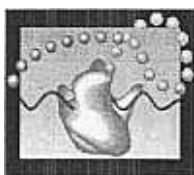


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21 June 1999.

Dr Toby St. George  
15 Tamarix Street  
Chapel Hill, 4069.

Dear Toby,

Thank you for your letter of 26 May 1999. I was again in Europe and thus the delay for my response.


Please find enclosed photocopy of everything I could find re seabird arboviruses.

As world leaders in the field of arbovirology, it was a great privilege to work with and learn virology from such competent researchers. I received invaluable tuition, assistance and collaboration from you, your research group (most notably Helen, Daisy and Steven) and that of Harry Standfast, Michael Uren and David Kemp, who were all active in the field of vectors and vector-borne disease at the time at Longpocket.

I would be most happy for the results of our collaborative efforts to be included in your up-coming collection of papers from the Longpocket Microbiology Section.

Trust this short missive finds you in good health and high spirits. Please give my regards to those from that time who may remember me.

Kindest regards,



Ian Humphery-Smith.  
Director

## Seroepidemiology of arboviruses among seabirds and island residents of the Great Barrier Reef and Coral Sea

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(Accepted 3 April 1991)

### SUMMARY

Duplicate neutralization tests were done on 401 avian and 101 human sera from island residents collected in the Coral Sea and on Australia's Great Barrier Reef against 19 known arboviruses. Antibodies to a potentially harmful flavivirus, Gadget's Gully virus, were equally present (4%) in both avian and human sera. Antibodies to another flavivirus, Murray Valley Encephalitis, and an ungrouped isolate, CSIRO 1499, were also present in both populations with non-significantly different incidences. Antibodies to Upolu, Johnston Atoll, Lake Clarendon, Taggart, Saumarez Reef and CSIRO 264 viruses were restricted to seabirds. Island residents with antibodies to Ross River and Barmah Forest viruses are thought to have been exposed to these viruses on the mainland as antibody to both viruses was absent among seabirds. These results indicate that consideration should be given to tick-associated arboviruses as potential public health hazards on islands where both seabird and human activities interact.

### INTRODUCTION

Attention has recently been drawn to the potential health risks posed by tick transmission of seabird-associated arboviruses to island residents of the South Pacific and, in particular, on Australia's Great Barrier Reef (GBR) [1–3]. These health risks apply equally to inhabitants of Micronesia and Polynesia, where islanders often participate in annual collections of seabird eggs or live close to large seabird colonies and must therefore regularly be attacked by seabird ticks. Elsewhere, Chastel [4] has reviewed the health problems posed by these arboviruses.

In the South Pacific, *Ornithodoros (Alectorobius) capensis* (Neumann, 1901) and *Amblyomma loculosum* Neumann, 1907 are the ticks most commonly found in association with seabird colonies, and both are known to bite humans. Humphery-Smith and colleagues [2] have reviewed the arboviruses already isolated from these two tick species. The development of hypersensitivity to the bites of *O. capensis* by residents of Heron Island at the southern extremity of the GBR is

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indicative of the frequency with which residents are attacked by ticks on islands inhabited by seabirds [3].

Coral and continental islands are often home to hundreds of thousands of seabirds. On these islands, one can encounter literally millions of seabird ticks. These ticks are located on birds, in their nests and scattered about the islands under coral rubble, in and on constructions of human origin and under the bark of trees or any suitable shelter. While awaiting the arrival of a suitable host *O. capensis* is able to survive for in excess of 3 years in the absence of a blood meal [5]. These South Sea islands have long been regarded as desirable places in which to live and increasingly are regarded as exotic holiday locations. Thus, human populations experience increasing contact with seabirds and their ticks.

Here we report the incidence of antibodies to known arboviruses found in 101 human sera taken from residents of Heron Island on the GBR and 401 avian sera collected from the same region and from atolls in the Coral Sea.

#### MATERIALS AND METHODS

Human sera were collected from 101 inhabitants of Heron Island, a coral cay situated in the Capricorn-Bunker Group at the Southern extremity of the Great Barrier Reef on the Tropic of Capricorn approximately 70 km from Gladstone, Australia. This island is home to some 350 persons who include tourists, resort staff, university researchers and National Parks officers. These individuals share the island (16.8 hectares) with an estimated 100 000 seabirds during the summer breeding season and millions of *O. capensis*. The island is characterized by central climax forest of *Pisonia grandis* and various seral stages occurring closer to the shore. An island resident was designated as someone who had spent at least one month on Heron Island during the seabird breeding season.

