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ESSAOUIRA AND KALA IRIS: TWO NEW ORBIVIRUSES OF THE KEMEROVO SEROGROUP, CHENUDA COMPLEX, ISOLATED FROM *ORNITHODOROS (ALECTOROBIUS) MARITIMUS* TICKS IN MOROCCO

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Summary. – Essaouira and Kala Iris viruses were isolated from *Ornithodoros (Alectorobius) maritimus* ticks parasitizing yellow-legged gulls (*Larus cachinnans)* on the coast of Morocco in 1979 and 1981, respectively. Serological evidence indicates that these two viruses are new members of the Chenuda complex within the Kemerovo serogroup of the genus *Orbivirus.* Ecological, pathological, morphological, and physicochemical properties are compatible with these findings. The infectivity of these viruses for man and animals, including seabirds, remains unknown.

Key words: Essaouira virus; Kala Iris virus; Orbiviruses; Ornithodoros maritimus; seabirds; Morocco; Kemerovo serogroup; Chenuda complex

Introduction

Moghreb („The Occident") embodies the geographic entity of Morocco, Algeria, and Tunisia in northwestern Africa. Each of these countries is divided into different climatic zones from the relatively temperate northern coasts to the pre-Saharan and Saharan areas in the south. The population is concentrated into cities; the rest of the region is cultivated or arid.

It was postulated that natural cycles of arboviral infections would be prevalent in such biotopes. Several serosurveys have demonstrated the active circulation of arboviruses (Porterfield and Ash, 1966; Nabli *et al.*, 1970; Chastel *et al.*, 1977; 1982; 1983). However, few viruses have been recovered from this area (Pilo-Moron *et al.*, 1970; Chastel *et al.*, 1981; Chastel, 1988).

In this paper, Essaouira and Kala Iris, two new orbiviruses of the Chenuda complex, are described. These viruses were isolated from *Ornithodoros (Alectorobius) maritimus* ticks collected from seabirds in Morocco in 1979 and 1981, respectively.

Materials and Methods

Tick collections. Ticks were collected on Essaouira Island, Morocco, between May 15 and June 22, 1979, and on Kala Iris Islet on May 25, 1981. Essaouira Island (31°31'N; 4°22'W) is located off the Atlantic coast and Kala Iris Islet (35°10'N; 4°22'W), near Al Hoceima Beach, off the Mediterranean coast of Morocco. Ticks, identified as *O. maritimus,* parasitized yellow-legged gulls (*Larus cachinnans* Pallas 1826) on both islands and were found in nests or on the gull chicks. A total of 255 ticks (140 nymphs, 48 males, 67 females) were collected on Essaouira Island and 122 ticks (8 larvae, 80 nymphs, 6 males, 28 females) on Kala Iris Islet. Ticks were sent to the Brest Virus Laboratory by air where they arrived alive.

Isolation procedures and virological studies. Ticks were triturated in pools of 4 to 16 individuals as previously described (Chastel *et al.*, 1983; 1985) and inoculated intracerebrally (ic) into 24–48 hrs-old suckling mice (SM). When paralytic signs occurred, the isolates were adapted to SM by serial ic passages. Reisolation attempts were made from the original material that had been stored at -70 °C.

Attempts were made to adapt the SM isolates to cell cultures and produce plaques in primary vervet monkey kidney and Vero cells propagated by standard methods (Shope and Sather, 1979).

Virological studies were performed in SM and 21 day-old weanling mice inoculated by ic and intraperitoneal (ip) routes or in cell cultures. End points of virus titrations were calculated in a standard way. Virus size was determined by filtration through Millipore filters with 220 nm pores. The effects of lipid solvents (diethylether), acidity (pH 3.0), and temperature (60 °C) were examined by incubating the virus with these chemicals for 1 hr (Main *et al.*, 1973).

Serology. Antigens were prepared from infected SM brains by sucrose-acetone extraction (Clarke and Casals, 1958). Haemagglutination of 24 hrs-old chick and goose erythrocytes was tested at pH 5.8 to 7.4 at 4 °, 20 °, and 37 °C.

