

Isolation of an English uukuvirus (family *Bunyaviridae*)

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SUMMARY

A virus of the Uukuniemi serogroup was isolated from various organs of a moribund kittiwake (*Rissa tridactyla*) and from ticks (*Ixodes uriae*) that were feeding on the bird. The kittiwake was found on Marsden beach, north-east England. This is the first virus in the family *Bunyaviridae* to have been isolated in England and only the second English arthropod-borne virus after louping ill virus, family *Togaviridae* (Smith & Varma, 1981). The possibility of infection of humans by uukuviruses is discussed.

Uukuniemi (UUK) virus was originally isolated in 1960 from the cattle tick, *Ixodes ricinus*, during a study of tick-borne viruses in Finland prompted by the occurrence of tick-borne encephalitis in certain southern areas of that country (Saikku & Brummer-Korvenkontio, 1973). This strain, S23, is the prototype of the uukuvirus genus (UUK serogroup) of the family *Bunyaviridae*. Since 1960, viruses of the UUK serogroup have been isolated from Palearctic, Oriental and Nearctic regions of the world (reviewed by Bishop & Shope, 1979). Uukuniemi virus has been isolated from several vertebrate species including passerine birds from south-east Finland (Saikku & Brummer-Korvenkontio, 1973; Saikku, 1974) and Azerbaijan, USSR (Gaidamovich *et al.* 1971). Other uukuviruses have been isolated from the 'seabird tick', *Ixodes uriae*, including Zaliv Terpeniya virus from both the Sea of Okhotsk, east USSR (Lvov *et al.* 1973) and Brittany, France (Chastel *et al.* 1981), and Oceanside and Oceanside-like viruses from the eastern seaboard of the USA (Yunker, 1975).

Nuttall *et al.* (1981) have reported the isolation of serologically identical strains of a virus of the UUK serogroup from both *I. uriae* ticks and the blood and brain of a juvenile kittiwake (*Rissa tridactyla*) collected in Scotland. This report describes the isolation of a virus of the UUK serogroup from a moribund kittiwake nestling and from engorging ticks found on it. The kittiwake was found at Marsden, Tyne and Wear in north-east England, on 22 July 1979, on the beach below cliffs on which kittiwakes were nesting. No gross pathology was observed on necropsy. Brain, liver, kidney, lung, spleen, gut and blood from the bird, together with the ticks, were stored at -70°C until they were processed for viruses.

Methods of virus isolation and characterization have been described (Nuttall *et al.* 1981). The ticks were examined as three pools: (1) 25 females, (2) six nymphs, and (3) a single mature female. Antigens for complement fixation tests (CFT) were

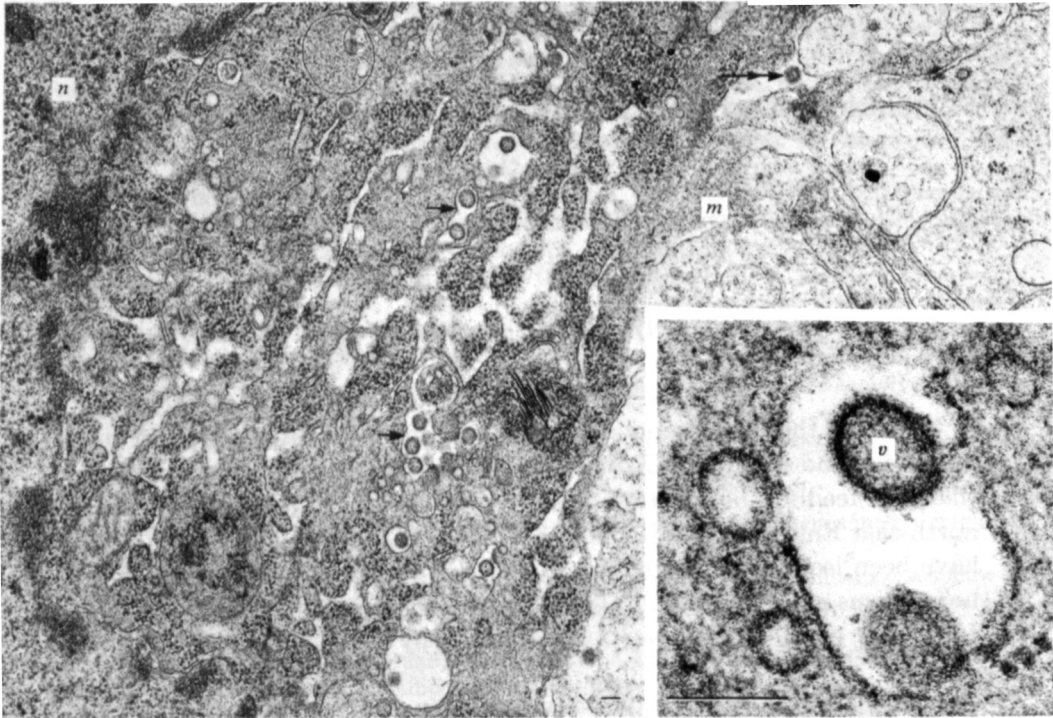


Fig. 1. Virus particles contained in vacuoles (arrowed) within a granular region of the cytoplasm situated adjacent to both the nucleus (*n*) and the cell membrane (*m*). Note particle in space between adjacent cells (double arrow). Inset shows an almost mature particle (*v*) budding into a vacuole. Bar in each case represents 100 nm.

prepared by sucrose–acetone extraction of mouse brain (Clarke & Casals, 1958) infected with the virus isolated from either kidney or tick pool 3.

