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# The ecology of Whataroa virus, an alphavirus, in South Westland, New Zealand

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## SUMMARY

The findings of a survey on the ecology of an alphavirus over the years 1964–9 are reviewed. Evidence is presented to show that wild birds constitute a vertebrate reservoir of the virus and that mosquitoes, primarily *Culiseta tonnoiri* and *Culex pervigilans*, which are both endemic New Zealand species, are responsible for summer transmission.

Serological evidence of infection was obtained in all years and evidence is presented to indicate that the virus is enzootic rather than being reintroduced each spring. The number of birds with antibody increased before mosquitoes became active in the spring and possible explanations of this are discussed.

The mean temperature in the hottest month in the study area is substantially below that in other areas with enzootic mosquito-borne viruses and experimental studies showed that Whataroa virus was able to replicate more rapidly in mosquitoes at low temperatures than any arboviruses previously studied.

The main natural focus of infection appeared to be in a modified habitat and the introduced song thrush (*Turdus philomelos*) to be the main vertebrate reservoir host.

## INTRODUCTION

In New Zealand a number of rural summer epidemics of influenza-like disease suggestive of an arbovirus aetiology have occurred. Because of this a serological survey covering a variety of climatic and biological zones throughout the country was initiated in 1959–60. This led to identification of areas on the west coast of the South Island, where the proportion of fowl sera containing low-titre haem-agglutination-inhibiting antibodies against group A and group B arboviruses indicated the existence of an enzootic infection (Maguire & Miles, 1960). In the 1961–2 summer several strains of an alphavirus (group A arbovirus) were isolated from mosquitoes in an area of South Westland near the small township of Whataroa and in due course this virus was named 'Whataroa Virus' (Ross, Miles, Austin & Maguire, 1964; Maguire, Miles & Casals, 1967).

The extreme complexity of continental ecosystems makes the study of the ecology of an arbovirus extremely difficult and laborious. The existence of an enzootic alphavirus in N.Z. enabled us to make a study of a mosquito-transmitted virus in the relatively impoverished fauna of a large island group in a temperate climatic zone.

South Westland appeared to be the area with the largest amount of infection and a serological survey carried out there in 1963 revealed that at that time 21% of wild bird sera contained neutralizing antibody against Whataroa virus. Laboratory studies showed that when non-immune birds were infected subcutaneously they circulated high titres of virus in the blood for some days.

The variety of mammals in the area was small. They included the usual domestic animals together with large numbers of the introduced Australian brush-tailed possum (*Trichosurus vulpecula*). No other wild mammals were very numerous, but stoats (*Mustela erminea*) were present in considerable numbers and from time to time there was a sharp rise in the number of rats, either *Rattus rattus* or *R. norvegicus*, and occasionally sharp increases in the number of house mice (*Mus musculus*). In our attempts to trap house mice we did not find large numbers in the bush and only caught one mouse per 36 trap nights. We had no success in trapping rats in the area. The very high rainfall in this area (about 4800 mm. per year) is probably the reason for the lack of a stable population of small burrowing mammals and for the complete absence of rabbits (*Oryctolagus cuniculus*). A very few hares (*Lepus europaeus*) were seen from time to time and deer (mainly *Cervus elaphus*) are increasing in the area, but are not yet numerous. Chamois (*Rupicapra rupicapra*) are in large numbers high in the mountains, but only come down to level ground during winter months. We did not find antibodies in sheep or cattle in areas in which we had obtained positive avian and human bloods. The titre of antibody in the serum of trapped possums was low and, when they were inoculated subcutaneously with virus in the laboratory, we were unable to demonstrate viraemia. Similarly, laboratory rats showed very little viraemia when inoculated subcutaneously with this virus (Dempster, 1964). Evidence obtained from these preliminary studies strongly suggested that the main reservoir of this virus was in birds and this seemed the more likely since the related viruses, Sindbis virus and Western equine encephalitis virus, are both regarded as having their main reservoir in avian hosts.

Laboratory studies have indicated that a blood titre of $10^7$ plaque forming units (p.f.u.) per ml. gave an ID 50 to engorging mosquitoes of the species tested (Austin, 1967). No titres of this order were found in any mammals other than suckling mice. Because of this we endeavoured to determine the role of different bird species in the ecology of Whataroa virus and studied birds in defined areas around the township of Whataroa at regular intervals over a period of 5 years. Certain other short term studies were made in special areas.

