

Arde Virus, a New Ungrouped Arbovirus Infecting *Amblyomma Loculosum* Ticks from Roseate Terns in the Seychelles*

Brief Report

By

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Summary

A previously undescribed arbovirus, proposed name Arde virus, was recovered from *Amblyomma loculosum* Neumann, collected from dead roseate terns *Sterna dougallii arideensis* Mathews in the Seychelles. Some important tick-virus-host associations are discussed.

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Irregularities in flights of marine birds, owing to injury, illness, or exhaustion, may be an important factor in the geographic distribution of certain arboviruses and tick parasites, some of which cause human illness (3). The exceptionally wide distribution of hard and soft ticks and their numerous host species, and the fact that humans visiting bird breeding colony areas are bitten and may become infected by virus infected ticks, emphasize the importance of studies on the identification and characterization of previously unknown, or suspected new viral agents.

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Herein we report the recovery of an apparently new arbovirus infecting the hard tick *Amblyomma loculosum* Neumann, which infests marine birds in the Seychelles, Indian Ocean. *A. loculosum* is recorded from 14 species and subspecies of marine birds from the Indian Ocean (Tanzania and islands in the Seychelles, Mauritius, and Cocos Keeling groups), and from islands in the Coral Sea off Queensland and near New Caledonia and in the Caroline Group in the Pacific Ocean (4). People visiting infested localities are frequently bitten by *A. loculosum*.

Three female *A. loculosum* were collected on 8 July 1973 by C. J. Feare from the feet of the 2 freshly dead roseate terns on Bird Island in the Seychelles, about 40 miles from Aride Island where numerous roseate terns breed. The collector was investigating a dieoff of thousands of sooty terns, *Sterna fuscata nubilosa* Sparmann, associated with a high population density of another tick species, *Ornithodoros (Alectorobius) capensis* Neumann, infected by Soldado virus of the Hughes serogroup (2).

Two of the 3 *A. loculosum* (HH37, 435) from the dead roseate terns were placed in a single pool and inoculated into 2 suckling white mice families. The third *A. loculosum* was preserved for taxonomic studies.

The virus recovery methods, virus characterization [filtration, sodium deoxycholate (SDC) sensitivity], serological procedures [hemagglutinin (HA), complement-fixation (CF), and neutralization (NT) tests], experimental infection in laboratory animals and neutralization index (NI) calculations were as described earlier (1).

A virus strain (EgArt 3088) was recovered and reisolated 9 months later from the original tick suspension. Primary virus passage in mice was variable, with 5 of 14 mice sick by day 8 (range 4 to 8 days). Second and third infected mouse brain (IMB) passage caused illness and paralysis in all 14 mice by day 4. Sixth passage IMB suspension log LD₅₀ (50 per cent lethal dose) was 10^{3.5}/ml intracerebrally (i.c.) and 10^{2.0}/ml intraperitoneally (i.p.); the average survival time was 5 days. The ICLD₅₀ titer following filtration through 0.45 and 0.22 μ millipore membrane filter was 10^{3.0} and 10^{1.2}/ml, respectively. In other studies EgArt 3088 demonstrated SDC sensitivity, i.e., 4.5 log reduction in titer.

In CF screen tests at NAMRU-3 and at YARU, using reference monovalent and polyvalent hyperimmune mouse sera to known arboviruses (Table 1), no serological relationship was established with any known arbovirus group, including Soldado virus, which was isolated from other ticks in the Seychelles (2). HA tests with sucrose-acetone extracted antigen of EgArt 3088 and goose erythrocytes at pH ranges 5.8–7.2 and 4° and 37° C were also negative.

Sixth passage IMB suspension caused paralysis and death in suckling mice by both i.c. and i.p. inoculation routes, but not in suckling hamsters, guinea pigs or rabbits. CF antibody production to EgArt 3088 virus was demonstrated in hamster and rabbit sera (1:4 and 1:32, respectively). No cytopathological reaction or virus multiplication were demonstrated in chick embryo fibroblast, Vero, BHK-21, embryonic hamster, or HeLa cell cultures using ten-fold dilutions (10² to 10⁸) of EgArt 3088 virus IMB suspensions.

