

Tick-borne Great Island Virus: (I) Identification of seabird host and evidence for co-feeding and viraemic transmission

M. A. NUNN^{1*}, T. R. BARTON^{2†}, S. WANLESS², R. S. HAILS¹, M. P. HARRIS²
and P. A. NUTTALL¹

¹NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford OX1 3SR

²NERC Centre for Ecology and Hydrology, Hill of Brathens, Banchory, Kincardineshire, AB31 4BW

(Received 26 May 2005; revised 1 August 2005; accepted 3 August 2005; first published online 11 October 2005)

SUMMARY

Great Island Virus (GIV) is an arbovirus present in the tick *Ixodes uriae*, a common ectoparasite of nesting seabirds. Common guillemot (*Uria aalge*) and black-legged kittiwake (*Rissa tridactyla*) are the preferred and most abundant hosts of *I. uriae* on the Isle of May, Scotland. As part of a study to understand the epidemiology of GIV, the ability of guillemot and kittiwake to support tick-borne transmission of GIV was examined. GIV was present in ticks feeding in isolated guillemot colonies and guillemots had virus-specific neutralizing antibodies demonstrating previous GIV infection. By contrast, only uninfected ticks were found in colonies inhabited solely by kittiwakes. GIV was isolated from kittiwake ticks in colonies which also contained breeding guillemots but no virus-specific neutralizing antibodies were present in blood samples of kittiwake on which infected ticks were feeding. Thus guillemots are the main vertebrate hosts of GIV on the Isle of May whereas kittiwakes do not appear to be susceptible to infection. Virus infection of adult ticks feeding on guillemots was highly efficient and may involve both viraemic transmission and transmission from infected to uninfected ticks feeding together on birds that do not develop a patent viraemia.

Key words: orbivirus, transmission, neutralizing antibody, tick, *Uria aalge*, *Rissa tridactyla*.

INTRODUCTION

Wildlife diseases normally only come to our attention if they have a direct effect on humans or domestic animals (e.g. West Nile flavivirus outbreak in humans in northeastern USA; Rappole, Derrickson and Hubalek, 2000), or if an epidemic occurs that causes dramatically increased mortality rates in a wild species (e.g. the 1988 epidemic of phocine distemper morbillivirus in North Sea harbour seals, *Phoca vitulina*; de Swart *et al.* 1995). The effects of most wildlife diseases, including arboviruses, are not so obvious, but nevertheless can affect population size and growth rates through modest increases in mortality and morbidity (Anderson and May, 1991; Grenfell and Gulland, 1995). An understanding of these effects requires quantitative analysis of pathogen transmission (Grenfell *et al.* 2002). Such studies have rarely been undertaken for tick-borne viruses. A notable exception is Louping Ill virus of red grouse vectored by *Ixodes ricinus* (Hudson *et al.* 1995).

Great Island Virus (GIV) is a tick-borne member of the genus *Orbivirus*, family *Reoviridae* (Bucher-

Osmond, 2003). It has 10 segments of double-stranded RNA (dsRNA) packaged in an icosahedral particle. Different strains of the virus are serologically distinct and have unique electrophoretic profiles of their dsRNA. *Great Island Virus* (GIV) is transmitted to avian hosts by the 3-host hard tick *I. uriae* (White, 1852) whose biology and more recently spatial genetic structure had been described (Eveleigh and Threlfall, 1975; Barton *et al.* 1996; McCoy and Tirard, 2002; McCoy, Tirard and Michalakis, 2003). GIV has been found in seabird colonies of both the northern and southern hemispheres, reflecting the bipolar distribution of *I. uriae* (Nuttall *et al.* 1981, 1984a; Doherty *et al.* 1975). However, the impact of GIV on its avian hosts has not been assessed. Indeed it is not known which seabird species actually supports the transmission of GIV. In this paper we identify guillemots as the principal host of the virus and provide evidence for viraemic transmission and also non-viraemic transmission (Jones *et al.* 1987; Lord and Tabachnik, 2002) between co-feeding ticks. In an accompanying paper (Nunn *et al.* 2006), we determine the most important parameters affecting GIV transmission rate between ticks and guillemots.

Our study was undertaken at the mixed seabird colony on the Isle of May, Scotland. At this site common guillemot (*Uria aalge*, Pontoppidan, 1763) and black-legged kittiwake (*Rissa tridactyla*, Linnaeus, 1758) are the main hosts for *I. uriae*,

* Corresponding author: NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford OX1 3SR. Tel: +44 (0) 1865 281630. Fax: +44 (0) 1865 281696. E-mail: amn@ceh.ac.uk

† Present address: University of Aberdeen, Lighthouse Field Station, George Street, Cromarty, Ross-Shire, IV11 8YJ.

since our earlier work showed these two relatively abundant species support far higher numbers of ticks than the other seabird species present on the island (Barton *et al.* 1996). The study system permits systematic sampling of ticks (sedentary) and birds (nesting) during the breeding season. The longevity and nest site fidelity of the birds also permits the same marked individuals to be recaptured and sampled year-after-year. To determine whether guillemots and/or kittiwakes are infected with GIV and are able to support transmission to ticks, we examined virus infection prevalence in ticks removed from birds and levels of virus-specific neutralizing antibody present in blood.