Avian sera were collected from the sites detailed in Table 1. Using a syringe, blood was collected from drops which formed following incision of the brachial artery; no birds were killed during this study. Sera were collected from the following species: 151 White Capped Noddies (*Anous minutus*); 21 Common Noddies (*A. stolidus*); 136 Wedged-tailed Shearwaters (*Puffinus pacificus*); 10 Lesser Frigatebirds (*Fregata ariel*); 1 Red-footed Booby (*Sula sula*); 32 Masked Boobies (*S. dactylatra*); and 50 Brown Boobies (*S. leucogaster*). All blood samples were allowed to clot at ambient temperature, which was often quite high, for 12–20 h and, following centrifugation at 4000 rev./min for 15 min, they were sterilized by filtration (0.2  $\mu\text{m}$ ) and stored at  $-20\text{ }^{\circ}\text{C}$ .

Duplicate neutralization tests were conducted in parallel in plastic micro-titration plates using Vero or BHK 21 cell cultures for the following arboviruses: Gadget's Gully, Saumarez Reef, Murray Valley Encephalitis (Flaviviridae); Ross River, Sindbis, Barmah Forest (Togaviridae); Upolu, Taggart, Peaton, Precarious Point, Thimiri (Bunyaviridae); Nugget (Reoviridae); Johnston Atoll, Lake Clarendon, Leanyer, CSIRO 264, CSIRO 976, CSIRO 1056, CSIRO 1499 (Ungrouped). All sera were diluted to 1/4 for use in neutralization tests. Thus positive results indicate the presence of specific antibody with a limiting titre of at least 4. Only inhibition of viral growth in both series of tests was taken to indicate the presence of neutralizing antibody against a given virus. Due to the

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Table 1. *Collection sites for avian sera situated in the Coral Sea to the north-east of mainland Australia*

Location	Longitude E	Latitude S
Lihou Reef	152° 03'	17° 08'
Flinders Reef	148° 25'	17° 44'
Marion Reef	152° 23'	19° 06'
Creal Reef	150° 29'	20° 31'
Fredrick Reef	154° 24'	20° 57'
Gannet Cay	152° 29'	21° 57'
Cato Island	155° 32'	23° 15'
Heron Island	151° 55'	23° 27'
Masthead Island	151° 43'	23° 32'
Musgrave Island	152° 24'	23° 55'

Table 2. *Arbovirus for which antibodies were detected*

Virus strain	Human sera*		Seabird sera*		P-value
	% + ve	No. tested	% + ve	No. tested	
Gadget's Gulley	4	101 (1/5)	4.0	300 (1/5)	N.S.
Saumarez Reef	0	101	0.5	380 (1/80)	N.S.
Murray Valley Encephalitis	5	101 (NT)	1.9	371 (NT)	N.S.
Ross River	9	101 (1/160)	0	149	< 0.001
Upolu	0	101	10.4	394 (1/16)	< 0.001
Johnston Atoll	0	101	3.8	367 (1/16)	< 0.05
Taggert	0	101	0.3	300 (1/6)	N.S.
Peaton	N.T.		1.2	261 (1/16)	
Lake Clarendon	0	8	2.7	300 (1/48)	N.S.
CSIRO 1499	1	101 (1/8)	3.2	401 (1/4)	N.S.
CSIRO 976	N.T.		1.2	261 (1/6)	
CSIRO 264	0	101	1.2	261 (1/64)	N.S.
Barmah Forest	2	101 (1/16)	0	149	N.S.

\* In parentheses, maximum antibody titre obtained.

N.T. Not tested.

N.S. Not significant.

limited volumes of serum obtained in many cases, serology could not be performed for all arboviruses for every serum sample collected. When the two neutralization tests were shown to be positive for the presence of antibody, relevant sera were subsequently titrated. Again, because of the limited size of serum samples, titrations were not performed in all cases. The presence of antibodies against Saumarez Reef and Ross River viruses was detected by haemagglutination-inhibition rather than neutralization tests. Chi-square tests were used to assess the relative incidence of antibodies in human and avian populations.

