

ARGASIDAE (ACARI : IXODIDA)
PARASITES OF WILD AND DOMESTIC ANIMALS IN SENEGAL:
2 — ARBOVIRUSES ISOLATION AND EPIDEMIOLOGICAL IMPLICATIONS.

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ARGASIDAE,
ARBOVIRUSES, BIRDS,
NESTING FAILURE,
SENEGAL

SUMMARY: Argasid ticks (2870 ticks divided into 226 pools) collected mainly from nests, chicken houses and burrows have been tested for viruses (155 *Ornithodoros (Ornithodoros) savignyi*, 1422 *Alectorobius (Alectorobius) capensis*, 567 *Alectorobius (Theriodoros) sonrai*, 16 *Argas (Persicargas) arboreus*, 150 *Argas (Persicargas) persicus* and 560 *Argas (Persicargas) near walkerae*). Two tick-borne viruses were isolated: 20 strains of Soldado from *A. (A.) capensis*, 12 strains of Bandia from *A. (T.) sonrai*. No strain of arbovirus was obtained from *A. (P.) arboreus*, *A. (P.) persicus*, *A. (P.) near walkerae* and *O. (O.) savignyi*. Soldado virus was obtained from numerous *A. capensis* of Langue de Barbarie National Park (PNLB) where it has caused, with its vector, nesting failure and non-egg hatching in terns' colonies. At the same time several *A. capensis* from which no arbovirus was obtained, have been involved in a desertion of pelican nesting site in Djoudj National Park of birds (PNOD). The Bandia virus was obtained from *A. sonrai* in Bandia.

ARGASIDAE,
ARBOVIRUS, OISEAUX,
PROBLÈMES DE NIDIFICATION,
SÉNÉGAL

RÉSUMÉ : Les argasidés (2870 tiques divisées en 226 lots) récoltés essentiellement de poulaillers, de nids et de terriers ont été contrôlés pour des isolements d'arbovirus, soit 155 *Ornithodoros (Ornithodoros) savignyi*, 1422 *Alectorobius (Alectorobius) capensis*, 567 *Alectorobius (Theriodoros) sonrai*, 16 *Argas (Persicargas) arboreus*, 150 *Argas (Persicargas) persicus* and 560 *Argas (Persicargas) proche de walkerae*. Deux espèces d'arbovirus ont été isolées : 20 souches du virus Soldado d'*A. (A.) capensis*, 12 souches du virus Bandia d'*A. (T.) sonrai*. Aucun arbovirus n'a été obtenu d'*A. (P.) arboreus*, d'*A. (P.) persicus*, d'*A. (P.) proche de walkerae* et d'*O. (O.) savignyi*. Le virus Soldado a été isolé à partir de nombreux *A. capensis* du Parc National de la Langue de Barbarie (PNLB) où il a causé, avec la tique qui l'héberge, l'abandon des couvées et la non-éclosion des œufs chez les colonies nicheuses de sternes. À la même période, plusieurs *A. capensis*, à partir desquels aucun arbovirus n'a été isolé, ont été impliqués dans la désertion d'une plate forme de nidification de pélicans au Parc National des Oiseaux du Djoudj (PNOD). Le virus Bandia a été obtenu d'*A. sonrai* provenant de Bandia.

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INTRODUCTION

Despite the importance of soft ticks in transmission of different affections (RODHAIN & PEREZ, 1985) studies in tick-borne viruses in Senegal primarily focused on Amblyommatidae (CAMILAS *et al.*, 1986; 1990). Only few investigations on arboviruses associated with Argasidae have been undertaken: CAMICAS *et al.* (1978) have shown that *Alestorobius (The riidoros) sonrai* (Sautet & Witkowski, 1943) was not involved in the ecology of the Chikungunya virus (CHIK); Crimean-Congo Hemorrhagic Fever (CCHF), Bandia (BDA) and Dugbe (DUG) viruses have been isolated from *A. sonrai* (ANONYME, 1998). The Soldado virus has been also isolated from *Alestorobius (Alestorobius) capensis* (Neumann, 1901) of Langue de Barbarie National Park (PNLB) (MAIN *et al.*, 1980).