MATERIALS AND METHODS

Materials

BHK21 cells and Vero cells were a gift from Steve Moss, CEH Oxford. Tissue culture plates (Corning), Eagles Minimum Essential Media (Sigma) and penicillin streptomycin antibiotics (Gibco-BRL) were purchased. Polyacrylamide for gel electrophoresis and all other chemical reagents were obtained from Sigma.

Four serologically distinct GIV strains named Broadhaven virus (BRDV), Colony B North virus (CBNV), Above Maiden virus (AMDV) and Colony virus (COYV) were used for plaque reduction neutralization tests. Filtered, high titre frozen (-70°C) stocks were derived from BHK-21 cells infected with 0.01 plaque forming units (pfu) per cell. BRDV was a gift from Steve Moss. It was derived from a fourth round plaque pick of FT363 virus. FT363 was isolated from a pool of 10 *I. uriae* engorged nymphs collected in 1975 from a seabird colony at St Abbs Head, Scotland (Nuttall *et al.* 1981) which lies 32 km south of the Isle of May. CBNV, AMDV and COYV were each isolated from single adult *I. uriae* collected on the Isle of May in 1994. Passage 2 stocks of these three viruses were used.

Study site

The study was carried out between 1993 and 1996 at the seabird colony on the Isle of May ($56^{\circ}11'\text{N}$, $2^{\circ}33'\text{W}$), Scotland. Approximately 12 000 pairs of guillemots and 6–7000 pairs of kittiwakes breed on the island. The two species nest in both discrete and mixed groups.

Capture and sampling of birds and ticks

Breeding kittiwakes and adult breeding and immature pre-breeding guillemots were captured with either a wire crook on the end of a 3 metre long bamboo pole, a monofilament noose fitted to a 5

metre fishing rod or in box traps. Birds not caught incubating eggs or brooding chicks were classified as breeders or pre-breeders on the basis of the presence or absence of a brood patch. Blood samples were taken (under licence) from the foot web (guillemot) or wing (kittiwake) using a sterile 23G needle and pressing a 4.25 cm^2 3 MM Whatman filter paper against the wound. Ethanol (80%) was used to clean the wound site before and after sampling. The filter papers were air-dried then stored at -70°C in sealed bags containing silica gel. In 1995, heparinized capillary blood samples were also taken. Each bird was searched for ticks by one of us (T.R.B.) using both sight and touch for 3 min. The search method detects nymphs and adult females but very few larvae. Ticks were removed using ethanol-sterilized forceps and placed in plastic tubes and stored alive at 4°C (≤ 2 weeks) before being frozen (-70°C). Before release, all birds were ringed with a unique British Trust for Ornithology serial metal ring and birds in the study area were ringed with unique colour ring combination rings for identification from afar.

Virus prevalence

Virus was isolated, titrated and plaque-purified from individual adult female ticks using BHK21 and Vero tissue culture cells according to the method of Nuttall *et al.* (1984b). In brief, virus isolation was from whole ticks that were homogenized in tissue culture media. After centrifugation, supernatants were added to cell monolayers that were observed microscopically for 6 days for signs of virus infection. Material containing infectious virus was stored at -70°C . Red blood cell fractions ($20\ \mu\text{l}$) from the heparinized capillary blood samples collected in 1995 were also used for virus isolation.

Purification, electrophoresis and silver-staining of viral dsRNA

BHK21 cells in a 25 cm^2 flask were infected with 0.1 virus particles/cell and harvested 24 h p.i. by pelleting ($10\ 000\ \text{g}$, 1 min) and resuspending in 0.18 ml of TRIzol (Gibco-BRL) solution by pipetting. Then 0.02 ml of chloroform was added and the mixture vortexed, chilled on ice for 5 min and microcentrifuged at $16\ 000\ \text{g}$ for 15 min. An equal volume of isopropanol was added to the upper colourless phase, which was vortexed, incubated at 4°C for 15 min and microcentrifuged at $16\ 000\ \text{g}$ for 15 min. The pellet was washed with 0.5 ml of 75% ethanol, then dried, resuspended in $12\ \mu\text{l}$ ddH₂O and stored at -20°C . The RNA concentration was estimated by measuring absorbance at 260 nm ($1A_{260}$ unit of dsRNA is equivalent to about $60\ \mu\text{g}/\text{ml}$).

