Seroprevalence of several sandfly-borne phleboviruses (Toscana, Sicilian, Arbia +/- Adana) in dogs from Greece and Cyprus using neutralisation demonstrate massive circulation of Sicilian virus

Sulaf ALWASSOUF,[1,2] Vasiliki CHRISTODOULOU,[3] Laurence BICHAUD, [1,2] Pantelis NTAIS,[4] Apostolos MAZERIS,[3] Maria ANTONIOU,[4] Remi N. CHARREL [1,2]

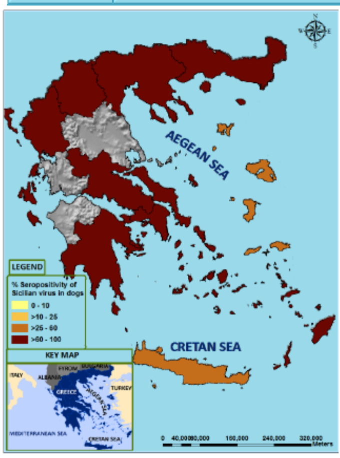
[1] Aix Marseille Université - IRD French Institute of Research for Development – EHESP, EPV UMR_D 190 "Emergence des Pathologies Virales", Marseille, France/ [2] IHU Méditerranée Infection, APHM Public Hospitals of Marseille, Marseille, France / [3] Veterinary Services of Cyprus, Nicosia, Cyprus / [4]; Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Faculty of Medicine, University of Crete, Voutes. Heraklion. Crete, Greece

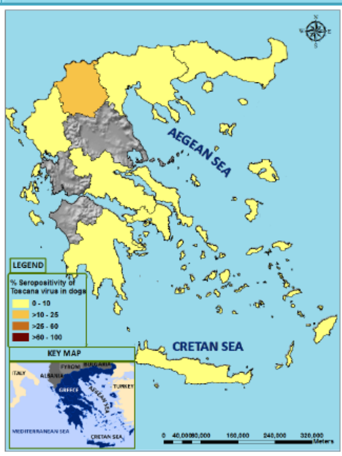
EDENext FP7- n°261504 EU project (www.edenext.eu)

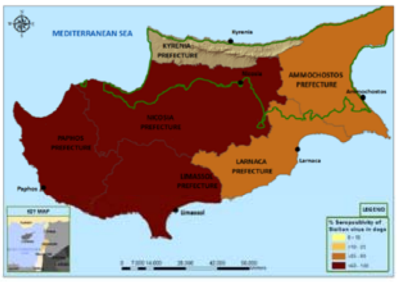
BACKGROUND: Sandfly fever, known to occur in Greece, Cyprus and neighboring countries, has been linked with phleboviruses transmitted by *Phlebotomus spp* flies. Human cases have been documented since 1990's, and are due to Sandfly fever Cyprus virus (closely related to Sandfly fever Sicilian virus and Corfu viruses) for the majority of cases; the circulation of Toscana virus, one of the 3 main causes of aseptic meningitis during the warm season has been recently described in Greece. Last, Adria virus (most closely related to the *Salehabad* species has been detected by PCR in a human case. At the outset of this study, there was neither seroprevalence data for non human vertebrates, nor nation-wide study.

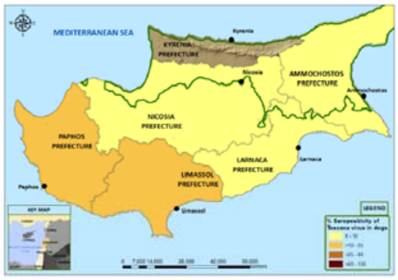
Materials & Methods: Dog sera were collected in regions listed in the Table. The virus microneutralisation (MN) assay (*Alkan et al. 2015, J Virol*) was done using the four viruses listed in the table. Twofold serial dilutions (1:20 to 1:160) were prepared for each serum and a volume of 50µL of each dilution was transferred into 96-well plates. A volume of 50µL containing 1000 $TCID_{50}$ of virus was added to each well except for the controls that contained PBS. The plates were incubated at 37°C for one hour. Then, a 100µL suspension of Vero cells containing approximately 2 x10^5 cells/mL of EMEM medium enriched with 5% fetal bovine serum and antibiotics was added to each well, and incubated at 37°C in presence of 5% CO2. After 6 days the microplates were read under an inverted microscope, and the presence (neutralization titre at 20, 40, 80 and 160) or absence (no neutralization) of cytopathic effect was noted.

