TUNIS VIRUS: A NEW *PHLEBOVIRUS* FROM *ARGAS REFLEXUS HERMANNI* TICKS IN TUNISIA

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Received May 17, 1994; revised July 11, 1994

Summary. – A new *Phlebovirus* provisionnally named Tunis virus has been isolated from *Argas reflexus hermanni* ticks parasitizing domestic pigeons. It is the first isolation of an arbovirus from Tunisia and the fourth tick-borne virus to be isolated from the Moghreb following Soldado, Essaouira and Kala Iris in Morocco. The pathogenic potential of this virus is briefly discussed according to the behaviour of its vector and previous serosurveys in the country.

Key words: Tunis virus; *Phlebovirus*; *Bunyaviridae*; *Argas reflexus hermanni*; domestic pigeons; Tunisia

Introduction

The current status of arboviruses in "the Moghreb", North-western Africa, has been recently reviewed as a part of the description of two new orbiviruses, Essaouria and Kala Iris, from Morocco (Chastel *et al.*, 1993). Although previous serosurveys in man and animals have evidenced the actual circulation of mosquito-, phlebotomus-, and tick-borne arboviruses in Tunisia (Nabli *et al.*, 1970; Chastel *et al.*, 1977; 1983) no isolation of such viruses has so far been reported from this country.

The authors report the isolation and characterization of an apparently new tick-borne virus belonging to the *Phlebovirus* taxon (genus), *Bunyaviridae* family, and provisionally named "Tunis virus" according to the location of its original isolation, the city of Tunis, Tunisia.

Materials and Methods

Tick collections. During the end of September and the beginning of October 1989, 214 specimens of ticks were collected by two of us (C.V. and A.B.) in burrows of small wild mammals from different biotopes near El Kef city, in the mountainous northern part of Tunisia, and from a colony of domestic pigeons (*Columba livia*) inhabiting dwellings of the Faculty of Medecine of Tunis (36.48 °N – 10.11 °E). Ticks were identified as follows: 136 *Ornithodoros erraticus*, 18 *O. normandii* and 55 *Argas reflexus* group: these later specimens were more precisely identified further as *A. reflexus hermanni* Audouin 1827 according to Hoogstraal and Kohls (1960). All the specimens were sent alive to the Brest Virus Laboratory by air and post.

Isolation and virological procedures. Ticks were triturated in pools of 1 – 30 nymphs or 2 – 24 adults. The diluent was MEM with 2% calf serum, antibiotics and amphotericin B. Pools were inoculated intracerebrally (ic) into 24 – 48 hrs-old suckling mice (SM). The only isolate we obtained, Brest/Ar/T2756, was adapted to SM by serial ic passages and a reisolation attempt was made from the original material stored -70 °C.

Virological studies were performed in SM and 21 day-old weanling mice inoculated by ic and intraperitoneal (ip) routes. End points of virus titrations were expressed in a standard way. Virus size was estimated by filtration through a Schleicher and Schuell filter with 200 nm pores. The effects of diethylether, pH 3.0 acidity and heat at 60 °C for 1 hr were tested in SM.

Serology. Hyperimmune ascitic fluid (IAF) was prepared against the Brest/Ar/T2756 isolate in weanling mice using Ehrlich sarcoma ascites. Antigen was prepared from infected SM brains by sucrose-acetone extraction (Clarke and Casals, 1958). Haemagglutination of 24 hrs-old chick erythrocytes was tested at pH 5.8 to 7.4 at 4 °C, 20 °C and 37 °C. T2756 complement-fixing antigen (CF) was screened against grouping and polyvalent IAF kindly supplied by NIAID, Bethesda, MD, USA.

For typing T2756, one-way CF tests and microprecipitation tests (Chan, 1965) were performed at the Brest Virus Laboratory while cross CF tests and plaque-reduction neutralization tests (PRNT) were done at the Department of Health and Human Services, Arbovirus Disease Branch, Fort Collins, CO, USA. PRNT were performed using Vero E6 cell cultures according to the procedure described by Lindsey *et al.* (1976).

Light and transmission electron microscopy (TEM). Light microscopy was done on coronal sections of infected SM brains whereas ultrathin sections and clarified negatively stained suspensions from the same material were examined by TEM.

Results

Tunis virus isolation

One strain of virus referred as to Brest/Ar/T2756 or "Tunis virus" was isolated from a pool of 24 partially engorged *A. r. hermanni* and reisolated 37 days after the original isolation. Another pool of 31 nymphs of the same tick and of the same origin proved negative as all the other tick suspensions tested.