Five ng purified viral dsRNA per lane was separated on 1 mm thick, 8 cm long upright 10% polyacrylamide slab gels at 42 mA for 4.5 h using a

Table 1. Mean virus prevalence in female ticks removed from breeding kittiwakes and guillemots inhabiting mixed colonies (present) or discrete colonies > 50 m apart (absent)

(The number of ticks (N_{tick}) and birds (N_{bird}) examined are shown.)

Host species	Tick infection prevalence (%)		% of birds with one or more infected ticks	
	N_{tick}	Mean \pm s.e.	N_{bird}	Mean \pm s.e.
Guillemot (kittiwake absent)	25	8 \pm 5	17	6 \pm 6
Guillemot (kittiwake present)	12	17 \pm 11	6	17 \pm 15
Guillemot total	37	11 \pm 5	23	9 \pm 6
Kittiwake (guillemot absent)	26	0	13	0
Kittiwake (guillemot present)	80	11 \pm 4	24	29 \pm 9
Kittiwake total	106	8 \pm 3	37	19 \pm 6

discontinuous Tris-glycine buffer system. Gels were fixed for 20 min in 30 ml of 10% ethanol, 0.5% glacial acetic acid. The fixative was removed and 30 ml of freshly made 0.1% (w/v) silver nitrate solution added. After 1 h the gel was washed 3 times in large volumes of ddH₂O then developed by adding 25 ml of 36% sodium hydroxide 1% (v/v) of 37% formaldehyde. The reaction was stopped by extensive washes with distilled water.

Detection of virus-specific neutralizing antibodies

Discs (1.8 cm²) were punched from the filter paper blood samples, and eluted overnight at 4 °C in 320 μ l of phosphate-buffered saline, 0.05% Tween 20, 50 μ g/ml penicillin/streptomycin (PBST). The protein concentration of the whole and eluted blood was determined by Bradford assay (Bio-Rad Laboratories GmbH). Eluted blood samples were diluted to 10 mg/ml with PBST; no sample was diluted more than 2-fold to achieve this concentration. Vero cells (2×10^5) in 0.5 ml of cell medium (RPMI 6% foetal bovine serum) were added to each well of a 24-well plate that was then placed in an incubator for 3 h. Meanwhile, 10^{-1} to 10^{-3} dilutions of each virus strain were made in PBST. Ten μ l of each eluted blood sample was mixed with 10 μ l of each virus dilution and incubated at 30 °C for 2 h before adding the entire mixture to a single Vero cell well. Thus the final dilution of virus on the plate was 10^{-3} to 10^{-5} . Blood samples were each assayed 4 times (duplicate wells on 2 plates). Cells were placed in the incubator for 1 h then overlaid with 0.5 ml of carboxymethylcellulose, and incubated for 4 days before fixing with formal saline and staining with crystal violet. Results were expressed as average fold decrease in virus titre relative to controls. The controls used were incubation of virus for 2 h in (1) PBST or (2) chicken blood eluted from filter paper. Samples that decreased virus titre more than 20-fold were regarded as seropositive. The cut-off point was 6 standard errors higher than the average value for chicken negative control samples. A 10-fold cut-off

point (ca. 90% plaque reduction) for seropositivity is commonly used in plaque reduction neutralization tests. However, its use increases the risk of false positive results, and has no effect on the overall conclusions of this work.

Statistical analysis

All data sets comprised unique individuals. Statistical analyses were performed using generalised linear modelling techniques (GLM; McCullagh and Nelder, 1989). In each analysis, the initial model, including all the explanatory variables (date, site, tick feeding stage and number/host, bird species and breeding status) was fitted and examined. Models were simplified taking account of previous analyses until the minimal adequate model was found. Normal model checking procedures were employed and appropriate transformations applied. Boxcox analysis indicated that the most appropriate transformation to normalize the neutralizing antibody data was $y = (\text{decrease in pfu})^{-y^2}$.

RESULTS

Virus prevalence in ticks and birds

Virus prevalence data were obtained from 37 female ticks feeding on 23 breeding guillemots and 106 female ticks feeding on 37 breeding kittiwakes. There was no difference in the mean infection prevalence of virus in feeding female ticks removed from breeding kittiwakes and guillemots (Table 1; $\chi^2_1 \approx 0.38$, $P=0.54$). However, GIV was only isolated from kittiwake ticks at sites that also contained breeding guillemots (Table 1). Virus prevalence did not show a significant year effect ($\chi^2_1 \approx 0.91$, $P=0.34$). Only 9 nymphs and no larvae were found on the kittiwakes that were captured so no attempt was made to analyse their infection prevalence. We were able to compare only on-host ticks from the two bird species because we did not find off-host (or questing ticks) in kittiwake nests. Virus prevalence data for off-host