A part of monitoring programs led by the Institut de Recherche pour le Développement and the Pasteur Institute concerns West Nile (WN), Yellow Fever (YF) and Rift Valley Fever (RVF) viruses which have been repeatedly isolated from mosquitoes in Sénégal (FONTENILLE *et al.*, 1993; ANONYME, 1998). It is known that West Nile virus can be hosted by *Argas* ticks when mosquitoes' peak decreases (MOREL, 1976). Furthermore, *A. capensis* could also play a role as reservoir of that virus CAMICAS (1980). Thus, we searched for WN in *Argas* ticks, in the ecological areas where this arbovirus has been isolated from and in ticks associated with migratory birds. RVF virus has been also isolated from *Aedes* mosquitoes in northern Senegal (FONTENILLE *et al.*, 1993). As PRETORIUS *et al.* (1997) suggested to check it with ticks associated with rodent burrows, we have investigated an eventual role of *A. sonrai* in the ecology of this arbovirus.

MATERIALS AND METHODS

Tick collections : *Argas (Persicargas) persicus* (Oken, 1818) Fischer & von Waldheim, 1823 and *A. (P.) near walkerae* Kaiser & Hoogstraal, 1969 parasite of domestic fowl were thoroughly searched for in the hen house. *A. (P.) arboreus* Kaiser, Hoogstraal &

Kohls, 1964 and *A. capensis* infesting marine and continental wild birds have been recovered from nests. The endophilic argasid tick, *A. sonrai*, has been recovered from rodent burrows using a motorized aspirator type Echo-Power Blower P. B. 210 E and the geophilous species *Ornithodoros (Ornithodoros) savignyi* (Audoin, 1827) has been collected from sandy soil a few minutes after presence of an host. Then ticks have been rinsed in tap water, and dried with blotting or filter paper, stored in Borel vials closed with gauze or Nalgen jars with screw-cap lids first opened in the centre and covered with fine gauze mesh.

Methods for arboviruses isolations : Inoculation method to newborn mice was used for arbovirus research. Identified and sorted ticks were pooled by sex and developmental stage; each triturated with a small pestle into its own mortar containing Hank's albumin media. After centrifugation at 2000 tr/mn for 5 minutes, the supernatant was kept. 0,02 ml of each inoculum was then injected by intracerebral route to newborn mice and to two continuous cell lines: Vero and A. P. 61 from *Aedes pseudoscutellaris*. Each inoculum was allotted to a litter of newborn mice who were placed into boxes with their mother. The brains of ill newborn mice were then collected for control. Detection of arboviruses growing in cell cultures was performed by indirect immunofluorescent analysis of reference mouse immune ascitic fluid pools previously described by DIGOUTTE *et al.* (1992). Identification of the viruses isolated on cell cultures and/or suckling mice was made using the complement fixation (CF) technique and confirmation was done using a seroneutralisation test (LENETTE *et al.*, 1979). Tests were performed at the World Health Organisation Collaborating Centre for References and Research on Arboviruses (CRORA) at the Pasteur Institute in Dakar.

RESULTS

Identification of arboviruses : A total of 2870 argasids divided into 226 monospecific pools were used to inoculate two days old new-born mice (TABLE 1). 138 pools of *A. capensis* collected in Langue de Barbarie National Park (PNLB) (15°50N, 16°30W) and sorted

Date	Species and developmental stage	Number of pools	Locality	Number of ticks	Viruses isolated	Number of strains
27/05/95	<i>Ornithodoros savignyi</i> ♂	1	Yang Yang (in soil)	10	-	0
	<i>Ornithodoros savignyi</i> ♀	1		10	-	0
	<i>Ornithodoros savignyi</i> N	1		10	-	0
27/05/95	<i>Argas persicus</i> ♂	1	Mboula (Hen house)	10	-	0
	<i>Argas persicus</i> ♀	1		10	-	0
	<i>Argas persicus</i> N	1		10	-	0
09/09/96	<i>Alectorobius capensis</i> ♂	25	PNLB (Terns' nests)	250	-	0
	<i>Alectorobius capensis</i> ♀	1		15	-	0
	<i>Alectorobius capensis</i> N	1		4	-	0
20/09/96	<i>Ornithodoros savignyi</i> ♂	1	Yang Yang (in soil)	20	-	0
	<i>Ornithodoros savignyi</i> ♀	1		20	-	0
	<i>Ornithodoros savignyi</i> N	1		11	-	0
19/11/96	<i>Alectorobius capensis</i> ♂	59	PNLB (Terns' nests)	590	Soldado	12
	<i>Alectorobius capensis</i> ♀	44		440	Soldado	8
	<i>Alectorobius capensis</i> N	3		30	-	0
19/11/96	<i>Argas arboreus</i> ♂	1	PNLB (Egrets' nests)	10	-	0
	<i>Argas arboreus</i> ♀	1		6	-	0
19/11/96	<i>Alectorobius capensis</i> ♂	3	PNOD (Pelicans' nests)	60	-	0
	<i>Alectorobius capensis</i> ♀	2		33	-	0
23/11/96	<i>Ornithodoros savignyi</i> ♀	1	Yang Yang (in soil)	3	-	0
	<i>Ornithodoros savignyi</i> N	6		71	-	0
19/11/1997	<i>Argas persicus</i> ♂	4	Pakéba (Hen house)	40	-	0
	<i>Argas persicus</i> ♀	4		40	-	0
	<i>Argas persicus</i> N	4		40	-	0
10/12/97	<i>Alectorobius sonrai</i> ♂	9	Bandia: Burrow of <i>A. niloticus</i>	180	Bandia	4
	<i>Alectorobius sonrai</i> ♀	12		240	Bandia	8
	<i>Alectorobius sonrai</i> N	6		120	-	0
28/03/98	<i>Alectorobius sonrai</i> ♂	1	R. Toll: Burrow of <i>X. erythropus</i>	10	-	0
	<i>Alectorobius sonrai</i> ♀	1		2	-	0
	<i>Alectorobius sonrai</i> N	1		15	-	0
00/10/1998	<i>Argas near walkerae</i> ♂	6	PK7 (Hen house)	120	-	0
	<i>Argas near walkerae</i> ♀	12		240	-	0
	<i>Argas near walkerae</i> N	10		200	-	0
	Total	226		2870		32