For complement-fixation (CF) tests, antigens were titrated against homologous and heterologous immune sera or ascitic fluids (IAF) obtained in weanling mice by methods previously described (Chastel *et al.*, 1985). CF antigens were screened against several grouping and polyvalent IAF supplied by the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA.

For typing the isolates, cross CF and plaque-reduction neutralization (PRN) tests were performed at the Yale Arbovirus Research Unit, New Haven, Connecticut, USA or in Brest using reference IAF kindly supplied by the World Health Organization Reference Center for Arboviruses, New Haven, Connecticut, USA.

Light and electron microscopy. Light microscopy was performed on coronal sections of infected SM brains. Isolates Brest/Ar/T222, Brest/Ar/T234, and Brest/Ar/T598 were examined by transmission electron microscopy of infected SM brains.

Results

Virus isolations

Nine strains of virus were isolated from the ticks collected in 1979 and 1981; three (Brest/Ar/T222, T234, T236) from Essaouira and six (Brest/Ar/T598, T599, T602, T603, T604 and T606) from Kala Iris (Tables 1, 2). The minimum field infection rate was four times greater at Kala Iris than at Essaouira. Reisolations succeeded 5–6 months after the original isolations.

Virological properties of isolates

Paralysis occurred after 11–16 days in all mice inoculated with tick homogenate Brest/Ar/T234 and after 6 days with other isolates. By the second serial passage, the survival time was reduced to 6 days for Brest/Ar/T234 and to 4 days for other strains. The third passage of all strains was pathogenic for SM by ic

Table 1. Virus isolation attempts from Ornithodoros maritimus collected on Essaouira Island and Kala Iris Islet, Morocco, during 1979 and 1981

	Essaouira Island (1979)				Kala Iris Islet (1981)			
	No. of tested	No. of pools	Isolations No.	MFIR	No. of tested	No. of pools	Isolations No.	MFIR
Larvae	0	–	–	–	8	1	0	0
Nymphs	140	12	0	0	80	6	4	1:20
Adult males	48	5	0	0	6	1	0	0
Adult females	67	10	3	1:22	28	4	2	1:14
Totals	255	27	3	1:85	122	12	6	1:20

MFIR = minimum field infection rate

Table 2. Virus isolations from Ornithodoros maritimus collected in Morocco during 1979 and 1981

Strain	Ticks	Location	Identification	
			Group	Virus
Brest/Ar/T222	10 F	Essaouira I.	Kemerovo	Essaouira
Brest/Ar/T234	7 F	"	Hughes	Soldado
Brest/Ar/T236	7 F	"	Kemerovo	?
Brest/Ar/T598	7 F	Kala Iris I.	"	Kala Iris
Brest/Ar/T599	7 F	"	"	?
Brest/Ar/T602	12 N	"	"	?
Brest/Ar/T603	12 N	"	"	?
Brest/Ar/T604	12 N	"	"	?
Brest/Ar/T606	16 N	"	"	?

F = adult females
N = nymphs

Table 3. The sensitivity of Brest/Ar/T222 and Brest/Ar/T598 to a lipid solvent, acidic pH, and heat

Strain	Virus titer (log)			
	Control	After treatment		
		Diethylether	pH 3.0	60 °C
T222	5.7	3.0	< 1.0	3.3
T598	3.0	< 1.0	< 2.0	1.0

Titer of T222 assayed in Vero cells and expressed per 0.1 ml.
Titer of T598 assayed in SM and expressed per 0.03 ml.