*Study areas*

The township of Whataroa is situated at lat. 43° 16′ S., long. 170° 22′ E., on the Whataroa flat, a river plain 16 km. long by 9 km. wide surrounded by forest-covered hills except for an outlet to the Tasman Sea (Fig. 1). Although much of it has been cleared for farming there are still very many large clumps of trees and areas of swamp where the hills merge into the plains. During the course of the study, birds were sampled regularly in four areas. These are described in detail elsewhere (Miles *et al.* 1971). They consisted of one area of rain forest, part of which

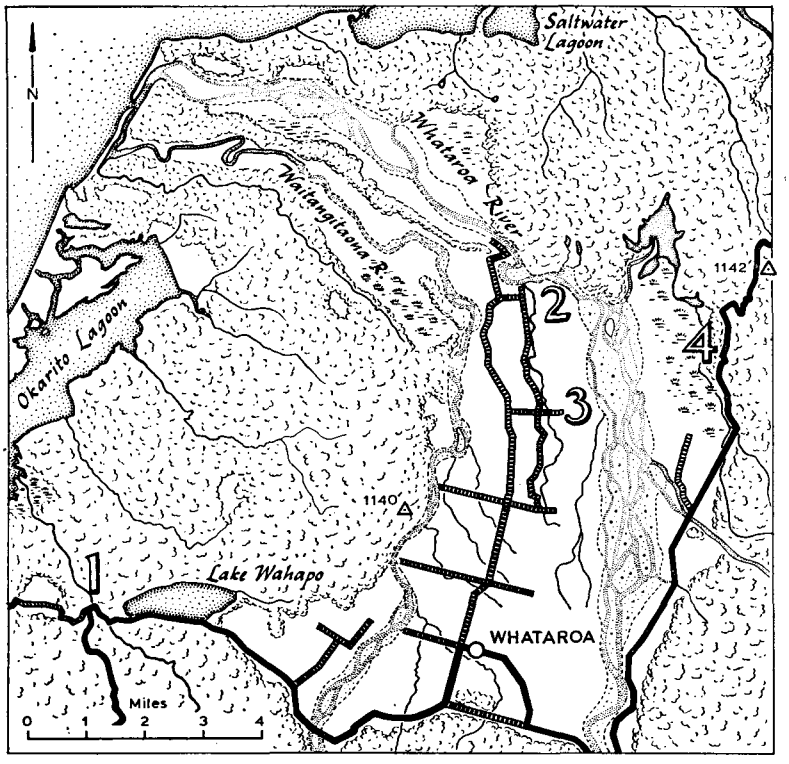


Fig. 1.

had been cut over and was in secondary growth, a fringe habitat between forest and swamp, an area of derelict swampy farm land close to forest and an area of improved land well away from the forest but including spinneys of thin bark totara (*Podocarpus hallii*).

*Okarito Lagoon*

A short-term survey was also made at Okarito lagoon, a coastal lagoon which carried a large permanent population of black swans (*Cygnus atratus*) and very substantial populations of migratory bar-tailed godwits (*Limosa lapponica*) in the summer and of other water birds. This area was abandoned quite quickly because few birds had antibody and the population of vectors was small and therefore it did not appear to be of any great significance in the ecology of our virus.

## MATERIALS AND METHODS

*Birds*

Birds were captured in mist nets, banded, bled from the jugular vein and released. The volume of blood taken varied from 0·05 to 0·4 ml. according to the size of the bird. Blood samples were diluted immediately to a total volume of 1 ml. and placed in a chilled insulated container. They were frozen to −70° C. within a few hours of collection and kept frozen until tested. Other methods of trapping were

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sometimes used and, during the open season, duck shooters were supplied with sterile bottles and asked to collect samples of blood from birds which they shot. Little reliance could be placed on the results obtained with these last specimens.

*Diluent*

Ten per cent calf serum in Hanks's balanced salt solution containing 10 units of heparin and 100 units of penicillin and streptomycin per ml. was used.

*Neutralization*

Plasma samples were tested for neutralizing antibodies by a plaque reduction test on monolayers of duck or chick embryo cells by the method described by Ross *et al.* (1964). The samples of blood were screened at the dilution at which they were collected and those containing virus neutralising antibody were titrated. Samples which neutralized at dilutions of 1:10 or greater were regarded as positive. When birds were sampled more than once a fourfold or greater change in antibody titre was regarded as a significant change.

