Acta virol. 34 : 582—591. 1990

ARBOVIRUSES IN THE IBERIAN PENINSULA

*ARMINDO R. FILIPE, H. REBELO DE ANDRADE

*Centre for Zoonoses Research, National Institute of Health, 2965 Aguas de Moura, and National School of Public Health, Lisbon, Portugal

Received June 13, 1989

Summary. — The information concerning the presence and activity of arboviruses in the Iberian Peninsula is very scanty. In Portugal serological studies have been done with sera from animals, wild birds and human population. The results have shown that some arboviruses have been active in some areas of the territory. West Nile (WN) virus was isolated from mosquitoes and Dhori and Thogoto viruses have been isolated from ticks. In Spain, in spite of the large territory, it's geographical contiguity to Africa and the existence of different kinds of climates and ecologic conditions, the information is reduced to some surveys done in some restricted regions of the country. In both countries African swine fever was frequent and recently African horsesickness has been introduced to Southern Spain. A straight liaison and cooperation among virology laboratories of both countries and also Morocco should be emphazised in order to establish epidemiological surveillance in Southern Europe.

Key words: *arbovirus*; *Iberian Peninsula*; *tick-borne orthomyxovirus*

Introduction

The knowledge we have concerning the presence and activity of arboviruses in the Iberian Peninsula is very scanty. However, this Peninsula occupies a very large geographical area of the European Continent. In fact, it has about 596 000 square kilometers, which represents close to 6% of the surface of Europe. It has a population around 45 million inhabitants distributed by the territories occupied by Portugal and Spain. Some migratory birds flying from Africa to Europe disperse through Spain and Portugal, and in many cases they cross these countries flying to the north of Europe (Carvalho, 1975; Bernis, 1980). Due to the ecological conditions, Africa is a very important reservoir of arthropod-borne viruses (Karabatsos, 1985), and in southwestern European countries the activity of the arboviruses, in the majority of cases, is very closely related to the survival and spread of those viruses in the North of Africa and in the Western coast of this continent.

Table 1. Summary of results of HI test for different arboviruses

Animal	Serum specimens (No.)	Serum reacting with antigens indicated									
		WN		ITME		TBE		Batai		Ťahyňa	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Bos taurus	1165	190	16	105	9	66	6	27	2	40	3
Ovis aries	129	4	3	2	2	1	0.7	—	—	—	—
Total	1294	194	19	107	11	67	5	27	2	40	3

The ecological changes in some areas of southern Iberia have resulted in better conditions for the survival of wild rodents and an increase in the population of arthropods. Mosquitoes, ticks, and phlebotomus (Tables 4, 5, 6) known as vectors for several arboviruses surving in the African continent and in some areas of Europe, have been identified in Portugal (Tendeiro, 1962; Leitão, 1971; Ribeiro *et al.*, 1977; Ribeiro *et al.*, 1977/78; Pires, 1979; Pires *et al.*, 1982; Karabatsos, 1985). The importance of arboviruses for the Iberian Peninsula can be summarized as follows: 1. Close geographical contiguity to the North of Africa; 2. Large movements of migratory birds between Europe (Carvalho, 1975) and Africa (Bernis, 1980) across the

Table 2. Serum samples examined by HI test with the WN virus antigen

Serum received from		Total of sera	% of positive sera	
Group 1	Serpa county	35	1	
	Cantanhede county	60	4	
	Evora county	64	6	
	Total	159	11	6.8
Group 2	Soldiers	1374	40	
	Nat. Inst. Health	116	4	
	Total	1490	44	2.9
	Total group 1 and 2	1649	55	3.3

Group 1 Counties where positive animal sera have been found previously.
Group 2 Serum samples which cover all the country.

Table 3. Wild birds with antibodies against arboviruses captured during summer 1969 and 1970

	Species	Sera	Virus group
		No. pos./No. tested	
Permanent Residents			
Greenfinch	*Chloris chloris*	0/7	Flavivirus
Haw finch	*Coccothraustes coccothraustes*	2/3	Flavivirus
Azure-winged magpie	*Cyanopica cyanus*	1/3	Flavivirus
Linnet	*Carduelis cannabina*	1/7	Flavivirus
Cattle agret	*Bubulcus ibis*	2/128	Flavivirus
Summer Visitors			
Swallow	*Hirundo rustica*	4/25	Flavivirus
Pied flycatcher	*Ficedula hypoleuca*	1/3	Flavivirus
Sand martin	*Riparia reparia*	1/30	Alfavirus

Iberian Peninsula; 3. Existence of large territories with reduced human population, where several viruses can be introduced and where they can survive without any visible marker for its presence and activity; and finally, 4. Areas with very favourable ecological conditions for introduction and survival of arthropods, mammals, and small rodents which serve as reservoirs of arboviruses.