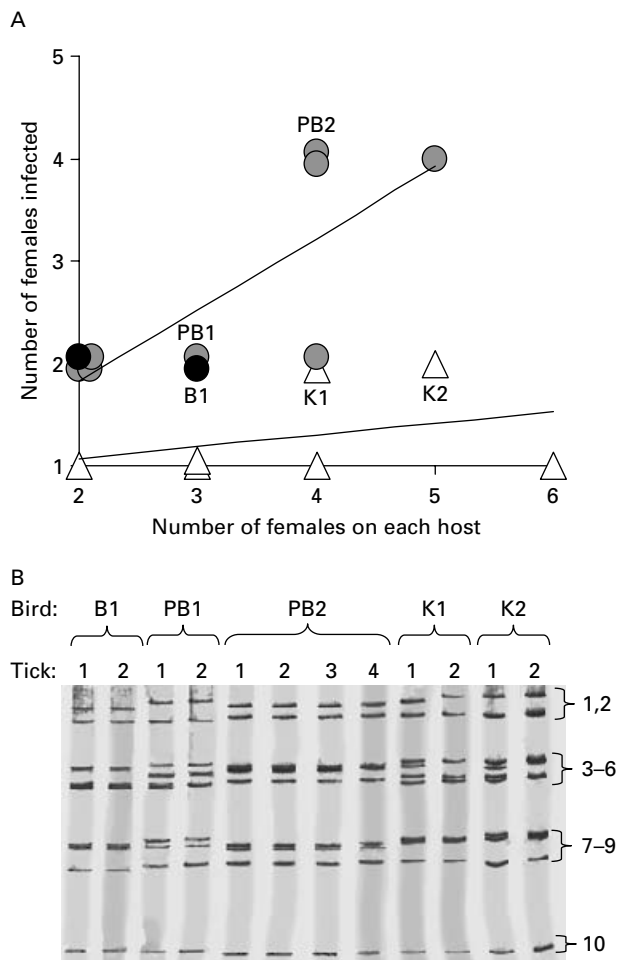


Fig. 1. (A) Association between number of female ticks on guillemot (filled circles: black breeders, grey pre-breeders) and breeding kittiwake (open triangles) and the number of these ticks infected with GIV. Linear regression lines are shown. R^2 values: guillemot 0.598, $P=0.052$; kittiwake 0.107, $P=0.664$. (B) Typical double-stranded RNA profiles (segment numbered at right) of GIV strains present in infected ticks obtained from a breeding guillemot (B1), 2 pre-breeding guillemots (PB1-2) and 2 breeding kittiwakes (K1-2).

ticks in guillemot colonies are reported in the accompanying paper (Nunn *et al.* 2005).

Few infected ticks were found feeding on guillemots so data for pre-breeding and breeding guillemots we pooled in subsequent analyses to increase infected tick sample size (no pre-breeding kittiwakes were caught). Virus prevalence was positively associated with the number of female ticks feeding on guillemots ($F_{1,72}=4.0$, $P=0.049$). Furthermore, among guillemots that had 2 or more female ticks, if 1 female was infected with virus, in most cases (6 out of 10 birds) all the other females on that bird were also infected (filled symbols, Fig 1A). Remarkably, all the infected ticks feeding on the same bird were infected with the same virus assessed by examination of viral RNA profiles (Fig. 1B). This did not appear to be due to contamination otherwise

we would expect at least 1 tick to have a relatively high virus titre (the source of contamination) and the others from the same bird to have low, or undetectable (i.e. <10 pfu), titres. However, at least 2 female ticks, from 7 of 10 guillemots with multiple ticks, had measurable virus titres (data from 4 of these birds are shown in Table 2).

The situation for kittiwakes (breeding alongside guillemots) contrasted markedly with that of guillemots. In most cases (5 of 7), only 1 of 2 or more female ticks co-feeding on kittiwakes was infected (open triangles, Fig. 1A). Furthermore, in both cases where 2 kittiwake ticks were infected, each tick harboured a different virus strain (Fig. 1B). A simple comparison of virus amplification in female ticks feeding on guillemots and kittiwakes is given by the number of infected ticks on birds divided by the number of birds that had infected ticks. That is: $28/10=2.8$ for guillemots and $9/7=1.3$ for kittiwakes (data in Fig. 1A).

Of 27 pre-breeding guillemots examined, blood from only 1 bird yielded virus in tissue culture; no ticks were found on this bird. Virus was not isolated from the blood of 27 breeding guillemots, or 16 kittiwakes breeding alongside guillemots. Eight guillemots (2 breeders and 6 pre-breeders) from which capillary blood samples were taken had 1 or more virus-infected female ticks ($n=17$) feeding on them (Table 2). None of the 8 blood samples yielded virus in tissue culture. Four of these guillemots had 2 or more ticks feeding on them (Table 2). Twelve of the 13 ticks from the 4 birds were infected and, as described above, ticks from the same bird were infected with the same virus. Detection of virus in the feeding ticks but not in the blood taken from the same bird shows that feeding ticks are not equivalent to blood samples. Thus sampling and comparing feeding adult ticks (which may have been infected as larvae, nymphs or adults) is the most sensitive way to look for differences in virus infection prevalence between kittiwakes and guillemots.