## RESULTS

The main investigation was continued over a period of 5 years from February 1964 to February 1969. Eight bird collections were made during the first 14 months, then from April 1965 till the project was concluded birds were collected and bled at approximately monthly intervals. The average number of plasmas from each collection tested was 90. During the 5 years in which birds were sampled, 4554 bird plasmas from 30 species were tested for Whataroa virus plaque neutralizing antibodies. Of these, 673 (15%) had significant levels of antibody. In the analysis considered here the collections were divided into 12-monthly periods starting at the beginning of spring on 1 August and finishing 31 July. In this analysis the plasma from birds caught before 1 August 1964 have not been included. Fig. 2 shows the proportion of positive plasmas in all areas together in each season. The high proportion of birds circulating antibody in the 1965–6 season suggests the occurrence of a Whataroa virus epizootic. During that period, birds were being caught at three sites (1, 2 and 3 in Fig. 1). The increased incidence of immune birds was observed in all areas.

Table 1 shows the incidence of immunity in each of six species most commonly caught in the area plus the pooled incidence in other species. It was found that each season about 20% of song thrushes (*Turdus philomelos*) were circulating Whataroa virus-neutralizing antibody with little variation from year to year. The proportion of blackbirds (*T. merula*) with antibody was the same as that in thrushes in 1964–5, but there was a steady decrease over the 5 year period. All other species showed the same pattern of low incidence in the 1964–5 season followed by a sharp increase in 1965–6 and then a decline to the previous low levels in the following seasons.

The variation in the proportion of positives at different times of year is of interest. In the epizootic season (1965–6) 34% of the birds tested between February and July were blackbirds and thrushes, but they included only 13% of the positive plasmas. In this season the proportion of positive plasmas increased more or less

Table 1. *Yearly incidence of Whataroa virus neutralizing antibody in individual bird species*

| Species | 1964–5 | | 1965–6 | | 1966–7 | | 1967–8 | | 1968–9 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No.* | %+ve | No. | %+ve | No. | %+ve | No. | %+ve | No. | %+ve |
| rush (*ırdus philomelos*) | 10/49 | 20 | 35/152 | 23 | 36/225 | 16 | 23/117 | 20 | 7/41 | 17 |
| ıckbird (*merula*) | 12/60 | 20 | 34/283 | 12 | 39/295 | 13 | 7/153 | 5 | 0/43 | 0 |
| ver-eye (*ıterops lateralis*) | 2/136 | 2 | 189/299 | 63 | 42/441 | 10 | 4/524 | 1 | 2/89 | 2 |
| affinch (*ringilla coelebs*) | 4/59 | 7 | 54/88 | 61 | 12/85 | 14 | 0/70 | 0 | 0/31 | 0 |
| dge sparrow (*unella modularis*) | 0/18 | 0 | 46/77 | 60 | 7/111 | 6 | 1/58 | 2 | 1/27 | 4 |
| lbird (*ıthornis melanura*) | 3/20 | 15 | 15/40 | 38 | 4/57 | 7 | 0/22 | 0 | 0/12 | 0 |
| ıer species | 1/90 | 1 | 67/156 | 44 | 10/238 | 4 | 2/145 | 1 | 0/56 | 0 |

* Number with antibody/number tested.

steadily from November through to the following March, when about 70% of all sera contained antibody. The findings in the enzootic seasons were quite different. The incidence of immune birds reached a peak in the early spring (September) followed by a drop in October and a second peak in November and December. Thereafter the incidence dropped to a very low level between February and July. During this period, 15% of the plasmas were from blackbirds and thrushes, yet these two species accounted for 50% of the positive plasmas.

On 540 occasions, birds were recaptured and bled after intervals from less than 1 month to 52 months. The antibody titre increased between bleedings on 43 occasions and decreased on 34 occasions. Eight birds had the same antibody titre in two plasma samples. The remaining 455 birds were free from antibody at both first and second bleedings.

All 43 birds which showed an antibody increase were exposed for all or part of the period from September to March. A drop in antibody titre was recorded after periods ranging from 2 to 3 weeks to 31 months. The birds which maintained their antibody titres were recovered after intervals ranging from 1 to 17 months (Table 2).

