ISTITUTO DI IGIENE DELL'UNIVERSITA' DI PALERMO
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INVESTIGATION ON ARBOVIRUSES IN WESTERN SICILY: INSECT COLLECTION AND VIRUS ISOLATION (*)

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In october 1968 a research program was initiated in this Institute in order to investigate the prevalence of arboviruses in Western Sicily. As part of this project, tick and mosquito specimens were collected in different localities and were processed for virus isolation attempts. Domestic and wild vertebrates serum samples were collected for serological studies.

The present paper describes the preliminary results of the insect collection and of virus isolation attempts.

Materials and methods

1. *Tick collection.* Tick specimens were captured in Madonie, a mountainous area of Western Sicily (see Map A). Most ticks were collected on cattle, sheep and goats: some were collected from white woolen blankets draged upon the ground, and a few samples were obtained from small wild mammals. Mostly adult ticks were collected. All arthropods specimens were stored alive in containers at +4°C until identified, usually within 1-2 days, after collection.

2. *Mosquito collection.* Mosquitoes were collected in a marsh near Mazara del Vallo (see Map B). Captures were made with manual electric aspirators, using

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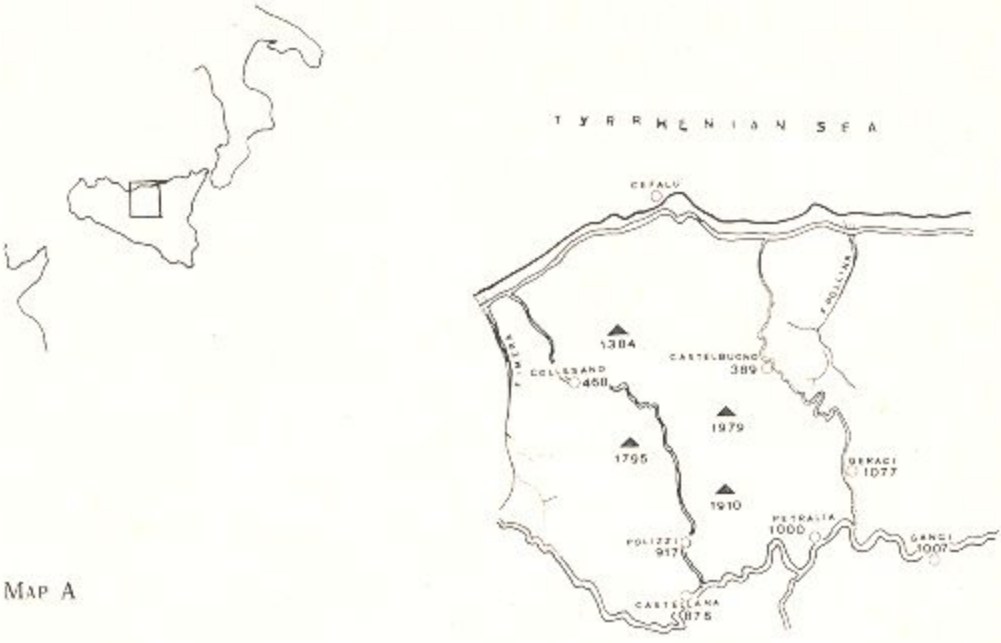
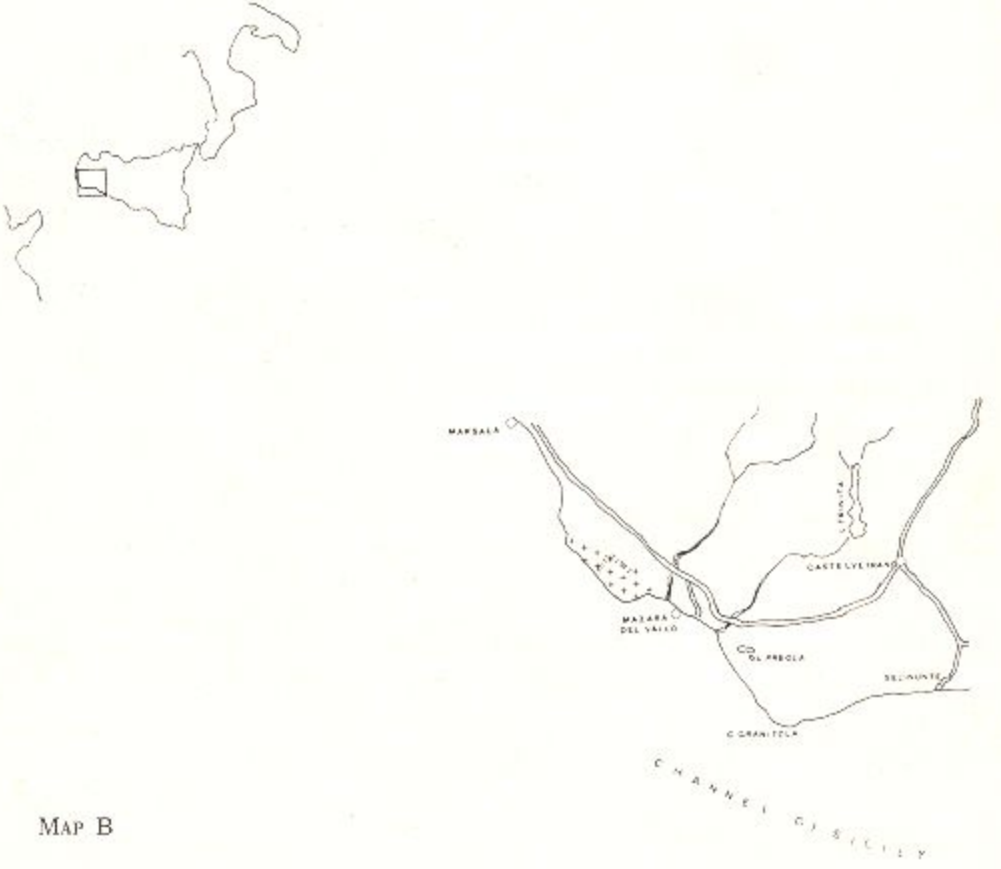