TABLE 1: Argasid pools and arboviruses isolated.

into males, nymphs and females were constituted, 20 strains of Soldado virus were isolated from 103 pools (TABLE 1). The Soldado virus strains were obtained from males and females collected in November 1996, while those of September from PNLB as those of the Djoudj National Park of Birds (PNOD) ($16^{\circ}25'N$, $16^{\circ}18'W$) tested negative.

— 540 *A. sonrai* sorted into 30 pools of nymphs, males and females were also inoculated. Twelve strains of Bandia virus were isolated from 21 pools of *A. sonrai* collected in Bandia ($14^{\circ}35'N$, $16^{\circ}59'W$) from

a burrow of *Arvicantis niloticus* in December, 10^{th} , 1997.

— No arbovirus was isolated from 2 pools of 6 females and 10 males of *A. arboreus* collected from a nest of *Egretta garzetta* in PNLB as from 155 *O. savignyi* collected between May and October 1996 and divided into 13 pools. 15 pools of *A. persicus* coming from different ecological areas in Senegal and 28 pools of *A. near walkerae* collected in Pk7 ($12^{\circ}36'N$, $12^{\circ}13'W$), from Kédougou to Dakar, en route were also negative for arbovirus research.

DISCUSSION : EPIDEMIOLOGICAL IMPLICATIONS

The Soldado virus, belonging to the Nairovirus genus, Bunyaviridae family and Hughes group, was isolated for the first time from nymphal *A. capensis* and/or *A. denmarki*. These ticks were collected from nests of a noody tern (*Anous stolidus*) on Soldado Rock Island (10°4N, 62°1W), Trinidad in the Caribbean (JONKERS *et al.*, 1993). Since this initial finding of the Soldado virus which is the prototype of a group of closely related arboviruses (Soldado and Soldado-Like), isolation of strains of this virus continue to be obtained in several parts of the world. CONVERSE *et al.* (1975) isolated it from *A. capensis* collected on the Seychelles in the Indian Ocean; HOOGSTRAAL *et al.* (1976) reported its isolation from specimens collected in lake Shalla (Ethiopia); KING *et al.* (1977a) obtained it from a breeding island of terns near Aransas National Wildlife Refuge, in Texas (USA). KEIRANS *et al.* (1976) isolated a related virus (Soldado-Like) from *A. maritimus* in Ireland; CONVERSE *et al.* (1976) recovered this virus from *A. maritimus* found on Puffin Island, CHASTEL *et al.* (1979) isolated it from *A. maritimus* in France. Soldado virus has been also isolated from a serum of a marine bird, *Rissa tridactyla*, in France (CHASTEL, 1990).