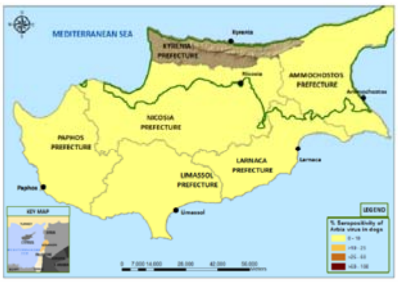
Country	Prefecture	Location	Nb of tested sera	Nb of interpretable sera	Toscana virus (%)	Sicilian virus (%)	Arbia virus (%)	Adana virus (%)
Greece	Attica	Attica	411	405	4.9	84.9	0.5	Nt
	Cent Macedonia	Chalkidiki,Serres,Thessaloniki,Veria	85	83	8.4	79.5	7.2	Nt
	Crete	Chania, Heraklion, Lassithi, Rethymno	288	267	1.1	52.4	3.7	Nt
	E. Macedonia &Thrace	Drama, Evros, Kavala, Komotini, Xanthi	98	87	2.3	70.1	4.6	Nt
	Epirus	Arta, Ioannina	25	25	4.0	72.0	4.0	Nt
	Ioanian Islands	Corfu island	57	56	5.4	67.9	1.8	Nt
	North Aegean	Chios island, Lesvos Island	62	57	7.0	49.1	3.5	Nt
	Peloponesse	Tripolis, Argos	59	51	5.9	76.5	3.9	Nt
	South Aegean	Rodos Island, Santorini, Syros	26	22	0.0	68.2	4.5	Nt
	Sterea Hellas	Chalkida, Evia, Karpenissi, Lamia	120	114	6.1	78.1	1.8	Nt
	West Greece	Agrinio, Patras	19	18	11.1	77.8	0.0	Nt
	TOTAL		1250	1185	4.4	71.9	2.6	Nt
Cyprus	Ammochostos		67	57	0.0	26.3	1.8	3.5
	Larnaca		27	20	5.0	40.0	5.0	20.0
	Limassol		97	70	11.4	61.4	5.7	11.4
	Nicosia		74	58	6.9	63.8	1.7	15.5
	Paphos		177	164	11.0	72.6	7.9	22.6
	TOTAL		442	369	8.4	60.2	5.4	16.3

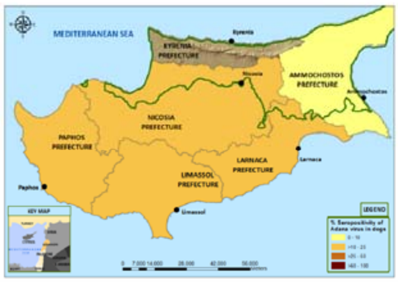


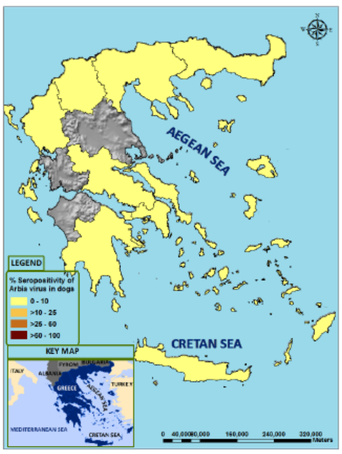


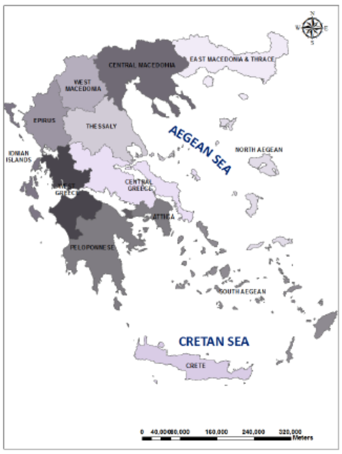












Results &Discussion : Sicilian virus, or close relatives, are circulating at very high level in the whole Greece, Crete and Cyprus. Then Adana virus, isolated in Mediterranean Turkey, that belong to the Salehabad / Arbia group is present is the whole Cyprus (11.4-22.6%), except for the eastern province (3.5%). Arbia virus is also present but at lower rate. Toscana virus is present in North Aegean Greece and in Western Cyprus, although prevalence in dogs is lower than for the other viruses. In conclusion, this study indicates that sandfly-borne phleboviruses belonging to 3 distinct genetic and antigenic groups are widely spread as showed from seroprevalence in dogs. It is needed to underline that there is no cross-reactivity between these viruses, thus demonstrating co-circulation in the studied regions. It is therefore important to perform the same type of study with human sera to confirm high exposure, and to develop diagnostic tests to be used for patients presenting with unexplained febrile illness and neuroinvasive infections.