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ISOLATION OF ORBIVIRUSES AND UUKUVIRUSES FROM PUFFIN TICKS

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Summary. — Two viruses were isolated from a pool of three female *Ixodes uriae* ticks found on a dead puffin *(Fratercula arctica)* on a beach at Arbroath, Scotland. Complement fixation tests showed that one of the viruses was an orbivirus belonging to the Kemerovo serogroup and was related to Cape Wrath virus. Cross-reactions did not occur in neutralisation tests with 4 Kemerovo group viruses previously isolated from *I. uriae* collected in British seabird colonies. The orbivirus was therefore named Arbroath virus. The other virus was of the Uukuniemi serogroup (family Bunyaviridae) and reacted in complement fixation and neutralisation tests with a virus isolated from *I. uriae* collected from a seabird colony at St Abb's Head, Scotland. Both the orbi- and the uukuviruses replicated in a tick *(Rhipicephalus appendiculatus)* cell line, RA-243.

Key words: Ixodes uriae; *Kemerovo*; *Uukuniemi*; *Arbroath virus*

Introduction

The hard tick *Ixodes (Ceratixodes) uriae* is a common ectoparasite of seabirds nesting in sub-polar and temperate regions (Clifford, 1979). At least 40 viruses, from four serogroups, have been isolated from *I. uriae* (Clifford, 1979; Nuttall, 1984). Kemerovo and Uukuniemi are the most common serogroups associated with viruses from *I. uriae* in the U.K. (Main *et al.*, 1976; Nuttall *et al.*, 1981, 1982). This paper reports the isolation of an orbivirus of the Kemerovo serogroup, and a virus of the Uukuniemi serogroup, from *I. uriae* ticks found on a dead puffin (*Fratercula arctica)* on a beach at Arbroath, Scotland.

Materials and Methods

Three engorged adult female *Ixodes uriae* ticks were found on a dead puffin on a beach at Arbroath, east Scotland (56°34′N, 2°35′W). The bird was ringed as a fledgling on the Isle of May (south-east Scotland) on 5th July 1977 and was found washed up on the shore on 8th August 1979.

A continuous cell line (RA-243) of *Rhipicephalus appendiculatus* was obtained from M. Pudney (London School of Hygiene and Tropical Medicine). The cells were maintained at 28 °C, and grown in Leibovitz's medium (L15) supplemented with 10% tryptose phosphate broth and 10% foetal calf serum. All other cell lines used and their conditions of growth have been described previously (Nuttall *et al.*, 1981).

Procedures for isolation of the viruses, electron microscopy, serological and physicochemical tests have been described previously (Nuttall *et al.*, 1981). The orbivirus and the uukuvirus were

plaque picked three times in Vero and *Xenopus* cell cultures, respectively. The orbivirus was designated ARB1 and the uukuvirus, ARB2. Immune ascitic fluids were prepared against two viruses isolated from ticks collected at St Abb's Head, Scotland, FT363, a Kemerovo group virus, and M349, an Uukuniemi group virus (Nuttall *et al.*, 1981), and two other Kemerovo group viruses, IF-2, isolated from 2 male *I. uriae* collected from Inner Farne, north-east England, and M325 from the Shiant Islands in the Outer Hebrides, Scotland (Nuttall *et al.*, 1982).

Results

Inoculation of mice and cell cultures

There was no evidence of virus replication in suckling mice during a period of 21 days following intracerebral inoculation with the pooled tick homogenate. However, inoculation of the pooled tick homogenate into primary chick embryo cell cultures yielded a virus that produced sickness in mice 5 to 6 days post-infection. The plaque-purified orbivirus (ARB1) also induced clinical signs 5 to 6 days after inoculation, whereas the plaque-purified uukuvirus (ARB2) showed no evidence of replication in suckling mouse brain (SMB).

The isolated viruses replicated in Vero, chick embryo fibroblast, chick embryo liver and *Xenopus* cell cultures, producing cytopathic effects. In contrast to the 'uncloned' isolate, the two plaque purified viruses only produced plaques in the cell line used for plaque purification after replication in either Vero or *Xenopus* cells. Both plaque purified viruses replicated in the *Rhipicephalus appendiculatus* continuous tick cell line, RA-243.

Table 1. Results of complement fixation and neutralization tests

Immune ascitic fluid (serogroup)[1]	Reciprocal of complement fixation titre of immune ascitic fluid, against the original isolate[2]	Reciprocal of neutralisation titre of immune ascitic fluid with plaque purified virus	
		ARB1	ARB2
Uncloned Arbroath	64	>256,<512	<16
Arbroath ARB 1	64	>256,<512	32
Arbroath ARB 2	<8	<16	64
St Abb's Head FT363 (KEM)	32	<8	ND[3]
Shiant Islands (KEM)	16	<8	ND
Farne Islands (KEM)	64	<8	ND
Cape Wrath (KEM)	32	ND	ND
Kemerovo (KEM)	16	ND	ND
St Abb's Head M349 (UUK)	<8	<16	64
Clo Mor (SAK)	<8	ND	<16
Avalon (SAK)	<8	ND	ND
Hughes group	<8	ND	ND
Gt Saltee Island (GS 80-3) (HUG)	<8	ND	<16

[1] Kemerovo (KEM), Uukuniemi (UUK), Sakhalin (SAK), Hughes (HUG).
[2] The antigen was prepared from infected mouse brains and used at a dilution of 1 : 8.
[3] Not done.