Table 4. Results of complement-fixation and neutralization tests comparing Essaouira with other Kemerovo group viruses

Virus	Strain	Essaouira Virus (Brest/Ar/T222)			
		Antigen		Antibody	
		CF	N	CF	N
Chenuda	EgAr 1152	32/128*	10/640	128/128	< 10/320
Baku	LEIV 46A	128/256	10/1280	64/128	10/320
Mono lake	CalAr 861	32/256	10/ < 640	32/128	< 10/320
Huacho	CalAr 883	8/128	10/–	8/128	–
Kemerovo	USSR R-10	8/256	< 10/–	< 8/128	–
Tribeč	original	16/64	< 10/–	< 8/128	–
Lipovník	Cz Lip 91	< 8/32	–	< 8/128	–
unnamed	FinV-808	< 8/128	< 10/–	< 8/128	–
unnamed	FinV-873	< 8/256	< 10/2560	< 8/128	–
unnamed	FinV-962	< 8/256	< 10/–	< 8/128	–
Tindholmur	DenAr 2	< 8/32	< 10/20	< 8/128	–
Mykines	DenAr 12	< 8/64	< 10/40	< 8/128	–
Cape Wrath	ScotAr 20	< 8/128	< 10/80	< 8/128	–
Great Island	CanAr 41	< 8/64	< 10/320	< 8/128	–
Bauline	CanAr 14	< 8/128	< 10/40	< 8/128	–
Yaquina Head	RML-15	< 8/128	< 10/128	< 8/128	–
Okhotskiy	LEIV 287ka	< 8/128	< 10/160	< 8/128	–
Nugget	AusMI-14847	< 8/256	< 10/–	< 8/128	–
Wad Medani	EgAr 492	< 8/32	< 10/–	< 8/128	–

CF = complement fixation
N = neutralization
*Homologous titer/Heterologous titer

route. All strains except Brest/Ar/T234 were pathogenic for SM by ip route and for weanling mice in 20 % by ic route. Brest/Ar/T234 was pathogenic only for SM inoculated ic. Thus, virological properties distinguished 2 types of strains among the 9 isolates: one (Brest/Ar/T234) from Essaouira with a relatively long incubation period and low pathogenicity in mice and 8 strains from both Essaouira and Kala Iris exhibiting a relatively short incubation period and more pronounced pathogenicity.

All isolates passed through a 220 nm membrane filter. Brest/Ar/T222 was relatively resistant to lipid solvents and high temperature, but sensitive to acidic pH (Table 3). Brest/Ar/T598 was relatively resistant to high temperature, but sensitive to lipid solvents and acidic pH (Table 3).

Serological properties of isolates

Haemagglutinins were not detected for any strains, but potent CF antigens and antibodies were obtained for all strains.

In CF tests, all strains except Brest/Ar/T234 cross-reacted with each other. Nine polyvalent or grouping fluids failed to react with 2 CF units of each antigen. Polyvalent 5 grouping fluid containing antibodies to Hughes, Soldado, Sawgrass, Matucare, and Lone Star viruses reacted at 1:32 with Brest/Ar/T234. This strain was identified as an „Old World" variant of Soldado virus (data not shown).

The 8 remaining strains reacted with a Kemerovo grouping fluid at titers of 1:32 to 1:256. Brest/Ar/T222 and Brest/Ar/T598 were selected as representative strains for further studies. By CF tests, Brest/Ar/T222 was shown to be a member of the Chenuda complex closest to Chenuda and Baku (Table 4). By PRN tests both Brest/Ar/T222 and Brest/Ar/T598 were distinct from each other and from Chenuda, Baku and other members of the serogroup (Table 4, 5).

Table 5. Plaque-reduction neutralization tests comparing Kala Iris (Brest/Ar/T598) with Essaouira (Brest/Ar/T598) viruses and other members of the Chenuda complex

Virus	Antibody						
	KI	ESS	CHEN	BAKU	ML	SGC	HUA
Kala Iris	160*	< 10	20	40	< 10	< 10	< 10
Essaouira	< 10	160	-	-	-	-	-
Chenuda	< 10	-	320	-	-	-	-
Sixgun City	< 10	-	-	-	-	80	-