Virus-specific neutralizing antibodies

The average protein concentration of whole blood taken from 8 guillemots was 151.4 mg/ml (range 132.1–176.4 mg/ml). The final concentration of the eluted blood sample (10 mg/ml) and virus is therefore equivalent to a serum dilution of about 1:30. Data were obtained on neutralizing antibodies present in blood samples from 23 breeding guillemots and 16 breeding kittiwakes. All 16 kittiwake blood samples were from birds breeding adjacent to guillemots of which 29% had 1 or more GIV infected female ticks feeding on them when captured (Table 1). Breeding guillemots had significantly higher levels of GIV neutralizing antibody than breeding kittiwakes against all 3 virus strains that were tested (BRDV $F_{2,37}=32.8$, $P<0.001$; CBNV

Table 2. Details of GIV infection for guillemot whose blood was tested for virus and which had 1 or more infected ticks when captured

(<10 plaque forming units (pfu) indicates virus was undetectable by plaque assay. Asterisked birds were assayed for virus-specific neutralizing antibodies.)

Status	pfu in blood	Number of ticks	Number of infected ticks	pfu in each tick
Breeder	<10	1	1	160
Breeder	<10	2	2	<10, <10
Pre-breeder	<10	1	1	2000
Pre-breeder*	<10	1	1	20 000
Pre-breeder	<10	1	1	240
Pre-breeder*	<10	2	2	220, 60
Pre-breeder	<10	4	4	8000, 10 000, 200, 16 000
Pre-breeder*	<10	5	4	<10, 50, 180, 100
Total		17	16	

$F_{2,37} = 20.94$, $P < 0.001$; AMDV $F_{2,37} = 15.63$, $P < 0.001$). Kittiwake had insignificant levels of neutralizing antibody comparable to controls (PBST or eluted chicken blood) (Fig. 2). BRDV, CBNV and AMDV were neutralized by 44%, 35% and 22% of the guillemot blood samples respectively (Fig. 3). Twenty-six percent of the guillemot blood samples neutralized 2 or 3 of the strains, 35% 1 strain and 39% none of the 3 strains tested. Only 10 of the blood samples were tested against COYV so data for this virus are not shown in Fig. 3. No detectable neutralizing antibodies against any of the virus strains were present in samples from 3 pre-breeding guillemot that yielded infected ticks but had no virus detectable in their blood (Table 2).

DISCUSSION

Both guillemots and kittiwakes attend breeding colonies as pre-breeders for at least 1 but usually 2–4 years before they recruit to the breeding population (Porter and Coulson, 1987; Harris, Halley and Swann, 1994). During this time they are exposed to parasitism by GIV infected ticks and, if susceptible to virus infection, a proportion of both species will be immune to GIV by the time they become breeders. In June, more female ticks feed on kittiwakes than on guillemots (Barton *et al.* 1996) and therefore a higher percentage of kittiwakes living alongside guillemots have 1 or more infected ticks feeding on them at this time of year. Despite this, neutralizing antibodies against GIVs were not detected in blood samples taken from breeding kittiwakes parasitized by infected ticks. The infected adult ticks feeding on kittiwakes were presumably infected, by feeding on guillemots, during an earlier life stage. This is supported by the observation that infected ticks on the same kittiwake contained different virus strains. In contrast to kittiwakes, 61% of the breeding guillemots had high titre ($\geq 1:30$)