Electron microscopy

Primary chick embryo liver and fibroblast cell cultures inoculated with the tick homogenate contained masses of virions resembling viruses of the Bunyaviridae (Fig. 1). Passage of this material once in mice, followed by inoculation of either Vero or BHK cell cultures, revealed orbivirus-like particles (Fig. 2). In contrast to infected vertebrate cell lines, RA-243 cells inoculated with the orbivirus (ARB1) contained few complete virions and a cytopathic effect was not apparent. However, large inclusions resembling viral matrix were observed within the cytoplasm (Fig. 3). The inclusions frequently contained fibrils but were rarely associated with virions. Virus particles were not observed in RA-243 cells infected with the uukuvirus (ARB2).

Physicochemical properties

Treatment of either ARB1 orbivirus (4.0 $\log_{10}$ PFU/ml) or ARB2 uukuvirus (3.0 $\log_{10}$ PFU/ml) with either ether, chloroform, 0.5% sodium deoxycholate or pH 3.0 buffer resulted in complete loss of infectivity.

Complement fixation and neutralization tests

The original isolate produced cross reactions in complement fixation tests with the Kemerovo serogroup. There was no evidence of an uukuvirus in this antigen (Table 1), and no cross reactions were produced with any of the other thirty immune ascitic fluids used. Immune ascitic fluid raised against ARB2 infected *Xenopus* cell cultures reacted with SMB antigen of M349 (Uukuniemi serogroup). In neutralisation tests, the orbivirus (ARB1) failed to react with immune ascitic fluids to four other Kemerovo group orbiviruses isolated in the UK (Table 1), and ARB1 ascitic fluid did not neutralise the corresponding Kemerovo group viruses. ARB2 cross-reacted with M349 virus of the Uukuniemi serogroup (Table 1). Neutralization was also observed between ARB2 virus and ARB1 ascitic fluid.

Discussion

A pool of three female *Ixodes uriae* found on a dead puffin *(Fratercula arctica)* on a beach at Arbroath contained both orbi- and uukuviruses. The orbivirus cross-reacted in complement fixation tests with the Kemerovo serogroup, which at present contains twenty viruses all isolated from ticks (Gorman *et al.*, 1983). Within this group there are four antigenic complexes (Main *et al.*, 1976; Gorman *et al.*, 1983); results from complement fixation tests indicated that the orbivirus isolate was related to the Great Island complex. The plaque purified orbivirus (ARB1) showed no evidence of neutralization with other Kemerovo group viruses of the Great Island complex isolated in the British Isles. These results indicate that ARB1 is a distinct serotype and should be named Arbroath virus.

There was no evidence of an uukuvirus in complement fixation tests with the original isolate, however, a positive reaction was obtained using ARB2 ascitic fluid with M349 virus isolated from St Abb's Head, Scotland. The plaque purified uukuvirus also produced cross-reactions in neutralisation tests with

M349 virus, a member of the Uukuniemi serogroup (Nuttall *et al.*, 1981). The uukuvirus (ARB2) showed no evidence of replication in SMB. The apparent absence of the uukuvirus in SMB inoculated with the original isolate was therefore not unexpected.

Physicochemical properties of the two isolated viruses, ARB1 and ARB2, were characteristic of orbiviruses and uukuviruses respectively. Sensitivity to lipid solvents (shown by ARB1) has been reported for several Kemerovo group viruses (Nuttall *et al.*, 1982). Both the orbivirus and the uukuvirus replicated in the *Rhipicephalus appendiculatus* tick cell line, RA-243. Electron microscopic examination of the cells, compared with control uninfected cells, did not reveal evidence of a cytopathic effect. A cytopathic effect has not been observed previously when arboviruses replicate in this cell line (Varma *et al.*, 1975).

The two viruses from Arbroath showed similarities to other viruses isolated from ticks collected at seabird colonies in north-east England and Scotland. It is unlikely that the movement of puffins contributes greatly to the spread of these viruses as the birds are rarely found more than 100 km from the place at which they were ringed as fledglings (Harris, 1983). There is, however, regular movement of puffins between colonies in east Scotland and north-east England (Harris, 1983), which could explain the similarities between the viruses isolated from Arbroath and viruses isolated from St Abb's Head.

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Explanation of Figures (Plates XXVI–XXVII):

Fig. 1. Chick embryo liver cell culture inoculated with a homogenized pool of *Ixodes uriae* ticks from Arbroath. Extracellular uukuvirus particles associated with cell membranes. Bar = = 100 μm.

Fig. 2. BHK cell cultures inoculated with suckling mouse brain infected with the original virus isolate from a pool of *Ixodes uriae* ticks. Intracytoplasmic viral matrix associated with orbivirus cores and virions. Bar = 100 μm.

Fig. 3. Rhipicephalus appendiculatus tick cell culture (RA-243) inoculated with Arbroath orbivirus (ARB1). Large intracytoplasmic viral matrix containing fibrils. Bar = 100 μm.