*90 % endpoints
(-) not done

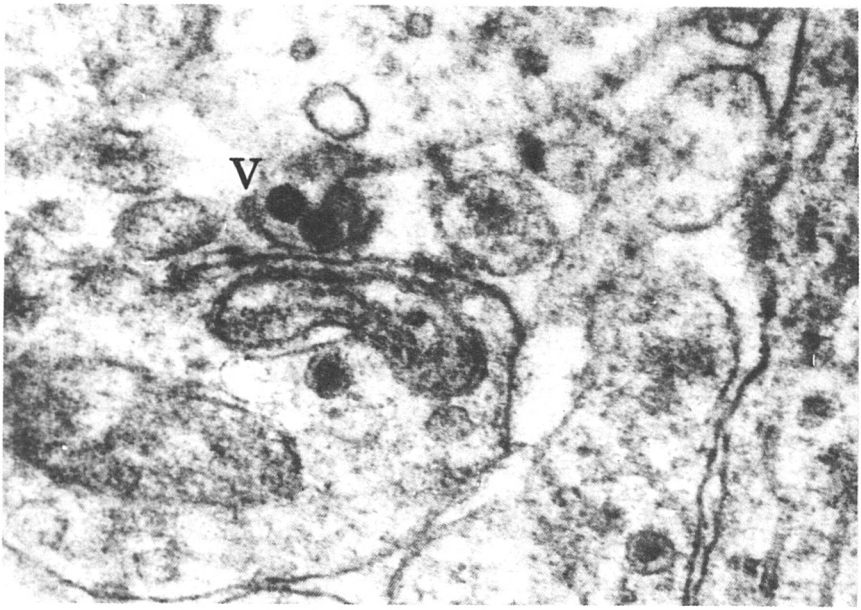

Fig. 1

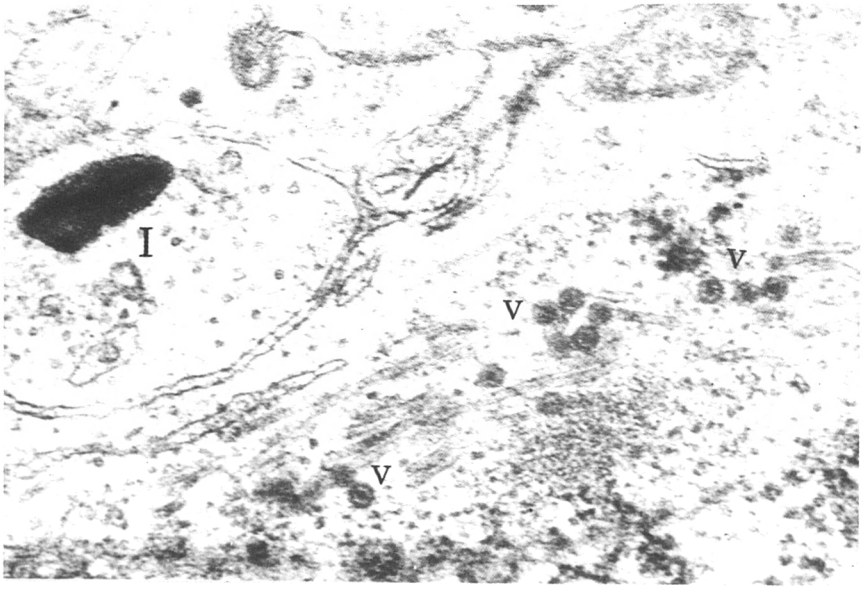

Fig. 2

For legend see page 490

Light and electron microscopy

Suckling mouse brain infected with Brest/Ar/T222 and Brest/Ar/T598 exibited signs of acute meningo-encephalitis by light microscopy. Signs included discrete meningeal infiltration with rounded cells, marked neuronal necrosis of the midbrain and Amon's horns, vasculitis and pericapillary cuffing. By electron microscopy, virus particles, compatible with the morphology of orbiviruses, were seen in SM brains infected with both Brest/Ar/T222 and Brest/Ar/T598 (Figs 1 and 2). They were rare, located in the cytoplasm of infected neurons and their diameter was 65 to 76 nm (mean 70 nm) for Brest/Ar/T222 and 65 to 82 nm (mean 77 nm) for Brest/Ar/T598. An electron-dense core was frequently seen (Fig. 1). Virus particles were not associated with a granular or tubular matrix but intracytoplasmic inclusion was occasionally found (Fig. 1).