In Senegal, Soldado virus was isolated for the first time from 223 *A. capensis* collected in nests of sterns in PNLB on January and February 1977 (MAIN *et al.*, 1980). It was isolated again, 19 years after, in November 1996, from 1030 *A. capensis* collected from the same site and sorted into 103 pools. In Northern Senegal, three parks of birds are located (PNLB, PNOD, Park of Gueumbeul). The PNLB is the breeding site of gray-headed gulls (*Larus cirrhocephalus*), several species of sterns: caspian terns (*Hydropogone caspia*), royal terns (*Sterna maxima albifrons*), gull-billed tern (*Sterna nilotica*) and egrets (*Egretta garzetta* and *Demigretta gularis*); the PNOD is the nidification site of cormorants (*Phalacrocorax africanus*), pelicans (*Pelecanus onocrotalus*) and ducks (*Plectropterus gambensis*) and the Park of Guembeul is recently known to be breeding site of the pink flamingo *Phoenicopterus roseus*. Ticks have been found in PNLB since 1977 but these are their first records from the PNOD. Numerous argasid ticks

have been collected on both parks every year from 1996 to 1998 (TABLE 1).

Lower densities of the stern colonies, abnormal development of young birds and non egg hatching have previously been reported by the services of the National Parks in Senegal and they hypothesize that the development of some creeping plants (*Ipomea asarifolia*) on the breeding zone was the main cause. Further prospecting done on the island revealed that argasid ticks of the *capensis* group (*A. maritimus* and *A. capensis*) heavily infested the breeding site. Enquiries led in PNLB on September and November 1996 have allowed us to identify *A. maritimus* and *A. capensis* from tern's nests, *A. arboreus* from nests of *Demigretta gularis* and *Egretta garzetta*. Detailed arboviral research carried out in the Pasteur Institute in Dakar revealed 20 strains of Soldado virus obtained from *A. capensis* in PNLB.

In the PNOD, desertion of a nesting site of pelicans and cormorants was observed for the first time in 1996. Birds left their first breeding site where few pelicans were only found breeding on soil in May, 1997. Another small island located in the backsoner of Khar (16°24N, 16°15W), in the same area, was then occupied for breeding. From the deserted nesting site, numerous *A. capensis* from which no strain of arbovirus was obtained, were collected, when it was not possible to record ticks from the new site.

The heavy infestation of argasid ticks is the only cause of breeding site desertion in PNOD, non hatched eggs and abnormal development of young birds in PNLB. Parents must probably desert the nest to escape the ticks themselves.

Similar cases of nest desertion and non hatched eggs have been reported from other parts of the world:

— a nesting colony of brown pelicans (*Pelecanus occidentalis*) has deserted a breeding island, Bird Key Stono, located in South Carolina, because of an infestation of *A. capensis* (KEIRANS *et al.*, 1992);

— KING *et al.* (1977a) also reported nest desertion of a seabird colony of brown pelicans in Aransas Bay, a lethal arbovirus being suspected causing death of young birds. Soldado and Aransas Bay viruses were also isolated from *A. capensis* infesting this colony (YUNKER *et al.*, 1979).

— CONVERSE *et al.* (1975), FEAR (1976) reported nest desertion caused by *A. capensis* infected by Soldado virus in Sooty terns, *Sterna fuscata* colonies in the Seychelles.

— Another case of nest desertion, due to *A. denmarki* has been observed by KING *et al.* (1977b) on the islands in the Gulf of California, Baja California, Mexico.

As from CONVERSE *et al.*, (1975), FEAR (1976), KEIRANS *et al.* (1992), KING *et al.* (1977a; 1977b), we can conclude that the heavy infestation of breeding sites with argasid ticks (*A. capensis*, *A. maritimus* and other *Argas* ticks) and transmitted virus are responsible for nesting failure. The Soldado virus presents the peculiarity of a wide geographic distribution and the migration of their host (marine birds, namely terns) assume the transport (ROBIN *et al.*, 1978). CLIFFORD (1979) speculates about ticks and related arbovirus exchange between Africa and Pacific regions. This virus is a cause of birds' mortality (CONVERSE *et al.*, 1975) and may be involved in human prurit complaint (CONVERSE *et al.*, 1975). The Bandia virus has been also isolated from *A. sonrai* collected from *Arvicantis niloticus'* burrow in December, 10th, 1997. This argasid is a proved vector of that virus (BRÈS *et al.*, 1967). Dugbe virus responsible of prolonged thrombocytopenia with febrile illness to humans (BURT *et al.*, 1996) has been isolated from *A. sonrai* in Senegal; Congo, Chikungunya and Koutango viruses were also isolated from the same tick in Senegal (ANONYME, 1998). Experimental vector incompetence for CCHF virus has been demonstrated with *A. sonrai* (DURDEN *et al.*, 1993); the argasid tick was not also capable of replicating Chikungunya virus under laboratory conditions (CAMICAS *et al.*, 1978).

No strain of arbovirus has been so far obtained from *O. savignyi* (KARABATSOS, 1985).

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