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LANDCARE RESEARCH
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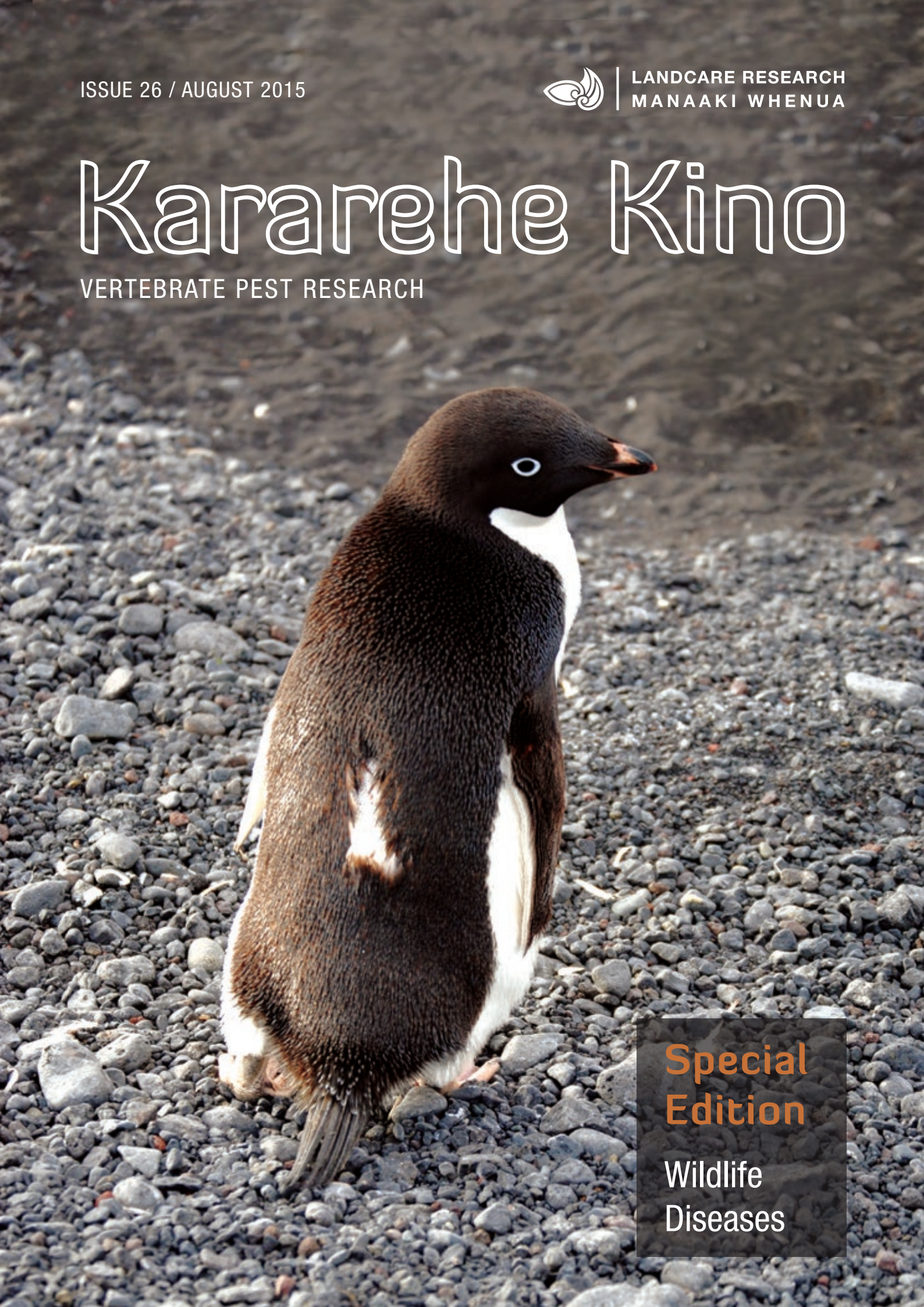
Kararehe Kino

VERTEBRATE PEST RESEARCH



Special Edition

Wildlife Diseases

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Photo credits:
Front cover: Adélie penguin at Cape Bird, Ross Island, Antarctica, showing feather loss on its back (see article on page 17) – Wray Grimaldi.