*Mosquitoes*

In the areas in which we have worked, two species of mosquitoes were dominant, while others occurred in special localities. In the rain forest, a large culicine *Culiseta tonnoiri* is the main mosquito, while in more open country the dominant is *Culex pervigilans*. In the fringe habitats in which we have been particularly interested and from which we obtained our virus isolations, both these species were present in proportions varying with the area, the rainfall in the recent past and the time of year. In the coastal areas they are replaced by the halophilic *Aedes australis*.

Table 2. *Changes in antibody titres of recaptured birds*

| Month of first sample | No. of birds showing a change in antibody titre between samples* | | | |
|---|---|---|---|---|
| | Increase | Unchanged | Decrease | Total |
| August, September, October | 16 (73%) | 0 | 6 (27%) | 22 |
| November, December, January | 17 (49%) | 5 (14%) | 13 (37%) | 35 |
| February, March, April | 5 (33%) | 3 (20%) | 7 (47%) | 15 |
| May, June, July | 5 (38%) | 0 | 8 (62%) | 13 |

* Plasma samples of 455 birds which were free of antibody on both occasions have been omitted.

Our isolations were made from *Culiseta* and *Culex* species, but owing to the difficulties of establishing them in our insectory, most of our studies on virus replication in mosquitoes were made on *Ae. australis*.

In our area the January isotherm is 16° C. and none of the mosquitoes of southern New Zealand will survive for a substantial period at 25° C. Our insectory was held at approximately 20° C., the highest temperature which allowed satisfactory survival of our mosquitoes.

Mosquitoes were allowed to feed on viraemic suckling mice and were then killed after varying intervals. The organs were disrupted by sonication and titrated by a plaque technique on chick-embryo monolayers. In 25% of mosquitoes, virus leaked into the haemolymph within a few hours of feeding. In a few (about 10%), substantial amounts of virus leaked out, probably because of damage of the wall of the intestine (Miles, Pillai & Maguire, 1973).

Apart from this, up to the 6th day, virus could only be demonstrated in the intestine. On the 6th day, 2 of 8 mosquitoes showed virus generalized through all their organs. In one of these the virus titre throughout was very high. Probably this was the result of a substantial early leak of virus into the haemolymph such as has been described above. Thereafter the proportion of mosquitoes with generalized virus increased until on the 17th day virus had generalized in 13 of 16 tested and these carried substantial amounts in the salivary glands. Although virus could be demonstrated in the salivary glands of most mosquitoes by the 8th day, only 1 of 31 tested for infectivity on day 14 succeeded in infecting a suckling mouse. However, on day 17, 6 of 10 mosquitoes which engorged on suckling mice transmitted infection. The results indicate that at least $10^4$ plaque forming units (p.f.u.) of virus must be contained in the salivary glands for transmission to take place.

Table 3. *Multiplication of Whataroa virus in* Ae. australis *engorged on viraemic suckling mice*

| Time after engorgement | Proportion of mosquitoes with virus in haemolymph | Proportion of mosquitoes with generalized virus in organs | Proportion of mosquitoes with $>10^4$ p.f.u. virus in salivary glands | Proportion of mosquitoes transmitting infection to suckling mice |
|---|---|---|---|---|
| 30 min. | *3/10 | — | — | — |
| 4 hr. | 1/7 | — | — | — |
| 1 day | 2/7 | — | — | — |
| 2 days | 3/10 | — | — | — |
| 5 days | — | 0/8 | 0/8 | — |
| 7 days | — | 2/8 | 0/8 | — |
| 9 days | — | 7/12 | 1/12 | — |
| 11 days | — | 8/11 | 1/11 | — |
| 14 days | 13/16 | 7/10 | 1/10 | 1/31 |
| 17 days | 4/8 | 13/16 | 10/14 | 6/10 |

— = Not tested.
* Number with specified infection/number tested.

This was only consistently present in mosquitoes with generalized virus which had been infected for 17 days, the longest period tested (Table 3).

After more success had been obtained in keeping *C. tonnoiri* in the insectory a limited number of experiments was carried out which showed that the susceptibility of this species was similar to that of *Ae. australis* and that multiplication of the virus in the two species at 20° C. was not greatly different.