Virological properties of Tunis virus

Paralysis occured in SM 16 – 21 days after ic inoculation of the original pool but during further serial ic passages the incubation period was reduced to 8 days. The third passage material was pathogenic for both SM and weanling mice but only by the ic route. Tunis virus passed through the 200 nm membrane filter and was found sensitive to diethylether, acidic pH and elevated temperature: ic titers in SM were reduced from 6.4 log units (for controls) to 2.6, <2.0 and <2.0, respectively.

Table 1. Brest/Ar/T2756 virus identification using CF test and UUK group antibodies[a]

Immune fluid	CF titers against T2756	Homologous titers
Oceanside	<4	1024
RML 105 355	16	256
Uukuniemi (S 23)	<4	128
Grand Arbaud	<4	256
Manawa	<4	256
Zaliv Terpeniya	<4	256
Brest/Ar/T260 (Cap Sizun)	8	512
Sumakh	<4	256
Brest/Ar/T2756	64	64

[a]Ponteves and Precarious Point antibodies not available.
Tests performed at the Laboratory of Virology, Faculty of Medicine, Brest.

Serological properties of Tunis virus

Haemagglutinin was not detected but CF antigen and antibody were easily obtained. In one-way CF tests, 14 polyvalent or grouping fluids failed to react with two CF units of Tunis antigen. Using 45 type-specific fluids, low CF titer reactions were demonstrated for two Uukuniemi group viruses, namely RML 105 355 and Brest/Ar/T260 (Table 1). These two viruses have been isolated from *Ixodes* (*C.*) *uriae* ticks parasitizing marine birds in Alaska and Brittany (Chastel, 1988). Antibodies to Precarious Point virus from Australia and Ponteves virus from Camargue, France, were not available.

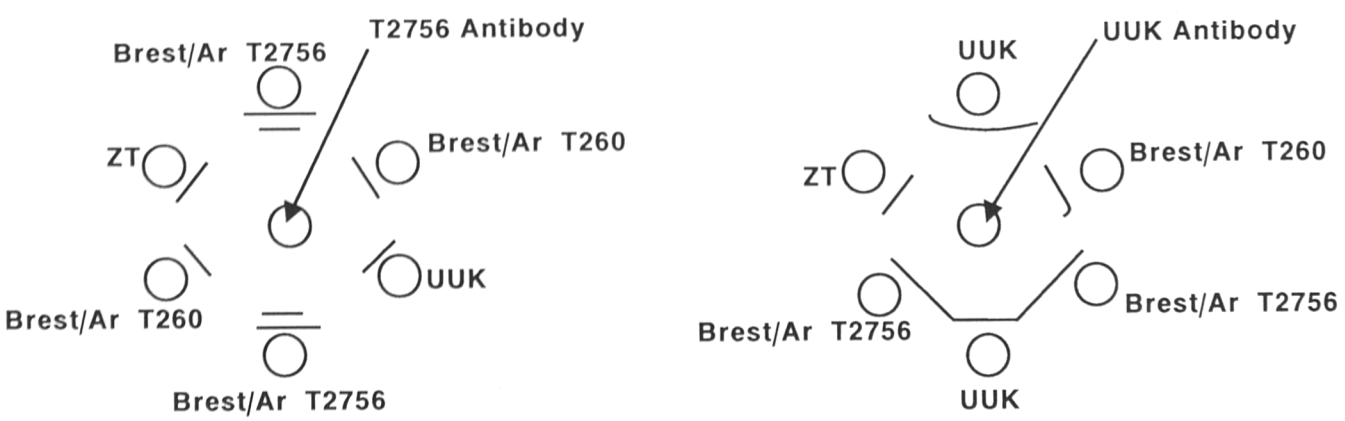

Fig. 1
Diagrams representing the results of microprecipitin tests performed between Tunis virus and Uukuniemi group viruses
Antibody in the central hole and antigens in the peripheral holes. UUK: Uukuniemi; ZT: Zaliv Terpeniya; Brest/Ar/T260: a ZT – like virus from France; Brest/Ar/T2756: Tunis.

Immunoprecipitin tests (Fig. 1) showed extensive cross reactions of Tunis virus in the Uukuniemi group viruses, i.e. Uukuniemi, Zaliv Terpeniya and Brest/Ar/T260, thus confirming the membership of Tunis virus in the Uukuniemi antigenic group.

Cross CF tests and PRNT, including Precarious Point antigen and antibody proved Tunis was definitely a member of the Uukuniemi antigenic group and, thus far, distinct from other recognized members of this group (Tables 2 and 3).