Page 2, 3: Gannet colony – Dan Tompkins; kākāpō – Bethany Jackson; rabbit – John Hunt; captured long-tailed bat – Kerry Borkin
Page 7: long-tailed bat – drawing by GH Ford, Wikipedia
Page 2, 14: rat photos – courtesy of Nga Manu Images

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St Arnaud forest – Caroline Thomson

In this issue:

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Editorial

Wildlife diseases: costs and benefits

When we talk about wildlife diseases, what tends to spring to mind are usually diseases such as rabies, avian influenza, West Nile virus, severe acute respiratory syndrome (SARS) or the latest, Middle East respiratory system (MERS) – diseases that originate in wild animals and now threaten people or New Zealand's agricultural industries. The global trend for people to increase their contact with wildlife, their habitats, and with domestic animals means it is more and more likely that new diseases will be transmitted between people and wildlife.

But the importance of wildlife diseases goes far beyond the threat to people and their livelihoods, as this issue of *Kararehe Kino* demonstrates. New Zealand's native biodiversity is also under significant threat from pathogens. First, diseases new to New Zealand are a potential threat. Dan Tompkins and his collaborators (page 5) have recently evaluated the risk of new diseases of wildlife and people arriving through the natural movement to New Zealand of migratory birds from Australian waters. Richard Hall and Dan White (page 7) have similarly been undertaking the first intensive survey of New Zealand bats for viruses. This work complements the Ministry of Primary Industries national wildlife surveillance programme. Second, the spread of existing diseases within New Zealand is also a concern. Avian malaria has been in New Zealand for many years and infection has been linked to deaths of a wide range of native bird species. Chris Niebuhr (page 8) has recently been investigating whether disease mortality is important at the population level, as impacts may worsen with the climate-change driven spread of the mosquito disease vectors and the disease.

Finding new diseases that may be potential threats has been greatly helped by new DNA-sequencing approaches (metagenomics). These approaches have also been applied recently to help find potential causes of new serious illnesses detected in native wildlife – Dan White (page 10) describes the hunt for the causative agent of a cloacal disease in kākāpō, and Wray Grimaldi (page 17) describes the hunt for the cause of feather loss in Adelie penguins. The difficulty when native wildlife is found with a new illness is that there is almost always very little information about naturally-occurring diseases, and so little information is available on which to base a diagnosis and decide on possible treatments.

The wildlife disease which receives the greatest attention and expenditure in New Zealand is bovine tuberculosis (TB), because of its potential economic impact. Possums are the main culprit infecting livestock, but other wildlife (deer, pigs , ferrets) are also involved through their interactions with possums and each other (alive and dead). As New Zealand moves towards TB eradication, understanding the combined role of all these wildlife species in maintaining TB will be critically important and has been investigated by Mandy Barron and her colleagues (page 15). Part of this research requires good estimates of transmission of Tb within species, which Carlos Rouco and his collaborators have recently provided with the first direct measure of Tb transmission rate between possums (page 12). They concluded that a better understanding of the conditions leading to high rates of transmission would allow better targeting of TB control efforts.

Wildlife diseases are usually thought of as causing unwanted harm, but in some cases that harm can be exploited for good, such as for pest wildlife control. Biological control of mammal pests using diseases has been around for many years – i.e., the control of rabbits with myxomatosis and, latterly, with Rabbit Haemorrhagic Disease (RHD). Host-pathogen systems usually involve a continuous process of co-evolution, as hosts develop immunity and pathogens alter their pathogenicity. Recent research by Janine Duckworth and colleagues (page 18) has clearly shown the evolutionary changes that have occurred in the RHD virus since its release in New Zealand, with virus strains in different parts of New Zealand now differing significantly in lethality to rabbits. Strain differences may in future allow better matching of strains to local conditions to enhance RHD efficacy. Biological control using wildlife disease may also exploit disease effects on the host more subtle than mortality. Infection of rodents with the parasite *Toxoplasma gondii* changes their behaviour and makes them more susceptible to trapping (and predation). Dan Tompkins and Clare Veltman (page 14) used computer models to assess how seeding rat populations with the parasite could contribute to improving their control with traps – a novel idea certainly worth following up given the very high costs of ongoing suppression of rat populations!

If New Zealand follows global trends then wildlife disease issues are going to become increasingly important, both for people and their livelihoods and for native biodiversity. While new technologies for disease discovery will help with early detection and diagnosis, the lack of good baseline information about pathogens in New Zealand wildlife will continue to be a major constraint on effective response to disease outbreaks.

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Seabird surveillance for evidence of past and present infectious agent incursion into New Zealand

Australasian gannet colony at Muriwai Beach.