TYRRHENIAN SEA
CEFALU'
1384
CASTELBUONO
389
COLLESANO
468
1979
1795
GERACI
1077
1910
PETRALIA
1000
POLIZZI
917
GANGI
1007
CASTELLANA
875

MAP A


MARSALA
MAZARA
DEL VALLO
CASTELVETRANO
SELINUNTE
C. GRANITOLA
CHANNEL OF SICILY

MAP B

gaseous CO_2 or dry ice as attractive. Insects were collected at dawn and sunset, and were transferred as soon as possible to the laboratory.

3. *Processing insect in the laboratory.* All arthropods were kept in the cold, identified, pooled and stored at —65°C. When mice litters were available, pools of 25-30 ticks or 100-150 mosquitoes respectively were ground in mortars, suspended in 1,5 ml of a solution made of buffered saline pH 7,8, 1% bovine plasma albumine (BPA), antibiotics and mycostatin, and centrifuged at 3,000 rpm in the cold for 1 hour. Supernates of these suspensions were used for mouse inoculation.

4. *Bacteriological control.* Each tick and mosquito suspension, newly prepared, was inoculated into thyoglicollate broth and blood agar tubes as bacteriological control, 0,1 ml per tube.

5. *Virus isolation attempts.* Litters of 1-2 days old Swiss mice were inoculated by intracerebral (IC) and sometimes also by intraperitoneal (IP) route, 0,03 per mouse. Each litter was checked twice daily for 21 days.

When suspected or clearly sick mice were found, brain and liver were harvested separately by sterile techniques. A 10% tissue suspension was prepared in buffered saline pH 7,8 containing 0,75% BPA and antibiotics. Suspensions were centrifuged at 2,500 rpm for 20 min in the cold, and supernates were inoculated IC into another group of suckling mice. Serial passages were made until definite isolations with high infectivity titers were obtained.

Results

Tick collection. Cattle, sheep and goats from which ticks were collected, live all year by grazing in wild pasture or in recently harvested wheat fields in various parts of the mountainous area of Madonie, from 400 to 1,800 meters above sea level. Many areas are covered by scrub and bush; others are beech and oak woods. Small numbers of unfed active ticks were captured by the blanket method and on hedgehogs. Collections carried out in October through December 1968 and April through December 1969 yelded 9,756 specimens of the following species: *Hyalomma marginatum* and *lusitanicum*, *Rhipicephalus bursa*, *R. sanguineus*, *Boophilus calcaratus*, *Haemaphysalis punctata* and *Ixodes exagonus*.