Viruses were isolated from the three tick pools and from all the organs of the kittiwake by intra-cerebral inoculation of two day-old mice and by inoculation of either *Xenopus laevis* or primary chick embryo liver cell cultures. They were re-isolated four months later in *Xenopus* cell cultures from all the samples except the blood. Inoculation of two-day-old mice resulted in a fatal encephalitis seven to 10 days post-inoculation. Necrotic lesions were observed on the surface of the cerebrum of moribund mice. Electron microscopic examination of thin sections of these lesions revealed densely staining granular extranuclear regions in the cytoplasm of several cells (Fig. 1). In these areas bunyavirus-like particles were seen both within and budding into cytoplasmic vacuoles (Fig. 1, inset). Virus particles observed in both infected mouse brain and cell cultures were approximately spherical, 92 ± 2 nm in diameter ($n = 32$), with a closely adherent membrane surrounding a granular core of variable density. Mature particles were usually contained within cytoplasmic vacuoles and often appeared to have regular fringes of projections.

The isolates produced plaques in *Xenopus* but not in Vero cell cultures. Plaques were faint, with a diffuse edge, and less than one millimetre in diameter when fixed and stained four days after infection. Treatment of four \log_{10} p.f.u. with either 50% ether, 0.5% or 0.05% sodium deoxycholate, or pH 3 buffer, resulted in complete

Table 1. Comparison of the different isolates by complement fixation (CF) and neutralization tests (NT)

Antigen	Antibody titre of immune ascitic fluids raised against						
	Tick isolate		Kittiwake isolate*		M349-St Abb's uukuvirus		Four viruses†
	CF	NT	CF	NT	CF	NT	
Tick derived	512‡	192§	64	128	512	128	64
Kittiwake derived	512	128	64	128	512	192	64
M349	512	128	64	96	512	128	64

* Originally isolated from kidney homogenate.

† A reference ascitic fluid (polyvalent 4) raised against Uukuniemi, Grand Arbaud, Nyamanini and Thogoto viruses.

‡ Reciprocal of ascitic fluid dilution resulting in 50% complement fixation, with antigen diluted 1 in 16 in each case.

§ Reciprocal of highest dilution of ascitic fluid causing a 50% reduction in plaque number.

|| Tick virus isolate of the Uukuniemi serogroup (Nuttall *et al.* 1981).

loss of infectivity. Infectivity was not affected by treatment with buffers at pH 5, 7 or 9.

The viruses isolated from tick pool 3 and from kittiwake kidney, and an uukuvirus, M349, were indistinguishable on the basis of CFT and neutralization tests (Table 1). In CFT using 29 reference ascitic fluids (described by Nuttall *et al.* 1981) obtained from the US National Institute of Allergy and Infectious Diseases (NIAID) (Bethesda, MD, USA) both antigens reacted only with polyvalent 4 (Table 1), which was raised against two viruses of the Uukuniemi serogroup (Uukuniemi and Grand Arbaud). M349 virus, isolated from seabird ticks collected at St Abb's Head, Scotland, was characterized previously as a member of the UUK serogroup on the basis of cross-reactions in CF with polyvalent 4 reference ascitic fluid, and with a reference Uukuniemi virus antigen obtained from NIAID.

The results expand the known distribution of the UUK serogroup in recording the first Uukuvirus from England; most earlier Scottish, Welsh and Irish isolates belonging to the family *Bunyaviridae* have been nairoviruses (Converse *et al.* 1976; Keirans *et al.* 1976). Isolation of virus from all organs of the bird probably reflects a viraemic phase; virus from the ticks may have been isolated from undigested blood meals. Although the kittiwake was apparently sick, the role of uukuviruses in causing disease is unknown.

Ixodes uriae will bite man when given the opportunity (Arthur, 1963; Mehl & Traavik, 1983; Nuttall, personal experience). Furthermore, serological evidence indicates that humans are susceptible to infection by Uukuniemi virus although clinical reactions have not been noted (Vasilenko, Iycks & Tamm, 1975). At Marsden beach, the site of origin of this, the first reported bunyavirus in England, a large population of kittiwakes nest on cliffs in close proximity (< 30 m) to sunbathing areas. Ticks that fall from the nests could find an alternative food source in sunbathers. The ability of *I. uriae* to transmit uukuviruses to humans therefore warrants investigation.

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