Border biosecurity is a real and present concern for New Zealand. Many infectious agents that impact on plant, animal and human health in other parts of the world are not currently found here, yet there is the risk that such agents could be introduced, in some cases with potentially devastating consequences. In response to this threat, much emphasis is currently placed on managing the risks of exotic agent incursion associated with human activities such as international air travel and container shipping. However, natural pathways of potential agent incursion into New Zealand also exist and should not be overlooked. In particular, many of our sea birds undergo long flights to other countries, and could bring back agents they are exposed to overseas. Some terrestrial birds also fall into this category, such as the shining cuckoo which migrates from the New Britain–Solomon Islands archipelago to New Zealand to breed.

Given that global change drivers, such as changes in land-use and climate, can alter bird dispersal and migration patterns, it is essential to understand the potential for agent incursion into New Zealand, and to be able to assess future change, which requires current baseline measures. To start to address these knowledge gaps, a team led by Dan Tompkins conducted surveys of seabirds at three locations – Muriwai Beach, Cape Kidnappers and Kaikoura Peninsula. Both Muriwai Beach and Cape Kidnappers are home to large colonies of Australasian gannets (photos), whose young cross the Tasman Sea within three months of hatching, remain in Australian waters until they are two to three years old, then return to their natal gannetries. The Kaikoura Peninsula is home to large populations of red-billed gulls (photo) and white-fronted terns. Red-billed gulls can move over 300 km after breeding and occasionally migrate across the Tasman Sea, while large numbers of white-fronted terns migrate from New Zealand to Australia (the farthest known movement of a banded bird being 2970 km from Kaikoura to South Australia).

For Dan's study, large numbers of these seabirds were captured at these three New Zealand locations, using hand nets for red-billed gulls and white-fronted terns, and shepherd's crooks for Australasian gannets. Blood samples were taken from individuals caught, and ticks (*Carios capensis* – formerly *Ornithodoros capensis* – and *Ixodes eudyptidis)* were also collected from birds captured at Kaikoura Peninsula.

The blood collected was centrifuged for serum extraction for serological testing, apart from single drops for thin blood smears and small amounts to go into buffer solutions for molecular diagnostics. Sera samples were generically screened for antibodies to flaviviruses; potential positives with sufficient

sera were re-tested, and also subjected to specific tests for Murray Valley encephalitis virus and Kunjin virus (flaviviruses of incursion concern from Australia). Testing for antibodies for specific alphaviruses (Ross River virus, Barmah Forest virus and Sindbis virus; arboviral agents of incursion concern also from Australia) was carried out on some samples (including some ticks). Smears were examined microscopically for blood parasites, with follow-up DNA sequencing to identify any infectious agents observed.

Babesia sp. parasites were detected in blood smears from Australasian gannets at both Muriwai Beach and Cape Kidnappers, and from both red-billed gulls and white-fronted terns (and their ticks) at Kaikoura Peninsula. Sequencing and phylogenetic reconstruction revealed three new unique sequence variants that were similar to two bird-derived variants already known. Two of the new variants were similar to *Babesia poelea* known from brown boobies in the Central Pacific, and the third was similar to *B. kiwiensis* from the North Island brown kiwi. The *Babesia* sequences identified in Dan's study showed no evidence of host specificity; hence ticks from seabirds are likely to be part of the incursion pathway by which *Babesia* parasites infect kiwi.

Antibodies to flavivirus were also detected in both red-billed gulls and white-fronted terns at Kaikoura Peninsula. This suggests that two flaviviruses (Saumarez Reef virus and an unidentified Hughes group arbovirus) previously isolated from birds and ticks at Kaikoura Peninsula are still present at this site. In addition, one Australasian gannet from Muriwai Beach was positive for antibodies to Ross River virus. This is most likely the result of exposure in Australian waters prior to returning to New Zealand. Mathematical models indicate that climate change will increase the capacity of mosquitoes in New Zealand to support Ross River virus outbreaks, and should birds return to New Zealand with this virus, they will provide a potential pathway for disease incursion into the country. As Ross River virus is a public health concern, causing debilitating polyarthritis in many people infected, more thorough surveillance should be carried out at Muriwai Beach to confirm its current status.

Serological testing was carried out by Cheryl Johansen's group at The University of Western Australia, and *Babesia* molecular diagnostics were carried out by Peter Irwin's group at Murdoch University, Melbourne, Australia. Funding was provided by the Foundation for Research, Science and Technology Cross Department Research Funding to Graham Mackereth (while at Biosecurity New Zealand).