Table I summarizes capture results, number of processed specimens and virus isolations.

Male *Rhipicephalus bursa* ticks appeared to be the most numerous in the area during June and July, disappearing after August. *Hyalomma marginatum* and *lusitanicum* species were also numerous and active adults were found during a longer period of the year. Many *Rhipicephalus sanguineus* specimens, captured on dogs living in the same region, were collected until June. A few *Boophilus calcaratus* and *Haemaphysalis punctata* specimens were found during some months. *Ixodes exagonus* samples were found only on a hedgehog.

TABLE I

Number of ticks collected and processed, 1968-1969.

Species	1968 Oct.-Nov.	1969				
		April-May	June	July	Aug.-Nov.	Total
Hyalomma marginatum and *H. lusitanicum*	101 (101)*	12 (12)	852 (492)	790 (612)	512 (311)	2,267 (1,528)
Rhipicephalus sanguineus	0	522 (522)	260 (116)	0	0	782 (638)
Rhipicephalus bursa	27 (27)	8	5,034 (2,081)**	1,393 (1,161)	0	6,462 (3,269)
Boophilus calcaratus	24 (24)	0	0	39 (24)	82 (35)	145 (83)
Haemaphysalis punctata	84 (84)	42 (42)	0	0	8	134 (126)
Ixodes exagonus	0	0	0	14 (14)	0	14 (14)
Total	236 (236)	584 (576)	6,146 (2,689)	2,236 (1,811)	602 (346)	9,804 (5,658)

* ( ) = Number processed for virus isolation.

** □ = Specimens contained viruses.

TABLE II

Number of mosquitoes collected and processed, 1968-1969 *.

Species	1968			1969			Total
	Oct.	Nov.	Dec.	July	Oct.	Nov.	
Aedes caspius	1,112	378	0	582	3,428	504	6,004
Aedes detritus	140	1,056	104	0	132	1,462	2,894
Theobaldia annulata	0	258	0	0	0	0	258
Culex pipiens	0	128	0	0	0	0	128
Total	1,252	1,820	104	582	3,560	1,966	9,284

* = All collected mosquitoes were processed for virus isolation.

Mosquito collection. Female mosquito specimens were collected from October to December 1968 and in July, October and November 1969. Captures were made in a marsh on the seashore near Mazara del Vallo in the southern coast of Sicily. The marsh vegetation is mostly reed thickets surrounding small ponds of stagnant brackish and fresh water. The area is inhabited by few animals; small wild vertebrates and migratory birds stop there occasionally.

Table II shows mosquito capture results. *Aedes caspius, Aedes detritus, Theobaldia annulata* and *Culex pipiens* were collected. *Aedes caspius* is the most abundant species in October, and collection of *Aedes detritus* continue during colder months. A few specimens of *Theobaldia annulata* and *Culex pipiens* were collected in November.

All mosquito pools were processed and inoculated into baby mice for virus isolation attempts.

Virus isolation. From 2,081 *Rhipicephalus bursa* specimens (captured in June-July 1969 from cattle, sheep and goats and the ground), six viral isolates were obtained. Another isolate, originally obtained from a pool of *Rhipicephalus sanguineus* ticks, captured on a dog living in an abbattoir of the Madonie in April, was lost by technical accident after four brain to brain passages. No viruses were obtained from the remaining 1,188 specimens of *Rhipicephalus bursa* species collected during other months. Negatives results were also obtained from the other species of tick specimens processed: 1,528 *Hyalomma marginatum* and *lusitanicum*, 126 *Haemaphysalis punctata*, 83 *Boophilus calcaratus* and

14 *Ixodes exagonus*. The remaining of *Rhipicephalus sanguineus* (637 specimens) were also negative for viruses.

No viruses were isolated from any of 70 mosquito suspension pools inoculated into suckling mice.