## RESULTS

The presence of neutralizing antibodies to known arboviruses found in human and seabird sera are shown in Table 2. Chi-square analyses conducted on the relative incidence of antibodies in human and avian populations are also presented in Table 2 together with the maximum limiting antibody titre obtained. Although these titres were in some cases quite low and the antibodies detected could be

genuine, they may in some instances mean that infection occurred a long time ago or represent cross reactions with related viruses (unknown or not tested). Because of the absence of antibodies in seabird sera to Peaton, Lake Clarendon and CSIRO 974 (known bird-associated arboviruses), these viruses were not tested in human sera.

No neutralizing antibodies in respectively human and avian sera (numbers in brackets) were observed against the following viruses: Sindbis (101/113); Nugget (18/113); Precarious Point (101/399); Thimiri (18/18); Leanyer (18:150); CSIRO 1056 (0/138). Where the number of sera tested is low, little validity can be given to the absence of antibodies.

#### DISCUSSION

Among the arboviruses examined, the risk of encephalitis in man is most pronounced among the Flaviviruses [7]. Of the three Flaviviruses examined, antibodies to each were present in seabirds. Most surprisingly, antibodies to Gadget's Gully virus were found to be circulating equally in both avian and human sera. This virus was isolated from the hard tick, *Ixodes uriae*, by St George and co-workers [6] from penguin colonies on Macquarie Island in the Southern Pacific Ocean. At present, no data are available on the pathogenicity of Gadget's Gully virus in vertebrates. These results are in contrast to the absence of antibodies to both Gadget's Gully and Saumarez Reef viruses from 14000 sera from mainland Australia [8]. Although not found to be present in this study from human sera, St George and co-workers [9] suggested that Saumarez Reef virus may have been responsible for febrile illness in meteorological workers.

Several authors have noted antibodies in MVE virus in avian fauna [10, 11]. Here, the incidence of antibodies to Murray Valley Encephalitis (virus) was not significantly different in human and seabird populations. Thus, even though the higher incidence in the former group may be indicative of a mainland origin, no conclusions can be drawn from these data as to the site of exposure to MVE virus for the humans in question.

Antibodies to Ross River virus are thought to be due to mainland exposure to these viruses because of the significant difference (Chi-square;  $P < 0.001$ ) observed between the incidence of antibodies to Ross River virus in human and avian populations. Although the results obtained for Barmah Forest virus were not significantly different in human and seabird populations, a similar origin is likely for antibodies to this virus due to their absence from seabird sera and the existence of known foci of infection on the mainland.

Antibodies to six arboviruses were present in seabird populations, but absent from the human population sampled at Heron Island. Although not conclusive evidence, these results suggest that Saumarez Reef, Upolu, Johnston Atoll, Lake Clarendon, Taggart viruses and CSIRO 264 viruses are restricted to seabirds, at least within our study area. Although these islands are sporadically home to mammals other than humans, the absence of freshwater means that these mammals cannot be considered as alternative maintenance hosts for the above viruses. These observations may also allay some public health fears, as both Saumarez Reef and Johnston Atoll viruses are closely related to known human pathogens [1].

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Finally, antibody to an ungrouped isolate CSIRO 1499 virus was found in both human and seabird populations (1.0 and 3.2% respectively of 101 and 401 sera tested). This virus was isolated from *Argas robertsi* on the Australian mainland from ticks associated with colonies of the cattle egret, *Bulbulcus ibis*. Experimentally, the virus is known to produce an antibody response in this avian host [12] and be capable of causing the death of birds (Standfast, pers. comm.). No data are available as to the pathogenicity of this virus in mammalian hosts.

In conclusion, the above results demonstrate the presence of antibodies to potentially harmful tick-associated arboviruses in human and avian populations on islands inhabited by seabirds. In the case of Gadget's Gully virus, these antibodies occur with equal incidence in human and avian populations. Thus as tourist activities increase in these remote locations, some attention should be given to the possible public health problems likely to be associated with some of these viruses. Furthermore, in island communities of Micronesia and Polynesia, these viruses may be responsible for unclarified human illness.