These conditions make the study of the arboviruses in the Iberian Peninsula very attractive and the obtained results confirm these ideas. However, in addition to that what has been done, there is an urgent need for basic biological research including the ecology of this group of viruses.

Arboviruses in Portugal

The study of arboviruses in the Iberian Peninsula goes back to 1967, when in Portugal a preliminary work was done in 1966 (Filipe, 1967). After very encouraging results, a programme has been designed to study a restricted zone of southern Portugal. The first surveys detected several areas with strongly positive serological reactions for WN virus (Tables 1, 2) as well as other arboviruses (Filipe and Pinto, 1969). At that time there was no reliable information about the distribution of arboviruses in this country, with exception of the studies done during the Blue Tongue virus epizooty, that occurred in 1957/58 (Estrella and Silva, 1956) and since the African swine Fever virus enzooty in 1960. In one region very close to the city of Beja in southern Portugal, it was possible to find several farms with animals showing high titre of antibodies against WN virus. At the same time an inquiry realized in the area permitted to discover that an epizooty of an encephalomyelitis of unknown origin occurred among different horses in the same region. A serological survey done with the sera from the surviving

Table 4. Mosquitoes identified in Portugal and arboviruses isolated from these arthropods in other continents

Species	Virus isolated in other continents
Anopheles atroparvus (*maculipennis*)	West Nile, Batai
Aedes capius	Issyl-Kul, Isfahan, Ťahyňa
Aedes vittatus	Babanki, Bunyamwera, Chikungunya, Middelburg, Ngari, Pongola, Saboya, Simbu, Sindbis, Yellow fever, Semliki
Culiseta annulata	Ťahyňa
Culex modestus	Ťahyňa, West Nile
Culex univittatus	Bagaza, Ingwavuma, Mossuril, M'Poko, Sindbis, Spondweni, Usutu, Wesselsbron, West Nile
Culex theileri	Germinston, Rift Valley, Sindbis, Shuni
Culex pipiens	Calovo, Flanders, Hart Park, Israel, Turkey, Japanese B, La Crosse, Olifantsvlei, Semliki, Sindbis, S. Louis Enc., Ťahyňa, Trivittatus, Turlock, Umatilla, Western Equi. Enc.

animals has shown that 29% of the studied horses had neutralizing antibodies against WN virus (Filipe *et al.*, 1973).

Following these studies it was possible to capture different species of mosquitoes. WN virus was then isolated from *Anopheles maculipennis* mosquitoes collected very close to the farm where the WN virus epizooty had occurred (Filipe, 1972). The successful results (Table 2 and 3) (Filipe, 1971; Filipe, 1974; Filipe, 1975; Filipe, 1980; Filipe, 1983) were followed by a programme for the study of tick borne viruses along with new viruses isolated in Portugal and in Europe. These new isolated viruses (Filipe and Casals, 1979; Filipe and Calisher, 1984) will be described in this survey.

In 1985 isolation of the Toscana virus, a sandfly virus was reported in Sweden from a patient who became ill after spending two weeks in the

Table 5. Ticks identified in Portugal and arboviruses isolated from these arthropods in Europe and Africa

Species	Virus isolated in other continents
Ixodes ricinus	Absettarov, Cent. Europ. Enc., Congo-CHF, Eyach, Hanzalová, Kumlinge, Lipovnik, Louping ill, RSSE, Tribec, Uukuniemi
Rhipicephalus sanguineus	Congo-CHF, Thogoto, Wad Medani
Rhipicephalus bursa	Congo-CHF, Thogoto
Haemaphysalis punctata	Bhanja, Cent. Europ. Enc., Congo-CHF, Tribec
Dermacentor marginatus	Bhanja, Congo-CHF, Dhori, Omsk Hem. Fev., Razdan, RSSE
Hyalomma marginatum	Bhanja, Congo-CHF, Dhori, Matrub, Manawa, Sindbis, Tamdy, Wanowrie, West Nile
Ornithodoros erraticus	African Swine Fev., Bandia, Qalyub

Table 6. Phlebotomus identified in Portugal and arboviruses isolated from these arthropods in Europe and Asia

Species	Virus isolated in Europe or Asia
Phlebotomus papatasi	Isfahan; Sandfly f. (Naples); Teheran
Phlebotomus perniciosus	Arbia; Toscana
Phlebotomus sergenti	
Phlebotomus ariasi	
Phlebotomus minuta	

South of Portugal (Ehrnst *et al.*, 1985). From previous work it seems that more arboviruses exist or have been active in some regions but for several reasons they have not been characterized. Further studies, in order to get more information about the existence of arboviruses in the centre and the northern territory of Portugal, are now being performed and are expected to be finished by the end of 1991. The recent situation over the territory of Portugal is summarized in Table 7.