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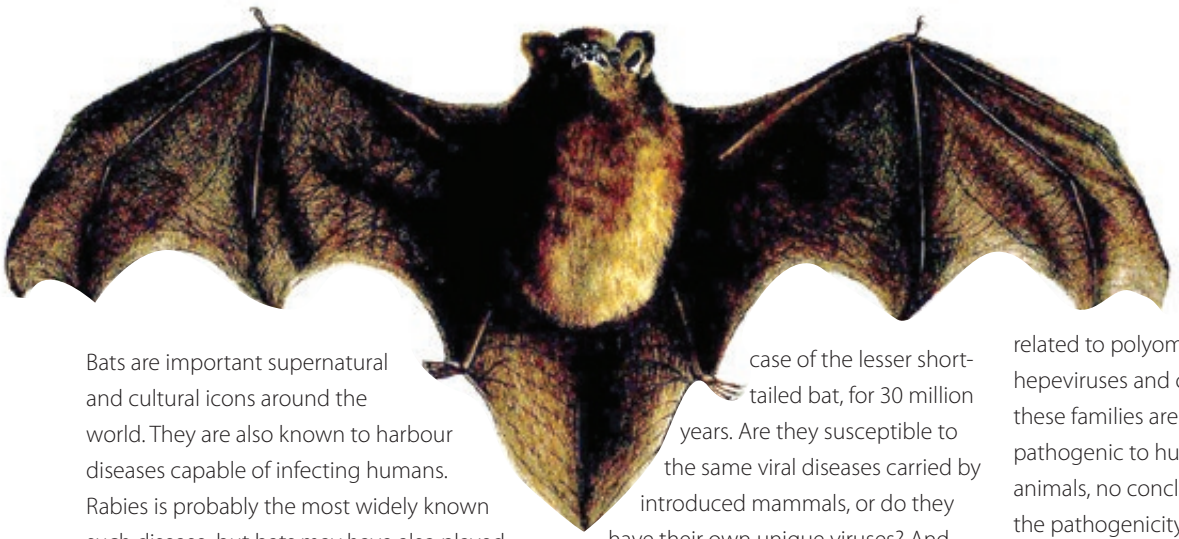
Red-billed gulls at Kaikoura Peninsula.

Dan Tompkins

Australasian gannets at Cape Kidnappers.

Viruses in New Zealand bats



Bats are important supernatural and cultural icons around the world. They are also known to harbour diseases capable of infecting humans. Rabies is probably the most widely known such disease, but bats may have also played host to SARS (a coronavirus), ebola virus and other viral hemorrhagic diseases. The fearsome reputation of these creatures for transmission of disease may be due to facets of their biology, but as they also comprise nearly 20% of all mammal species, the chances are, some will be reservoirs of diseases that affect humans. Bats have also received a great deal of attention in recent times because researchers are interested in predicting where new animal reservoirs of disease may exist. It is hoped that such studies may predict, or even prevent, the emergence of future catastrophic pandemic diseases.

Studies of bat disease not only look to see what viruses may pose a risk to humans, but also to the bats themselves. Many bat species are threatened and have special conservation status, which is the case for New Zealand bats. New Zealand has only three terrestrial native mammals, and all three are bats. One species is recently extinct, but the other two species are widely distributed around New Zealand, albeit in small, concentrated populations; they are the long-tailed bat (*Chalinolobus tuberculatus*), and the lesser short-tailed bat (*Mystacina tuberculata*). A curious aspect about New Zealand bats is that prior to the arrival of humans and introduced mammals, they have been isolated from all other mammals for well over one million years, and in the case of the lesser short-tailed bat, for 30 million years. Are they susceptible to the same viral diseases carried by introduced mammals, or do they have their own unique viruses? And what are the risks posed by these viruses, both to human health and to the health of the bat populations in New Zealand?

Richard Hall at the Institute of Environmental Science and Research (ESR) and colleagues at Landcare Research and the Department of Conservation have started to answer these questions, applying specialist next-generation sequencing technologies (see article by Daniel White on page 10 for details of this technique) to identify viruses in guano samples of the New Zealand lesser short-tailed bat, collected from Codfish Island.

Next-generation sequencing instruments are capable of sequencing all DNA (or RNA) present in a sample. For the last 30 years, scientists have used Sanger-sequencing (a processive biochemical method for sequencing small, individual pieces of known DNA), which requires some prior knowledge of the DNA being searched for. The new sequencing technologies are unbiased and allow researchers to discover new micro-organisms, and in particular, new viruses. A single experiment will provide millions of DNA sequences from one sample and each sequence can then be compared to genomic databases using a similarity search. In this way, it is possible to catalog most of the organisms present within a sample, even those which are quite novel.