That disease induced by arboviruses, and particularly flaviviruses, has not previously been suspected among the resident or tourist populations of Heron Island is not surprising because (1) the tourist population, (largely international) visiting the island is extremely mobile and thus follow-up studies are virtually impossible; and (2) the number of long-term residents on the island is very limited as the turn-over rate of staff is quite high. Furthermore, the number of residents acting as sentinels for arbovirus detection is relatively low (101 with at least one month's exposure during the seabird breeding season) when compared to the known attack rates for other flaviviruses. For example this is in the order of 1-800/100 000 for St Louis Encephalitis. Also the ratio of apparent to inapparent infection should be considered. This is 1:200 to 1:300 for Japanese Encephalitis, for which the case fatality rate can vary during epidemics from 20-70% in underdeveloped countries to 2-11% among American troops [7]. The latter figures might tend to render insignificant any potential health risk if it were not for the fact that in excess of two million tourists visit the GBR annually. The total number holidaying in the South Pacific is larger and ever increasing.

## ACKNOWLEDGEMENTS

We wish to thank Marine Sciences and Technologies, P & O Resorts (Australia) Pty. Ltd, and CSIRO/University of Queensland for financial support. This study was made possible by the kind cooperation of the captain and crew of the L.H.S. Cape Don and the staff and management of the Heron Island Resort and the Heron Island Research Station. Seabirds were collected under a permit from the Queensland National Parks and Wildlife Service.

## REFERENCE

1. Humphery-Smith I, Cybinski DH. Health risks from tick-transmitted arboviruses on Australia's Great Barrier Reef. *Med J Aust* 1987; **146**: 606-7.
2. Humphery-Smith I, Cybinski DH, Moorhouse DE, Dale D. Arboviruses and zoonotic infections on the Great Barrier Reef and in the Coral Sea. In: St George TD, Kay BH, Blok J, eds. *Arbovirus research in Australia*, Brisbane: Commonwealth Scientific and Industrial Research Organisation and Queensland Institute of Medical Research, 1987: 209-17.

3. Humphery-Smith I, Thong YH, Moorhouse D, Creevey C, Gauci M, Stone B. Reactions to argasid tick bites by island residents on the Great Barrier Reef. *Med J Aust* 1991. In press.
4. Chastel C. Tick-borne virus infections of marine birds. In: Harris KF, ed. *Advances in disease vector research*. Vol 5. New York: Springer-Verlag, 1988: 25-60.
5. Humphery-Smith I, Moorhouse DE. Host acquisition by *Ornithodoros capensis* Neuman (Ixodoidea: Argasidae). *Ann Parasitol Hum Comp*, Paris 1981; **56**: 353-7.
6. St George TD, Doherty RL, Carley JG, et al. The isolation of arboviruses including a new Flavivirus and a new Bunyavirus from *Ixodes* (Ceratiixodes) *uriae* (Ixodoidea: Ixodidae) collected at Macquarie Island, Australia, 1975-1979. *Am J Trop Med Hyg* 1985; **34**: 406-12.
7. Monath TP, Flaviviruses. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL; Monath TP, Roizman B, eds. *Virology*. New York: Raven Press, 1990: 763-814.
8. Hawkes RA, Boughton CR, Naim HM, Wild J, Chapman B. Arbovirus infections of humans in New South Wales. Seroepidemiology of the Flavivirus group of Togaviruses. *Med J Aust* 1985; **143**: 555-61.
9. St George TD, Standfast HA, Doherty RL, Carley JG, Fillipich C, Brandsma J. The isolation of Saumarez Reef virus, a new Flavivirus, from bird ticks *Ornithodoros capensis* and *Ixodes euryptidis* in Australia. *Aust J Exp Biol Med Sci* 1977; **55**: 493-9.
10. Boyle DB, Marshall ID, Dickerman RW. Primary antibody responses of herons to experimental infection with Murray Valley Encephalitis and Kunjin viruses. *Aust J Exp Biol Med Sci* 1983; **61**: 665-74.
11. Kay BH, Hall RA, Fanning ID, Young PL. Experimental infection with Murray Valley Encephalitis virus: galahs, sulphur-crested cockatoos, corellas, black ducks and wild mice. *Aust J Exp Biol Med Sci* 1985; **63**: 599-606.
12. Standfast HA, Muller MJ, Cybinski DH. Cattle egrets and ticks as hosts of arboviruses. In: St George TD, Kay BH, Blok J, eds. *Arbovirus research in Australia*. Brisbane: Commonwealth Scientific and Industrial Research Organisation and Queensland Institute of Medical Research, 1987: 218-20.