Virus isolate EgArt 3088 appears to be a previously undescribed, ungrouped arbovirus. The dead roseate terns carrying the infected ticks probably became infested and apparently infected in their breeding grounds on Aride Island. We propose the name Aride virus for this agent.

Table 1. Reference virus-immune serum tested by complement-fixation with Aride virus EgArt 3088 at NAMRU-3 and YARU

Abu Hammad (EgArt 1194)	Pathum Thani (EgArt 753)
Abu Mina (EgArt 4996-63)	Powassan
Acado (Ar 1846-64)	Pretoria (EgArt 3089)
Arumowot (Ar 1284-64)	Punta Salinas (CalAr 888)
Bahig (EgAnB 90)	Qalyub (EgArt 237)
Bandia (IPD/A)	Quaranfil (EgArt 1113)
Bhanja (Ig 690)	Royal Farm (EgArt 371)
Bunyamwera	Sakhalin (56300-86)
Burg El Arab (An 3782-62)	Sandfly Fever (H 5202)
Chenuda (EgArt 1152)	Sandfly Fever (AR 443-61)
Chobar Gorge (707100-8)	Sawgrass (Ta 14-64A-1247)
Colorado Tick Fever (Condon)	Silverwater (M 3737)
Congo (Ug 3010)	Simbu (An 6165-31)
Dengue-1	Sindbis (Original)
Dera Ghazi Khan (JD 254)	Soldado (Tr 52214)
Dhori (Ig 61-1313, EgArt 930)	Thogoto (EgArt 734)
Dugbe (IgAr 1792)	Tribev (Original)
Farallon (CalAr 846)	Uganda S
Ganjam (Ig 619)	Upolu (C 5581)
Grand Arbaud (EgArt 363)	Uukuniemi (An 1825-61)
Hazara (JC 280)	Wad Medani (EgArt 492)
Huacho (CalAr 883)	Wanowrie (Ig 700, EgArt 865)
Hughes (Dry Tortugas)	West Nile (Eg 101)
Johnston Atoll (LBJ)	Yellow Fever (17D)
Jos (IbAr 17854)	Zika
Kaisodi (G 14132)	Zirqa (Por 7866, HRT 619)
Kao Shuan (EgArt 904)	Q 3255
Kemerovo (EgAn 1169-61)	EgArt 427
Keterah (Hooper 1361)	EgArt 1147
Lagos Bat	EgArt 1475
Langat (TP 21)	EgArt 2771
Lanjan (TP 94)	EgArt 3080
Lipovnik (Lip 91)	EgArt 3091
Lone Star (TM 1381)	EgArt 3608
Manawa (PakAr 462)	EgArt 3716
Matariya (EgAn 1477-61)	EgAr 1169-64
Matruh (EgAn 1047-61)	EgAr 1225-64
Matucare (Maru 21343)	EgAnB 12502
Mono Lake (CalAr 861)	EgAnB 13383
Nyamanini (EgArt 1304)	EgAnB 1398-61
Obodhiang (Ar 1275-64)	

Aride virus is ecologically associated with marine birds and ticks occupying restricted habitats where birds breed or rest and virus circulation may be seasonal or continue throughout the year (3). Presently there is no proof of transstadial or transovarial transmission of Aride virus in *A. loculosum*, nor that other species of marine birds are susceptible. Antibody to Aride virus has yet to be demonstrated in the marine birds or humans.

It is noteworthy that Aride virus, characterized here, was isolated from fully fed female ticks taken from the feet of 2 dead roseate terns, *Sterna dougallii arideensis* Mathews, in full adult breeding plumage, on an island about 40 miles from the nearest breeding colony. The fed ticks were capable of ovipositing, and

larvae hatching where the terns fell might easily have found new hosts among the tens of thousands of other marine birds nesting nearby. Some of the frequently documented but usually unexplained deaths of numerous marine birds in many parts of the world may be caused by poorly-known arbovirus infections. The number of reports of *Amblyomma loculosum* attacking humans on islands in the Indian and Pacific oceans and in the Coral Sea (4) enhance interest in the epidemiology of Aride virus.

The lack of knowledge of the ecology and infectivity of Aride virus indicates the need for further work to establish the importance of this agent and its epidemiology in nature.

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