Table 2. Complement-fixation relationship of Brest/Ar/T2756 virus to members of the Uukuniemi antigenic group

Viral antigens	Results of CF grid titrations using antibodies to					
	UUK	GA	MWA	PP	ZT	Brest/Ar/T2756
Uukuniemi (UUK)	64/128					<8/<16
Grand Arbaud (GA)		128/1024				<8/<16
Manawa (MWA)			64/1024			<8/<16
Precarious Point (PP)				128/1024		<8/<16
Zaliv Terpeniya (ZT)					64/512	<8/<16
Brest/Ar/T2756	128/32	64/32	32/32	64/32	64/32	64/32

[a]Optimal antigen titer/antibody titer at optimal antigen dilution. Tests performed at the Department of Health and Human Services, Arbovirus Disease Branch, Fort Collins.

Table 3. Neutralization relationships of Brest/Ar/T2756 virus to members of the Uukuniemi antigenic group

Virus	PRNT titer of antibody to					
	GA	MWA	PP	UUK	ZT	Brest/Ar/T2756
Grand Arbaud (GA)	640					<20
Manawa (MWA)		ND				
Precarious Point (PP)			160			<20
Uukuniemi (UUK)				320		<20
Zaliv Terpeniya (ZT)					ND	
Brest/Ar/T2756	<20	<20	<20	<20	<20	320

Tests performed at the Department of Health and Human Services, Arbovirus Disease Branch, Fort Collins. PRNT: plaque-reduction neutralization test; Titer: reciprocal of highest dilution of antibody giving >90% neutralization; ND: not done

However, PRNT were partly incomplete since suitable plaque assays were missing for both Manawa and Zaliv Terpeniya viruses (Table 3). In addition, it was not possible to compare Tunis with Ponteves virus because this agent previously isolated in southern France from *Argas reflexus* (Hannoun *et al.*, 1970) was apparently lost and no Ponteves antibody was available in any reference center.

Light and electron microscopy

SM brains infected by Tunis virus exhibited marked histological signa of acute encephalitis including extensive necrosis of neurons, diffuse vasculitis and perivascular cuffing.

By TEM, rare viral particles exhibiting a typical *Bunyaviridae* morphology were seen in extracellular spaces of the neuropil (Figs. 2 and 3). Their mean diameter was 81 nm and their surface exhibited large spikes as currently seen with Uukuniemi group viruses (Von Bonsdorf *et al.*, 1970; Von Bonsdorff and Pettersson, 1975; Chastel *et al.*, 1979, 1981). In spite of a careful survey of neurons, no budding was detected into Golgi complexes although they were dilated and they represent selective site for Uukuniemi virus group maturation (Kuismanen *et al.*, 1982). Negative staining of clarified viral suspensions showed rare deformed particles with a mean diameter of 92 nm (details not shown).

Discussion

Serological results clearly showed that Tunis virus, strain Brest/Ar/T2756, belongs to the *Phlebotomus* genus of the *Bunyaviridae* family and strongly suggests that this virus is

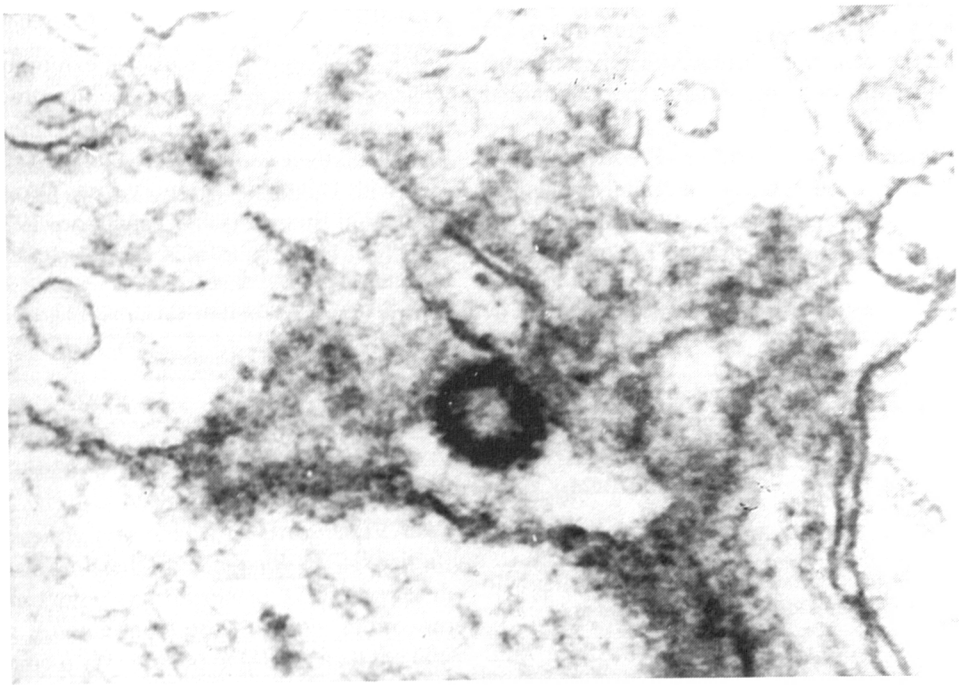

Fig. 2
TEM of suckling mouse brain infected by Tunis virus
A typical virus particle with well-defined surface projections between elements of the neuropil (magn. 140 000 x).

