), California, Congo (Congo, Hazara, Ganjam, Dugbe, Bhanja), Kemerovo, Palyam (Palyam, Vellore, Kasba, Corriparta, Acado, Eubenangee, Pata, D'Aguilar), Phlebotomus Fever, Quaranfil (Quaranfil, Johnston Atoll, Qalyub, Bandia, Kaisodi, Lanjan, Silverwater), Simbu, vesicular stomatitis, Tacaribe, rabies (Rabies, Lymphocytic choriomeningitis, Vaccinia, Herpes simplex, Newcastle Disease), and individual West Nile, Powassan, Sindbis, Venezuelan equine encephalitis, Silverwater, Thogoto, Uukuniemi, Kemerovo, Soldado, Bhanja, Nyamanini, Aransas Bay, Tete, Lone Star, Colorado Tick Fever, Chenuda, Sakhalin, Sawgrass, Tribec, and Enseada.

Mice 5 to 7 days after being inoculated with clarified supernatant fluids from two of the four pools of ticks. The mice died very suddenly, often appearing well when examined and then being found dead 2 to 4 hr later. With passage in suckling mice, the average survival time stabilized at two days. Crude antigens of both strains (PoTi 503 and PoTi 509) reacted in CF tests only with antibody to Thogoto virus and NIH-4, which contains antibody to Thogoto virus. A virus dilution-Thogoto antibody neutralization test in suckling mice confirmed this finding. Therefore, strains PoTi 503 and PoTi 509 are strains of Thogoto virus or a closely related virus.

Prototype Thogoto virus (strain IIA) was isolated from a pool of *Boophilus decoloratus*, *Rhipicephalus appendiculatus*, *R. simus*, and *R. evertsi*, collected in September 1960 from cattle in the Thogoto forest near Nairobi, Kenya (Haig *et al.*, 1965). Subsequent isolations from Cameroon and Central African Republic (Sureau *et al.*, 1976), Nigeria (Berge, 1975; Causey *et al.*, 1969; East African Virus Research Institute Report, 1968; Ibadan Arbovirus Annual Report, 1969; Kemp *et al.*, 1973; Moore *et al.*, 1975), Uganda (East African Virus Research Institute Report, 1968), Italy (Albanese and Bruno-Smiraclia, 1972), Egypt (Williams *et al.*, 1973), Ethiopia (Wood *et al.*, 1978), and Iran (Sureau *et al.*, 1980) suggest that Thogoto virus is a tick-borne virus with wide geographic distribution. Its isolation from humans, one with optic neuritis and another with a fatal meningoencephalitis (Moore *et al.*, 1975) emphasizes its potential importance in public health.

Thogoto virus strains have been isolated from humans, domestic cattle, and camels or from ticks removed from these hosts, sheep, or goats in central, east central, and northeast Africa; the Middle East; and southern and western Europe. The ticks from which these isolates were obtained (*Rhipicephalus*, *Boophilus*, *Amblyomma*, and *Hyalomma* species) feed almost exclusively, as adults, on herbivores, including domestic cattle and wild ruminants (Hoogstraal and Aeschlimann, 1982). *Boophilus decoloratus* and *annulatus* are one-host ticks; *Rhipicephalus*, one- or two-host ticks; and *Amblyomma*, three-host ticks. Whereas, *Amblyomma* and *Hyalomma* ticks may utilize wild birds as hosts, *Rhipicephalus* and *Boophilus* species do not. It is thus possible that the relationship between certain ticks and migrating birds is

responsible for the dissemination and wide distribution of Thogoto virus but that, once introduced, foci of Thogoto virus are found in ticks feeding principally on wild and domesticated ruminants. It is, however, possible but unlikely that tick-infested domestic animals are responsible for the introduction of certain viruses to Portugal.

Antigenic and other comparisons of strains of Thogoto virus from different geographic areas may shed some light on this problem. In fact, it has already been recognized (Srihongse *et al.*, 1974) that the Sicilian strain of Thogoto virus is a subtype of the prototype. More recently, Clerx *et al.* (1983) have demonstrated that Thogoto virus possesses structural characteristics similar to those of accepted orthomyxoviruses. Given that Thogoto virus is a human pathogen, further studies with this virus are necessary.