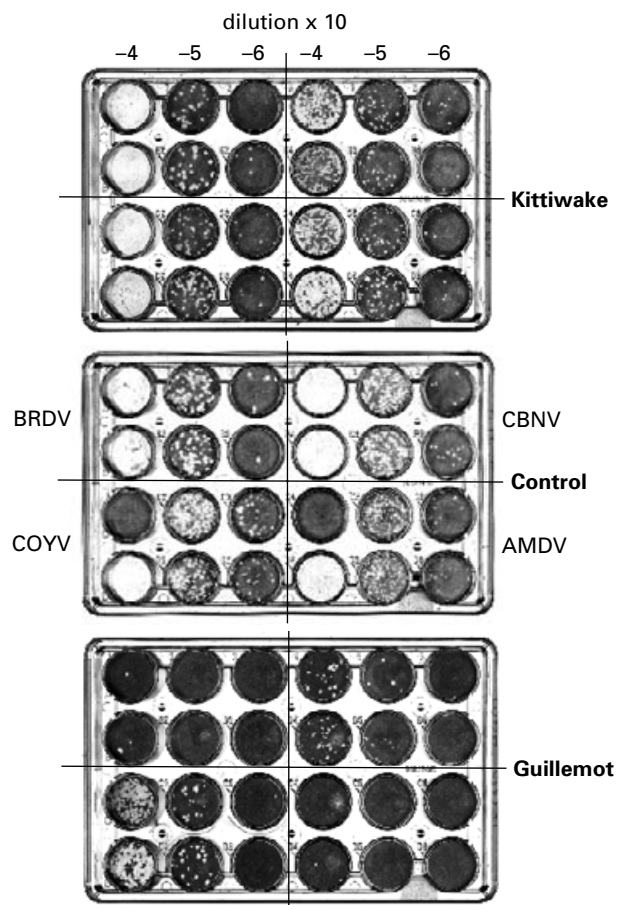


Fig. 2. Typical plaque reduction neutralization result for breeding kittiwake and guillemot. As indicated for the control (adult chicken blood), 1 virus strain was assayed in each quarter of the plate. Two wells on the control plate (COYV-4 and AMDV-4) had no virus added to them. These are results for birds 12 (kittiwake) and 20 (guillemot) in Fig. 3.

antibodies that neutralized one or more of the virus strains by $\geq 95\%$, demonstrating past infection by GIV. All 3 GIV strains infect guillemots. However,

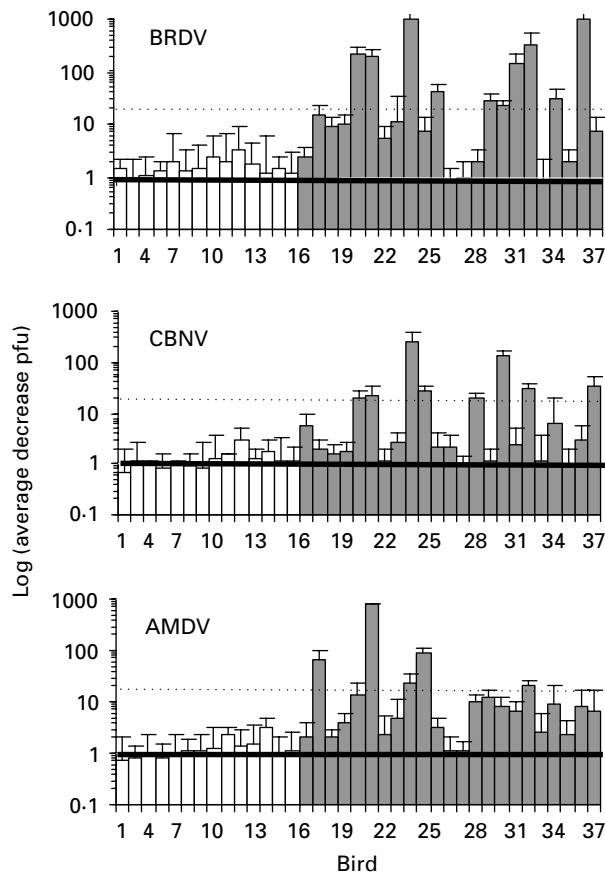


Fig. 3. Neutralization of 3 GIV strains by blood from breeding kittiwakes (white) and breeding guillemots (grey). All kittiwake samples were from birds inhabiting colonies immediately adjacent to guillemot colonies. Lower solid line indicates point of no increase or decrease compared to control. Upper dotted line indicates cut-off point for seropositivity (see main text for explanation).

differences in the percentage of birds that were immune to each strain suggests there may be temporal changes in strain abundance.

Virus infection prevalence in ticks feeding on birds also supports the hypothesis that guillemots, but not kittiwakes, are infected by GIV. No GIV infected ticks were present in colonies inhabited solely by kittiwake, whereas in isolated guillemot colonies as well as mixed kittiwake/guillemot colonies, a similar percentage of female ticks were infected with GIV. GIVs have also been isolated from ticks feeding exclusively on guillemots on Great Saltee Island, Eire (Nuttall *et al.* 1984*a*). Guillemots can develop a detectable viraemia since GIV was isolated directly from the blood of one pre-breeding guillemot during this study. The number of kittiwake blood samples tested for virus is too small ($n=16$) to conclude that they never develop a viraemia, although previous studies report the isolation of GIVs from tissues and blood of auks (including guillemots and Atlantic puffins, *Fratercula arctica*) but not kittiwakes (Nuttall, 1985). GIVs that can infect kittiwakes may exist but show no cross-neutralization with the

strains we tested by virus neutralization assay. However, the most parsimonious explanation of our results is that guillemots, but not kittiwakes, are susceptible to GIV infection. It is an open question whether the presence of kittiwakes increases (by supporting greater numbers of *I. uriae*) or reduces (not productive hosts for GIV) the rate of virus transmission, but the steep decline in breeding kittiwake numbers on the island between 1993 and the present day (S.W., personal communication) may well have affected GIV transmission rates in mixed kittiwake/guillemot colonies. By contrast to kittiwakes, the number of breeding guillemots has remained fairly constant and data from 2004–2005 indicate virus prevalence and mean number and distribution of ticks ($n=148$) feeding on guillemots ($n=189$) are similar to the values observed between 1993 and 1996 (M.A.N., unpublished data).