Of the 6 isolates obtained from ticks, three different serological and pathogenic types of 2/ea were obtained. Reisolation attempts from original pools of 2 isolates, which seemed to belong to the same type, were successful after 13 months storage at —65°C. Original tick suspensions of other isolates were not available for reisolation procedures.

Serological identification of these isolates are in progress, using viral reagents supplied by Dr. ROBERT E. SHOPE of the Yale Arbovirus Research Unit (YARU). Some of the isolated strains were also submitted to YARU for further studies.

DISCUSSION

Conditions in Sicily favorable to the maintenance of arboviruses in nature include: 1) A subtropical climate suitable for life cycles of insect vectors, similar to those found in other areas where arboviruses have been frequently isolated. 2) The presence of domestic and small wild animals in fairly large numbers in certain parts of the island, such as in Madonie. 3) The presence of migratory birds, which may be a link between Sicily and Africa, where numerous arboviruses are present (D'ALESSANDRO and DARDANONI, 1956 [2]). Recently, arboviruses have been isolated both from ticks and from mosquitoes in Central and in North Italy (BALDUCCI et al., 1968 [1]; VERANI et al., 1970 [3]). Since the classical epidemics of Sicilian sandfly fever during the World War II, there were no intensive studies of this and other arboviruses in Sicily prior to our present investigation.

Our initial surveys in Madonie, a hilly cattle-raising area 100 km Southwest of Palermo, have been fruitful for virus studies, at least among ticks collected from domestic animals. Although systemic insect collections have not been carried out on a year-round basis, several species of ticks were found during the summer months of June to August. Among species so far tested, *Rhipicephalus bursa* was the only species found infected with viruses. This species represents the most prevalent tick found in the study area.

Although final identification of viruses isolated from ticks is still in progress, some of their characteristics led us to believe that they belong to the arbovirus group. Serological surveys of man and animals, in certain regions of Sicily, are being performed. Preliminary

results show that at least one of the viruses isolated during this investigation have infected a high percentage of domestic animals in those areas.

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SUMMARY

ALBANESE, M. - BRUNO-SMIRAGLIA, C. - DI CUONZO, G. - LAVAGNINO, A. and SRIHONGSE, S. — INVESTIGATION ON ARBOVIRUSES IN WESTERN SICILY: INSECT COLLECTION AND VIRUS ISOLATION.

An investigation on the presence of arboviruses in Western Sicily was initiated in 1968. Several species of ticks and mosquitoes were collected for virus isolation attempts. Six viral isolates, belonging to three different serotypes, were obtained from *Rhipicephalus bursa* ticks while 5 other tick species were negative for viruses. Final identification of these viruses are in progress. No isolates were obtained from 4 species of mosquitoes so far studied.

RIASSUNTO

ALBANESE, M. - BRUNO-SMIRAGLIA, C. - DI CUONZO, G. - LAVAGNINO, A. e SRIHONGSE, S. — RICERCHE SUGLI ARBOVIRUS NELLA SICILIA OCCIDENTALE: CATTURA DEGLI INSETTI ED ISOLAMENTO DI VIRUS.

Vengono riferiti i risultati di un'indagine iniziata nel 1968 sulla presenza di arbovirus nella Sicilia occidentale. Da zecche della specie *Rhipicephalus bursa* sono stati isolati sei agenti virali appartenenti a tre sierotipi, di cui è in corso l'identificazione. I tentativi d'isolamento da altre cinque specie di zecche e da quattro specie di zanzare sono risultati negativi.

RÉSUMÉ

ALBANESE, M. - BRUNO-SMIRAGLIA, C. - DI CUONZO, G. - LAVAGNINO, A. et SRIHONGSE, S. — RECHERCHES SUR LES ARBOVIRUS EN SICILE OCCIDENTALE: CAPTURE DES INSECTES ET ISOLEMENT DES VIRUS.

Ont été présenté les résultats de recherches sur la présence d'arbovirus en Sicile occidentale, commencées en 1968. Ont été isolé de tiques *Rhipicephalus bursa* six souches de virus qui appartiennent à

trois sérotypes et dont l'identification est en cours. Les essais d'isolement de virus de cinq autres espèces de tiques et de quatre espèces de moustiques ont donné des résultats négatifs.

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