Tick-borne viruses in Portugal

It is well known that infected ticks can be transported and introduced by migratory birds into distant geographical areas (Balducci *et al.*, 1973; Ernek *et al.*, 1977; Karabatsos, 1985). After 1971 we studied the distribution of tick-borne viruses to investigate their presence, prevalence, and public health importance in Portugal. As first result of this work the Dhori virus was isolated from a pool of ticks identified as *Hyalomma marginatum* (Filipe and Casals, 1979). Following this it was possible to identify another tick-borne virus, pathogenic for the laboratory mice, that was later identified as Thogoto virus. This virus was isolated from *Rhipicephalus sanguineus* ticks collected in 1978 (Filipe and Calisher, 1984). Structural characterization of these two viruses showed that they were very closely related to the family *Orthomyxoviridae* (Clerx *et al.*, 1983). Recent research (Davies *et al.*, 1986; Davies *et al.*, 1987) has involved adaptation of these orthomyxoviruses to arthropods in their natural habitat.

A study was undertaken to investigate the possible differences or similarities between Thogoto virus strains isolated in a variety of geographical areas, but no antigenic differences could be found. No antigenic differences could be seen when comparing strains isolated in areas as distant as Kenya, Portugal, Nigeria, Italy or Iran (Calisher *et al.*, 1987). However, biological differences related to the virulence and pathogenesis of Thogoto virus for laboratory mice could be detected. No association of Dhori and Thogoto viruses with human disease has been reported in Portugal, in contrary to the available information obtained in other regions, where these viruses were recognized as human pathogens (Moore *et al.*, 1975; Butenko *et al.*, 1987).

Meanwhile humans and domestic animals with antibodies against these two viruses have already been found in Portugal (Filipe *et al.*, 1985; Filipe

Table 7. Arboviruses in Portugal

Virus	Virus isolation (Source and year)	Antibodies
Sindbis	—	Birds
Chinkungunya	—	Man, domestic animals
West Nile	*Anopheles maculipennis* (1969)	Man, birds, domestic animals
Tick-borne encephalitis	—	Man, domestic animals
Ťahyňa	—	Domestic animals
Batai	—	Domestic animals
Sicily	—	Man
Toscana	Man (in Sweden, 1983)	Man
Dhori	*Hyalomma marginatum* (1971)	Man, domestic animals
Thogoto	*Rhipicephalus sanguineus* (1978)	Man, domestic animals
Bhanja	—	Domestic animals
Congo-Crimean haemorrhagic fever	—	Man
Blue tongue	Sheep (1957/58)	—
African swine fever	Swine (1957/1960—1)	—
African horse sickness	Horse (1989—)	

and Morais, 1986). The presence and activity of both viruses is associated with tick bite, and most probably the human disease has been diagnosed by the physicians as some kind of "tick-borne fever". Thogoto virus is an orthomyxovirus adapted to the replication and transmission by ixodid ticks in their natural habitat. In laboratory mice Dhori and Thogoto viruses have shown a high degree of hepatotropism and cytopathology associated with replication in the liver (Albanese *et al.*, 1973; Filipe *et al.*, 1986). It seems that these two viruses may be interesting models for the study of virus-induced liver pathology. Later serological surveys have shown that several domestic animals had neutralizing antibodies against Bhanja virus (Filipe *et al.*, 1985). At the same time, using the immunofluorescence test, three human sera showed antibodies against Congo-Crimean Haemorrhagic Fever virus (Filipe *et al.*, 1985; Filipe and Morais, 1986). In addition, further not yet characterized viruses have already been isolated.