The other most common haemophagous insect in the area is a simuliid, *Austrosimulium ungulatum*. Austin (1967) carried out a series of experiments on the vector potential of this species. He found that virus injected into the haemocoele would replicate, but that no concentration in the salivary glands took place. Further the insects could not be infected by feeding, but could transmit virus mechanically during 24–48 hr. after feeding on a viraemic suckling mouse. Thus this species might be able to transmit virus mechanically at the peak of an epidemic when many birds are viraemic, but could not be the main vector under normal enzootic conditions.

*Feeding preferences*

The available information on feeding preferences of New Zealand mosquitoes is limited. We know from experience that man is attractive to all species. Horses make excellent bait and chickens and rabbits attract approximately the same number of mosquitoes when used in the same trap on alternate nights. Apparently all species in the study area are indifferent feeders. They appear to be equally ready to bite birds and mammals.

*Viraemia in birds*

A series of experiments has been carried out to find the approximate ID 50 for some species of birds which may be ecologically important for Whataroa virus, and

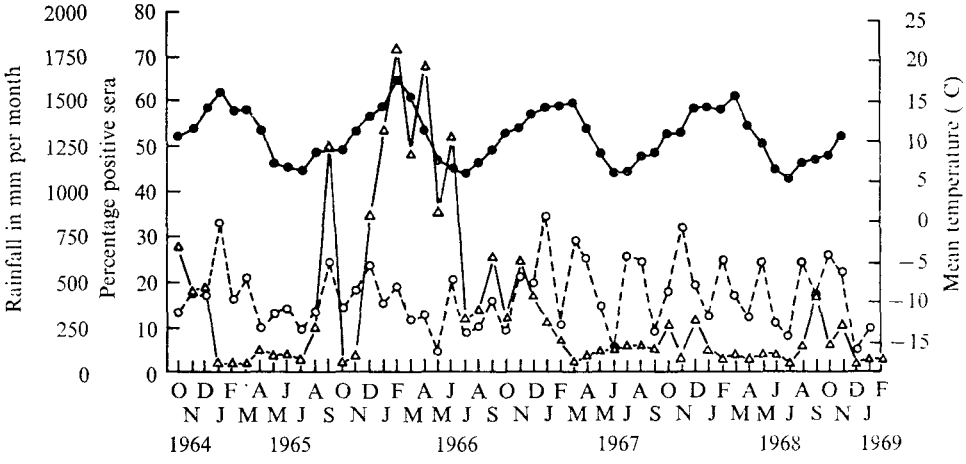


Fig. 2. Mean temperatures and rainfall and percentage of captured birds with antibody. ●, Mean temperature (°C); ○, total rainfall (mm/month); △, percentage of birds with neutralizing antibody.

to find the extent and duration of viraemia. The birds were collected in an area where there is good evidence that Whataroa virus circulates rarely if at all, and none possessed antibody before infection. The ID 50 for the three species for which an estimate was possible was between $10^{3\cdot0}$ and $10^{3\cdot3}$ p.f.u. of virus. This requirement of quite a substantial dose of virus before regular infection occurs probably explains why, although a high proportion of mosquitoes have virus in their salivary glands from the 8th day on, regular transmission is not obtained until 17th day. The maximum viraemia found varied from $10^{7\cdot1}$ to $10^{8\cdot2}$ p.f.u./ml. (F. J. Austin, in preparation). It has been estimated for some species of mosquito that the average amount of blood taken at a feed is about 0·003 ml. Our figures are not greatly different from this. Therefore a high proportion of mosquitoes feeding on birds with this level of viraemia would be expected to become infected.

## DISCUSSION

The information obtained from this longitudinal survey of Whataroa virus infection of wild birds in South Westland indicates that the virus was present in the area throughout the study period. For most of the time the incidence of wild-bird plasmas with Whataroa virus-neutralizing antibody was less than 25%, suggesting an enzootic situation. In September 1965 the proportion of plasmas with antibody rose sharply and remained above 30% almost continuously until June the following year. Since there was no evidence of illness or death among the birds this may be regarded as a clinically inapparent epizootic situation.

The reasons for the occurrence of the epizootic in 1965–6 are obscure. The total annual rainfall of 4200 mm. was somewhat below that in other years of the study and the mean January temperature at 17° C. slightly above, but the climatic conditions at the time of the first antibody peak in September 1965 were in no way abnormal and the conditions in the late summer when the number of birds showing evidence of infection was very high were in no way different from those in the previous year when the proportion of birds with antibody was very low (Fig. 2).