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References

Albanese, M., and Bruno-Smiraclia, C. (1972): Isolation of Thogoto virus from *Rhipicephalus bursa* ticks in Western Sicily. *Acta virol.* **16**, 267.

Berge, T. O. (Ed.): *International Catalogue of Arboviruses*, 2nd ed., DHEW Publ. No. (CDC) 75-8301, 1975.

Casey, H. L. (1965): Standardized diagnostic complement-fixation method and adaptation to microtest, part II, pp. 31—34. Adaptation of LBCF method to micro technique. *Public Health Monogr. No.* **74**, Public Health Service Publication No. 1228, U.S. Government Printing Office, Washington, DC.

Causey, O. R., Kemp, G. E., Madbouly, M. H., and Lee, V. H. (1969): Arbovirus surveillance in Nigeria, 1964—1967. *Bull. Soc. Path. exot.* **62**, 249—253.

Clarke, D. H., and Casals, J. (1958): Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am. J. trop. Med. Hyg.* **7**, 561—573.

Clerx, J. P. M., Fuller, F., and Bishop, D. H. L. (1983): Tick-borne viruses structurally similar to orthomyxoviruses. *Virology.* **127**, 205—219.

East African Virus Research Institute Report (1968) **18**, 32.

Filipe, A. R., and Casals, J. (1979): Isolation of Dhori virus from *Hyalomma marginatum* ticks in Portugal. *Intervirol.* **11**, 124—127.

Haig, D. A., Woodall, J. P., and Danskin, D. (1965): Thogoto virus: A hitherto underscribed agent isolated from ticks in Kenya. *J. gen. Microbiol.* **38**, 389—394.

Hoogstraal, H., and Aeschlimann, A. (1982): Tick-host specificity. *Bull. Soc. ent. Suisse* **55**, 5—32.

Ibadan Arbovirus Annual Report (1969), 188.

Kemp, G. E., Causey, O. R., Moore, D. L., and O'Connor, E. H. (1973): Viral isolates from livestock in northern Nigeria: 1966—1970. *Am. J. vet. Res.* **34**, 707—710.

Moore, D. L., Causey, O. R., Carey, D. E., Reddy, S., Cooke, A. R., Akinkugbe, F. M., David-West, T. S., and Kemp, G. E. (1975): Arthropod-borne viral infections of man in Nigeria, 1964—1970. *Ann. trop. Med. Parasit.* **69**, 49—64.

Srihongse, S., Albanese, M., and Casals, J. (1974): Characterization of Thogoto virus isolated from ticks (*Rhipicephalus bursa*) in Western Sicily, Italy. *Am. J. trop. Med. Hyg.* **23**, 1161 to 1164.

Sureau, P., Ravisse, P., Germain, M., Rickenbach, A., Cornet, J. P., Fabre, J., Jan, Ch., and Robin, Y. (1976): Isolement du virus Thogoto a partir de tiques *Amblyomma* et *Boophilus* en Afrique Centrale. *Bull. Soc. Path. exot.* **69**, 207—212.

Sureau, P., Klein, J. M., Casals, J., Digoutte, J. P., Salaun, J. J., Piazak, N., and Calvo, M. A. (1980): Isolation of Thogoto virus, Wad Medani virus, Wanowrie virus and Crimean-Congo hemorrhagic fever virus from ticks of domestic animals in Iran. *Ann. Virol.* (*Paris*) **131**, 185—200.

Williams, R. E., Hoogstraal, H., Casals, J., Kaiser, M. N., and Moussa, M. I. (1983): Isolation of Wanowrie, Thogoto and Dhori viruses from *Hyalomma* ticks infesting camels in Egypt. *J. med. Ent.* **10**, 143—146.

Wood, O. L., Lee, V. H., Ash, J. S., and Casals, J. (1978): Crimean-Congo Hemorrhagic Fever, Thogoto, Dugbe, and Jos viruses isolated from ixodid ticks in Ethiopia. *Am. J. trop. Med. Hyg.* **27**, 600—604.