Discussion

It has been reported that orbiviruses within the Kemerovo serogroup can be divided into four antigenic and ecological subgroups: (1) the Kemerovo/Tribeč/Lipovník complex infecting mammals and birds in the Palearctic Region and transmitted by *Ixodes ricinus* and *I. persulcatus;* (2) the Cape Wrath/Great Island/Bauline/Yaquina Head/Okhotskiy/Nugget complex infecting seabirds in the coldest areas of both hemispheres and transmitted by *I. uriae*; (3) the Chenuda/Baku/Mono Lake/Huacho complex infecting land-, water- and seabirds worldwide and transmitted by soft ticks of the genera *Argas* and *Ornithodoros;* (4) the Wad Medani/ Seletar complex infecting large mammals in tropical and subtropical areas and transmitted by hard ticks of the genera *Boophilus, Hyalomma, Amblyomma,* and *Rhipicephalus* (Main *et al.,* 1976). The results of serological assays show that all strains isolated from *O. maritimus* in Morocco, except Brest/Ar/T234 (Soldado virus: Hughes group) (Chastel *et al.*, 1983), were members of the Chenuda complex.

Essaouira and Kala Iris viruses conform to serological, geographical, and biological properties of this complex. The close antigenic relationships of Essaouira to Baku virus is of particular interest because Baku was reported from *O. (A.) capensis* and *O. (A) coniceps* in Azerbaijan (Lvov *et al.*, 1971; Gromashev-

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Fig. 1

Brest/Ar/T222 infected SM brain

Virus particles (V) with a central core and a mean diameter of 70 nm are disseminated in the cytoplasm of a neuron. A distant intracytoplasmic inclusion (I) (46 000 x).

Fig. 2

Brest/Ar/T598 infected SM brain

A cluster of few virus particles (V) with a mean diameter of 77 nm is located in a small process of neuropil (61 000 x).

sky *et al.*, 1971). Hoogstraal and coworkers (1979) suggest that the *O. capensis* of Russian workers corresponds to *O. maritimus* of western workers.

It has been demonstrated in other laboratories that Essaouira and Kala Iris have RNA and protein electrophoretic profiles characteristic of orbiviruses, but different from those of other Kemerovo group viruses isolated from Ireland, England, Faroe Islands, Norway, and California (Jacobs *et al.*, 1986; Black *et al.*, 1986). Electrophoretic profiles of Essaouira and Kala Iris viral proteins were similar while the RNA profiles were unique, thus corroborating the distinction of these two agents (Black *et al.*, 1986).

Essaouira shares CF antigens with Nugget virus. The latter is associated with *I. uriae* and penguins on Macquarie Island in the Australian Antarctic (Doherty *et al.*, 1975). The RNA and proteins of Nugget exhibit properties unique among the orbiviruses (Gorman *et al.*, 1984). Genomic reassortments might occur in nature among orbiviruses from different complexes when simultaneously infecting the same seabird host.

Essaouira, but not Kala Iris, was relatively resistant to diethylether. Relative resistance to lipid solvents is not universal among orbiviruses (Buckley, 1972). Some strains of Wad Medani were sensitive (Borden *et al.*, 1971), as were strains of Bauline virus (Main *et al.*, 1973). The infectivity of both Essaouira and Kala Iris viruses was destroyed by exposure to acidic pH, a property shared by orbiviruses (Borden *et al.*, 1971).

Limited information is available on the pathogenicity of Essaouira and Kala Iris viruses. Scientists bitten by *O. maritimus* larvae on Essaouira Island exhibited fever, malaise, rhinopharyngitis and pruritus; however, antibodies to Soldado, but not Essaouira, were detected in these persons (Chastel *et al.*, 1981). Among 128 small rodents and insectivores tested from northern Morocco in 1977, CF antibody to Essaouira was demonstrated in one field mouse (*Apodemus sylvaticus)* (Chastel *et al.*, 1982). The infectivity of the two Moroccan orbiviruses for vertebrates, including gulls and other birds, remains unknown.

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