During the enzootic years the highest proportion of immune birds occurred in the spring or early summer. There was an initial peak in September followed by a drop in October and a second peak in November or December. This was succeeded by a steady decline in frequency of antibody carriers to very low levels during the late summer, autumn and winter. Further evidence for the major spread of Whataroa virus in the spring and early summer is provided by the recovery data. All of the birds which developed antibody between captures were exposed for all or part of the period between September and March and presumably were infected during that time or immediately before their first capture. Those birds which showed a decrease in antibody between bleedings must have been infected before they were first bled. A similar decrease in antibody has been observed by South African workers who, on the basis of their field observations and experimental studies, concluded that some birds responded to Sindbis virus infection with only a transient antibody response (McIntosh, McGillivray, Dickinson & Taljaard, 1968; McIntosh, Dickinson & McGillivray, 1969; McIntosh, Madsen & Dickinson, 1969).

This pattern of high antibody incidence in birds in the early summer followed by a decrease about midsummer differs from that reported by workers who have studied the activity of other mosquito-borne alphaviruses in wild birds. Reeves & Hammon (1962) showed that in California the incidence of Western equine encephalitis (WEE) virus antibodies in house finches and English sparrows remained at summer levels at least until September. In a study in Alabama covering a period of 14 months Stamm (1968) showed that the highest incidence of both Western and Eastern equine encephalitis antibodies was found in wild birds bled in the late summer and winter and that the lowest frequencies occurred in midsummer.

Our pattern may be affected by the migration of birds from the mountain areas to the plains at the end of the breeding season. During the months of August to January the average number of silver-eyes captured was 3·4 per day and the proportion of birds netted which had been previously captured was 20%. These figures clearly indicate a resident population. From February to April the captures were 12·1 per day and the recovery rate fell to 3·3%. The introduced finches show a similar pattern. Assuming that these unbanded birds came from higher ground where the virus was not enzootic they would be expected to dilute the proportion of positive sera greatly except when virus circulation was so intense that they would also rapidly become infected as in the epizootic of 1965–6.

All the evidence we have obtained indicates that Whataroa virus exists in a bird–mosquito cycle, but the early spring rise in the proportion of birds with serum neutralizing antibodies is difficult to interpret on the basis of spread by mosquitoes. These are inactive in the winter and early spring and, although they have been observed to engorge indoors in the warm as early as July, this has not been seen in the open air. Substantial numbers of mosquitoes did not appear before October.

The possibility of some other vector has been considered. Studies carried out in the United States in the early 1950s make it seem improbable that bird mites are importantly involved, and a limited study failed to reveal infected mites in nests or viraemia in nestling thrushes (F. J. Austin & J. A. R. Miles, unpublished). Ticks

have only been found in the coastal areas in Westland and none have been proved to be natural vectors of alphaviruses, although Whataroa virus can replicate in *Ornithodoros capensis* (Ross, 1971). Austin's studies have shown that the only simuliid in the area can carry Whataroa virus mechanically, but not biologically. It would therefore only be likely to be effective in transmitting this virus at the peak of a very intense epizootic.

The annual reintroduction of the virus by migrating birds seems improbable. There are only two migrant species commonly seen inland in the study area. These are the shining cuckoo (*Chalcites lucidus*) and the long-tailed cuckoo (*Eudynamis taitensis*). These arrive too late in the season to explain the rise in the number of sero-positive birds in September. Bar-tailed godwits (*Limosa lapponica*) begin to arrive in mid-August and reach substantial numbers in September. However, they are only found on the coast and around coastal lagoons where they are relatively little exposed to mosquitoes, although very exposed to simuliids. They are unlikely to be responsible for a regular reintroduction of virus. The one trans-Tasman migrant, the double banded dotterel (*Charadrius bicinctus*), is rare in South Westland and could not be significant in the virus cycle.