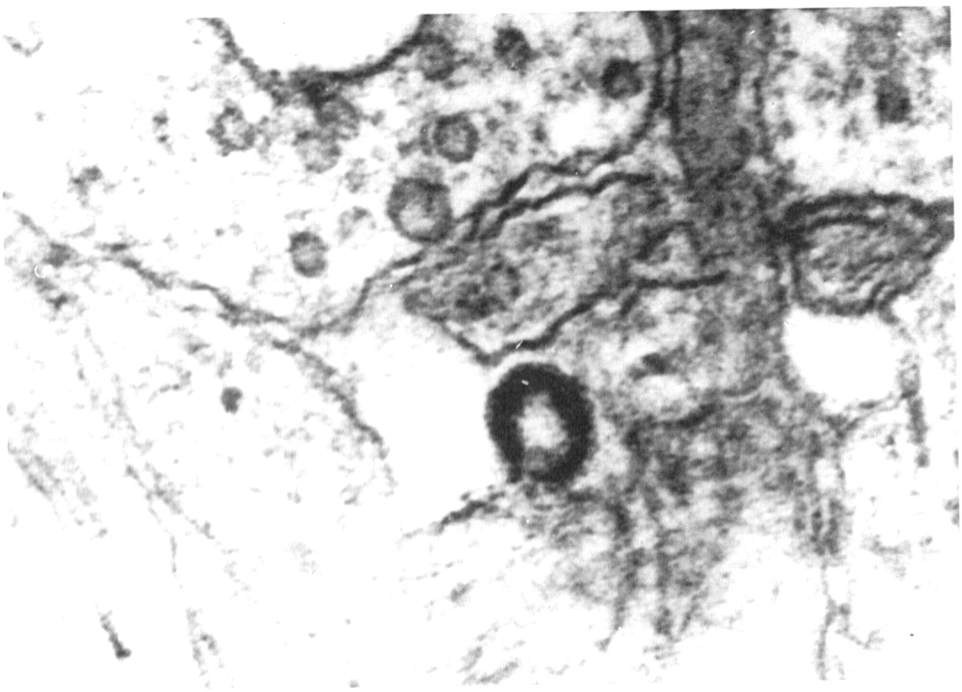

Fig. 3
The same preparation as in Fig. 2
Another typical viral particle possibly at the end of budding process (magn. 140 000 x).

a new serotype in the Uukuniemi antigenic group. Results of TEM are compatible with such a classification.

Tunis virus is the first arbovirus to be isolated from Tunisia and the fourth tick-borne virus from the Moghreb following Soldado, Essaouria and Kala iris viruses (Chastel *et al.*, 1993).

The *Argas reflexus* group of ticks has previously yielded five arboviruses: West Nile, (Flavivirus), Chenuda (Orbivirus) and Quaranfil (Arenavirus-like agent) all from *Argas r. hermanni* in Egypt and Afghanistan, and Grand Arbaud and Ponteves, two Uukuniemi group viruses from *Argas r. reflexus* in southern France (Karabatsos, 1985). All these viruses are serologically distinct from Tunis virus, including Grand Arbaud.

We are unaware of the pathogenic potential of Tunis virus for birds, other animals or man. However, the *A. r. hermanni* colony that yielded Tunis virus seems established in dwellings of the Faculty of Medecine in Tunis for many years and no medical incident has been so far reported. Moreover, *A. r. hermanni* is essentially a bird-feeder and does not commonly attack man. Thus, infection of human beings appears unlikely although an indirect mechanism of contamination has been postulated to explain infections of children by Quaranfil virus in Egypt: the accidental crushing of infected *Argas arboreus* ticks walking on the back of infested trees and the penetration of the virus through an excoriation of the skin (Taylor *et al.*, 1966). This may also occur in Tunisia with *A. r. hermanni* and Tunis virus.

During human serosurveys in Tunisia, detection of antibody to Uukuniemi group viruses was not attempted (Nabli *et al.*, 1970) but CF antibody to Uukuniemi virus, strain S23, was demonstrated in sera of wild rodents (Chastel *et al.*, 1977). However, we can not say whether that CF antibody corresponds to Tunis or (more probably) to another member of the same serogroup. In fact, the pathogenic potential of Tunis virus remains to be determined.

Acknowledgements. The excellent technical assistance of Mrs Odermatt, Brest and the financial support of "Foundation Langlois", Rennes, France, have been much appreciated. The competent technical assistance of Mrs D. Martin, Fort Collins is gratefully acknowledged.

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