Horizontal transmission to uninfected ticks requires that they either feed on a viraemic host or co-feed with infected ticks (Jones *et al.* 1987). During co-feeding (or non-systemic) transmission, tick-borne viruses are transmitted between ticks, usually feeding in close proximity, in the absence of detectable viraemia (Labuda *et al.* 1997). In our system, kittiwakes do not seem to support virus transmission, whereas transmission to female ticks feeding on guillemots appears to be highly efficient. Given the present data we can ask whether the transmission between ticks feeding on guillemot is via a viraemic or non-viraemic co-feeding mechanism. It seems likely that viraemic transmission occurs since GIV was isolated directly from guillemot blood and an engorged female tick found off-host in the box traps used to capture pre-breeding guillemots contained 820 000 plaque-forming units of virus which is 30 times more virus than any other titrated tick (M.A.N., unpublished data) suggesting it had fed on a viraemic host. Two other observations indicate that co-feeding transmission may also occur. First, the positive association between virus prevalence and the number of female ticks feeding on adult guillemots is most readily explained by a co-feeding mechanism. Under viraemic transmission, virus prevalence and number of feeding ticks should be independent unless some guillemots have consistently higher tick burdens than others, or ticks prefer to feed on viraemic birds. Second, 4 guillemots with multiple ticks had no detectable viraemia but all the ticks on each of the birds were infected with the same virus strain. Notably, more than 95% of female ticks are clustered on the head and neck of guillemots (T.R.B., unpublished observation) and proximity appears to increase the efficiency of co-feeding transmission (Labuda *et al.* 1997).

Thus GIV transmission probably occurs via both viraemic and non-viraemic routes. Experimental verification of these routes in wild guillemots is ongoing. The experimental observation that co-feeding

transmission can occur on hosts that have neutralizing antibody to a virus (Jones *et al.* 1997; Labuda *et al.* 1997) is particularly significant for GIV, since it may often encounter conditions where its sedentary tick vector feeds exclusively on breeding guillemots whose high levels of virus neutralizing antibody may prevent viraemic but not non-systemic transmission. Co-feeding transmission also appears to have a critical role in the survival of other arboviruses. It is required for the persistence of louping ill virus (Hudson *et al.* 1995; Jones *et al.* 1997; Norman *et al.* 2004) and tick-borne encephalitis virus (Randolph, Gern and Nuttall, 1996; Randolph *et al.* 1999) in nature, under conditions where they would otherwise become locally extinct. Non-systemic transmission has now also been demonstrated for insect-borne arboviruses (Lord and Tabachnik, 2002) and may be a general feature of arbovirus transmission. If co-feeding transmission proves widespread, it has profound consequences for arbovirus control since non-viraemic (but co-feeding competent) hosts may be difficult to identify and vaccination may not prevent virus amplification by non-viraemic transmission.

We would like to thank Chris Wernham for assistance in the field and Scottish Natural Heritage for permission to work on the Isle of May National Nature Reserve. This work was funded by the Natural Environment Research Council.