We did not succeed in devising a technique for trapping godwits in our area, but we did make a short-term study of water birds around Okarito Lagoon where godwits were most numerous. Duck and swan bloods obtained from shooters in the 1963 winter had given a high proportion of positive virus neutralization tests. These results were suspect because the trauma of shooting often leads to release of non-specific virus-neutralizing substances into bird blood (Scherer, Hardy, Gresser & McClure, 1964). In the late summer of 1964 when the black swans (*Cygnus atratus*) were moulting we captured approximately 100 and obtained blood specimens from the wing veins. Only two gave positive tests. In the following summer none of 12 silver gulls (*Larus novaehollandiae*) and few swans had virus-neutralizing antibody. If godwits were important in the introducing of virus each year, one would expect a higher infection rate in birds using the same feeding grounds.

We are inclined to favour an alternative view that the endocrine changes and other physiological stress at the beginning of the breeding season lead to a recrudescence of a latent infection and thus to stimulation of antibody production. Our failure to demonstrate any evidence of sero-conversions from negative to positive between April and August and the observation of birds with the same antibody titre in plasma samples taken up to 17 months apart encourage us to think that latent infections may be important in the enzootic survival of virus in the area. We have no direct evidence as yet, either field or experimental, for or against this theory.

In the epizootic season the proportion of positive sera continued to rise until the end of the summer when about 70% of all bird plasmas contained significant antibody titres. This is the same scale of involvement as that reported by Stamm (1963), who observed that at the end of an arbovirus transmission season up to 70% of the birds in a local population may be circulating virus-neutralizing antibodies. Although bird plasmas and mosquito extracts collected during this period

were inoculated into suckling mice and onto duck embryo cell cultures, no arbovirus isolations were made. This is surprising considering the high infection rate of birds, but is probably due to the poor conditions under which the specimens were sometimes held in the field.

In the first enzootic season 20% of the thrushes and blackbirds were circulating Whataroa antibodies, but few of the other birds were. In the following season there was a considerable increase in the number of birds with antibody. The increase occurred in all species except thrushes and blackbirds. Subsequently the antibody rate decreased for all species except thrushes and in the last season of the survey *T. philomelos* was the only species in which significant numbers of immune birds had been detected. In a small series collected early in October 1972 a high proportion of thrushes was again shown to carry neutralizing antibody against the virus (J. A. R. Miles & F. J. Austin, unpublished).

Because of these serological findings we think that it is probable that *Turdus* species are important for the maintenance of the virus in the area. If this is correct it is interesting that a virus showing distinct differences from related viruses in other countries should be maintained in a reservoir dominated by species introduced into New Zealand only about 100 years ago. However, the areas in which we have evidence that the virus is enzootic are habitats modified by man in such a way as to give a considerable advantage to introduced birds of fringe habitats and farm lands. Our findings demonstrate that a new cycle of a mosquito-borne arbovirus can become established in such a modified habitat using introduced species as reservoir hosts. The unmodified habitats which were studied did not appear to contain a similar natural enzootic focus, but were only involved in epizootic situations.

*Mosquitoes*

The effects of temperature on the cycle of Japanese encephalitis virus in mosquitoes have been studied and Huang (1957) found that in *Culex pipiens* only 3 of 21 mosquitoes held at 18–22° C. became infectious compared with 17 of 20 kept at 31° C., a temperature similar to that found in the epidemic season in China and Japan. Hess, Cherubin & La Motte (1963) reported that St Louis virus, related to Japanese encephalitis virus, had similar temperature requirements, but that WEE virus became epidemic at rather lower ambient temperatures. However, the mean temperatures in the areas where WEE is epidemic are not below 21° C. in the epidemic season. Chamberlain & Sudia (1955) found that for another alphavirus, eastern equine encephalitis virus, in *Ae. triseriatus* reducing the temperature from 26·5 to 21° C. increased the time for virus to reach peak transmissibility from 17 to 34 days.

The mean temperature in the hottest month in our study area of 16° C. is lower than that of any other area with an enzootic mosquito-borne virus of which we have heard. Also the extrinsic incubation time of 17 days at 20° C. in *Ae. australis* and a minimum extrinsic incubation in *C. tonnoiri* of 10 days at the same temperature indicates a much more efficient replication in the insect host at low temperatures than in other systems which have been reported.

These findings suggest a high degree of adaptation in this virus to replication in mosquitoes at the relatively low temperatures found in our study area and indicate that strains of virus have been enzootic there for a considerable period. It is then the more interesting that all the evidence points to the main natural focus of this virus being now in a modified habitat with one or two passerine bird species introduced into the area not more than 100 years ago as the main vertebrate reservoir.

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