Arboviruses in Spain

Information about the activity of the arboviruses in Spain is very limited. For unknown reasons the study of this group of viruses has never been encouraged. However, the large dimensions of the territory occupied by Spain, and the ecological conditions prevailing there, are very favourable for the survival and dissemination of the arthropod-borne viruses. In fact, the first knowledge about arboviruses in this territory is related to a serological survey done with a group of sera from the human population living in Valencia at the Mediterranean coast. The results are not very conclusive but the authors reported that they have found sera with antibodies against WN, Ťahyňa and Sandfly viruses (Sanchis-Bayarri Vaillant, 1974; Sanchis-

Table 8. Results of haemagglutination-inhibition tests for Flaviviruses with human sera from northern province of Spain

Province	Number of sera		% positive
	tested	positive	
La Coruna	362	62	17.1
Orense	14	1	7.1
Pontevedra	179	35	19.5
Leon	61	7	11.4
Asturias	85	11	12.1
Total	701	116	16.5

Bayarri Vaillant and Sanchis-Bayarri Lahoz, 1975). At the same time, another survey has been done with serum samples from the population living in northwestern Spain. Very high titres and a very large percentage of positive sera against WN virus have been found among the human population studied (Table 8) (Garea Gonzalez and Filipe, 1977). The survey has revealed that in the sixties some WN virus epidemics occurred in this region. This was in agreement with the WN epidemics that has been very active all over the Mediterranean area at that time.

Some years later studies were performed using samples of sera collected in the region of the National Park of Doñana, southwestern Spain, not very far from Gibraltar. Several sera had been found with antibodies against the

Table 9. Results obtained with serum samples from the human population living in the delta del Ebro area and tested by HI

Village	Total of sera	Positive sera						% Positive sera
		Antigenic group						
		Alpha	%	Flavi	%	Bunya	%	
Amposta	353	—	—	1	0.2	—	—	0.2
Aldea	1	—	—	—	—	—	—	—
Camarles	45	—	—	—	—	—	—	—
S. Carlos	56	—	—	1	1.7	—	—	1.7
Ampolla	105	6	5.7	31	29.5	1	0.9	36.1
Jésus y Maria	107	9	8.4	2	1.8	—	—	10.2
La Cava	41	—	—	—	—	—	—	—
S. Jaime	188	24	12.7	25	13.2	2	1.0	27.1
Montells	139	4	2.8	23	16.5	1	0.7	20.1
Masdenverge	2	—	—	—	—	—	—	—
Total	1037	**43**	**4.1**	**83**	**8.0**	**4**	**0.3**	12.5

Table 10. Arboviruses in Spain

Virus	Virus isolation	Antibodies
Sindbis	—	Man
Semliki	—	Man
Chikungunya	—	Man
West Nile	—	Man, wild rodents
Bunyamwera	—	Man
Ťahyňa	—	Man
Sicily	—	Man
Uukuniemi	—	Wild rodents
Bhanja	—	Wild rodents
Blue Tongue	Sheep	—
African swine fever	Swine, ticks	—
African horse sickness	Horse	—

arboviruses used in the battery of antigens, but the most interesting result was the high percentage of sera with antibodies against Chikungunya virus (Lozano Olivares, 1980) that has been found. In 1978/79 a group of French researchers reported that small rodents captured in different areas of Spain had antibodies against WN, Uukuniemi, Bhanja, and Ťahyňa viruses (Chastel *et al.*, 1980).

One of the areas that have been studied very intensively was the delta of the river Ebro, southwest of Barcelona. The results obtained with this survey were the most interesting from all the studies already done in the Iberian Peninsula. As a matter of fact, among the population in the delta antibodies against flaviviruses were found in high titres (Table 9). In some villages more than 30% of collected sera had antibodies against these viruses. Probably some epidemics of "influenza-like" disease occurred in the area just before 1980 (Lozano Olivares and Filipe, 1986).

With the exception of Blue Tongue, African Swine fever and African horsesickness viruses, to our knowledge, no other arboviruses have been isolated on the Spanish territory of the Iberian Peninsula. The situation as it is now known in Spain, and the results of the serological surveys already mentioned are presented in Table 10, where the situation in Spain is summarized.

Conclusions

After all that has been written it seems necessary to increase the basic research in all areas of biological importance for the study of the arbovirus ecology in the Iberian Peninsula. Basic studies should include the study of the populations of small mammals, migratory birds, entomology, and ecology. They constitute main information for the studies with arthropod-borne viruses. The experience obtained with the study of the arboviruses in different countries has shown that introduction of new viruses or arthropod-vectores to a certain geographical area is permanently possible. For this

reason arboviruses represent a group of pathogens for which it is necessary to maintain a continuous epidemiological surveillance. A straight liaison and cooperation among the virology laboratories of both countries of the Iberian Peninsula should be emphasized.

Acknowledgements. This work has been supported by research grant from the JNICT, Lisbon, Portugal — grant 87 136/IMU; by the National School of Public Health, Lisbon, Portugal and the Centre for Zoonoses Research from the National Institute of Health, Águas de Moura, Portugal. We thank Mrs. Ines Perinas and Miss Olga Costa for their cooperation during the preparation of the manuscript.

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