REFERENCES

- Anderson, R. M. and May, R. M.** (1991). *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press: East Kilbride, Scotland.
- Barton, T. R., Harris, M. P., Wanless, S. and Elston, D.** (1996). The activity periods and life-cycle of the tick *Ixodes uriae* (Acari: Ixodidae) in relation to host breeding strategies. *Parasitology* **112**, 571–580.
- Bucher-Osmond, C.** (2003). The Universal Virus Database ICTVdB. *Computing Science and Engineering* **5**, 16–25.
- De Swart, R. L., Harder, T. C., Ross, P. S., Vos, H. W. and Osterhaus, A. D.** (1995). Morbilliviruses and morbillivirus diseases of marine mammals. *Infectious Agents and Disease* **4**, 125–130.
- Doherty, R. L., Carley, J. G., Murray, M. D., Main, A. J., Kay, B. H. and Domrow, R.** (1975). Isolation of arboviruses (Kemerovo group, Sakhalin group) from *Ixodes uriae* collected at Macquarie Island, Southern Ocean. *American Journal of Tropical Medicine and Hygiene* **24**, 521–526.
- Eveleigh, E. S. and Threlfall, W.** (1975). Bionomics of *Ixodes (Ceratiixodes) uriae* White, 1852 on auks (Alcidae) from Newfoundland. *Canadian Journal of Zoology* **53**, 82–86.
- Grenfell, B. T. and Gulland, F. M. D.** (1995). Ecological impact of parasitism on wildlife host populations. *Parasitology* **111** (Suppl.) S3–S14.
- Grenfell, B. T., Amos, W., Arneberg, P., Bjornstad, O. N., Greenman, J. V., Harwood, J., Lanfranchi, P., McLean, A. R., Norman, R. A., Read, A. F. and Skorpington, A.** (2002). Visions for future research in wildlife epidemiology. In *The Ecology of Wildlife Diseases* (ed. Hudson, P. J., Rizzoli, A. P., Grenfell, B. T., Heesterbeek, H. and Dobson, A. P.), pp. 151–165. Oxford University Press, Oxford.
- Harris, M. P., Halley, D. J. and Swann, R. L.** (1994). Age of first breeding in common murre. *The Auk* **111**, 207–209.
- Hudson, P. J., Norman, R., Laurenson, M. K., Newborn, D., Gaunt, M., Jones, L., Reid, H., Gould, E., Bowers, R. and Dobson, A.** (1995). Persistence and transmission of tick-borne viruses: *Ixodes ricinus* and louping-ill virus in red grouse populations. *Parasitology* **111** (Suppl.) S49–S58.
- Jones, L. D., Davies, C. R., Steele, G. M. and Nuttall, P. A.** (1987). A novel mode of arbovirus transmission involving a non-viraemic host. *Science* **237**, 775–777.
- Jones, L. D., Gaunt, M., Hails, R. S., Laurenson, K., Hudson, P. J., Henbest, P. and Gould, E. A.** (1997). Transmission of louping ill virus between infected and uninfected ticks cofeeding on mountain hares. *Medical and Veterinary Entomology* **11**, 172–176.
- Labuda, M., Kozuch, O., Zuffova, E., Eleckova, E., Hails, R. S. and Nuttall, P. A.** (1997). Tick-borne virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology* **235**, 138–143.
- Lord, C. C. and Tabachnik, W. J.** (2002). Influence of nonsystemic transmission on the epidemiology of insect-borne arboviruses: a case study of vesicular stomatitis epidemiology in the western United States. *Journal of Medical Entomology* **39**, 417–426.
- McCullagh, P. and Nelder, J. A.** (1989). *Generalised Linear Models*, 2nd Edn. Chapman and Hall, London.
- McCoy, K. D. and Tirard, C.** (2002). Reproductive strategies of the seabird tick *Ixodes uriae* (Acari: Ixodidae). *Journal of Parasitology* **88**, 813–816.
- McCoy, K. D., Tirard, C. and Michalkis, Y.** (2003). Spatial genetic structure of the ectoparasite *Ixodes uriae* within breeding cliffs of its colonial seabird host. *Heredity* **91**, 422–429.
- Norman, R., Ross, D., Laurenson, M. K. and Hudson, P. J.** (2004). The role of non-viraemic transmission on the persistence and dynamics of a tick-borne virus – Louping ill in red grouse (*Lagopus lagopus scoticus*) and mountain hares (*Lepus timidus*). *Journal of Mathematical Biology* **48**, 119–134.
- Nunn, M. A., Barton, T. R., Wanless, S., Hails, R. S., Harris, M. P. and Nuttall, P. A.** (2006). Tick-borne Great Island Virus: (II) Impact of age related acquired immunity on transmission in a natural seabird host. *Parasitology* **132** (in this issue).
- Nuttall, P. A., Carey, D., Reid, H. W. and Harrap, K. A.** (1981). Orbiviruses and bunyaviruses from a seabird colony in Scotland. *Journal of General Virology* **57**, 127–137.
- Nuttall, P. A., Kelly, T. C., Carey, D., Moss, S. R. and Harrap, K. A.** (1984a). Mixed infections with tick-borne viruses in a seabird colony in Eire. *Archives of Virology* **79**, 35–44.
- Nuttall, P. A., Moss, S. R., Jones, L. D. and Carey, D.** (1984b). Comparison of mice and cell cultures for

- isolation of tick-borne viruses. *Journal of Virological Methods* **9**, 27–33.
- Nuttall, P. A.** (1985). Tick-borne viruses in seabird colonies. *Seabird* **7**, 36–42.
- Porter, J. M. and Coulson, J. C.** (1987). Long term changes in recruitment to the breeding group, and quality of recruits at a kittiwake *Rissa tridactyla* colony. *Journal of Animal Ecology* **56**, 675–690.
- Randolph, S. E., Gern, L. and Nuttall, P. A.** (1996). Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. *Parasitology Today* **12**, 472–479.
- Randolph, S. E., Miklisova, D., Lysy, J., Rogers, D. J. and Labuda, M.** (1999). Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology* **118**, 177–186.
- Rappole, J. H., Derrickson, S. R. and Hubalek, Z.** (2000). Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerging Infections and Diseases* **6**, 319–328.