Richard and his team have had some unexpected results. The team discovered not only a novel coronavirus, but also sequences that are related to polyomaviruses, papillomaviruses, hepeviruses and caliciviruses. While all of these families are known to contain viruses pathogenic to humans, the bat host or other animals, no conclusions can be drawn about the pathogenicity of the New Zealand bat viruses. However, this does show that New Zealand bats are likely to be infected with viruses, just as other mammalian species are. The types of viruses detected in our bats are similar to the profiles observed for exotic bats in other studies. This is quite surprising, given the long isolation that the lesser short-tailed bat has experienced. The results even challenge current theory on the evolution of coronaviruses, which were thought to be only 10,000 years old. This work raises the possibility that they may be much older. Or perhaps the bats have contracted these viruses since the arrival of introduced mammals in New Zealand.

A great deal more surveillance work needs to be conducted in New Zealand on our bat species and many questions remain unanswered. However, this work does illustrate that New Zealand bats can play host to similar viral species to those observed in bats overseas.

This work was funded by the core research fund from ESR and Landcare Research, provided by the Ministry for Business, Innovation and Employment.

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Dan White

Is *avian malaria* playing a role in native bird declines in New Zealand?

Clare Cross

Collecting blood via brachial venipuncture from a silvereye for screening for the presence of avian malaria.

Emerging infectious diseases, defined as disease-causing agents that rapidly increase in geographic range, host range, or prevalence, pose a serious threat to the conservation of global biodiversity. Avian malaria is one such disease that may affect New Zealand avifauna, considered to be the most extinction-prone in the world. Avian malaria is caused by protozoan parasites of the genus *Plasmodium* and is vectored by mosquitoes. In Hawaii, avian malaria is vectored by the exotic mosquito, *Culex quinquefasciatus*, and has been a major factor in the population declines of native forest bird species, limiting many species to higher elevations.

Of the approximately 3500 species of mosquitoes in the world, 15 species are found in New Zealand, three of which are introduced. *Culex quinquefasciatus* was introduced to New Zealand over a century ago, but in recent decades has expanded its range from its introduction sites in Northland and Auckland to as far south as Christchurch. New Zealand's native *C. pervigilans* is also suspected of playing a role in malaria transmission, complicating our understanding of local transmission dynamics.

The presence of malarial parasites has been confirmed in native New Zealand avifauna and has been linked with the deaths of birds of multiple species, including the mōhua *(yellowhead)*, kererū, great spotted and brown kiwi, New Zealand dotterel, and saddleback. The presence of malarial parasites has also been confirmed in multiple non-native species of birds in New Zealand, including the blackbird, song thrush, house sparrow, and starling, leading to suggestions that these non-natives may be acting as reservoir species from which spillover to native species may occur. Although this mosquito-borne disease has impacted on both captive populations and wild individuals in New Zealand, the impact on wild populations of native birds is still unclear.

During the last 30 years, declines in abundance have been reported for five native bird species in the Nelson Lakes region: bellbird, rifleman, grey warbler, New Zealand tomtit, and tūī. Avian malaria is one possible cause of these declines, since the declines have generally occurred at lower altitudes where there is greater abundance of non-native bird species (potential reservoirs of malaria) and likely also higher mosquito densities.

Dan Tompkins and University of Otago PhD candidate Chris Niebuhr are investigating the causes of these declines at Nelson Lakes National Park. Blood samples were taken from native (n =134) and non-native (n =126) forest birds along an elevational gradient (650 m to 1400 m) during three summers (2012–13 to 2014–15), complemented with mosquito sampling. All samples are currently undergoing testing for the presence of malarial parasites using a Polymerase Chain Reaction assay, with *Plasmodium* sp. identity being confirmed through DNA sequencing.

Although blood samples are still being processed, initial results show the prevalence of avian malaria in non-native species is 10 times that in native species — 13.5% (126)

and 1.5% (134) respectively. Additionally, mosquito surveys show the presence of the native *C. pervigilans* in the region, but not the exotic *C. quinquefasciatus*. Also, a malaria-positive song thrush was found as high as 1400 m (tree-line) while mosquitoes were not detected above 1000 m. Thus, pathogen reservoirs exist at higher elevations, with only the mosquito vector lacking for the transmission cycle to be complete. Although the results are still being analysed, any supporting evidence for disease impacts could help appropriate management to be developed and put in place, while a lack of evidence would enable resources to remain focused on the other issues facing our native species (e.g. habitat restoration, pest control, translocations and genetic management).

The study, expected to be completed by the end of the year, will also integrate mathematical modelling with empirical data to better investigate avian malaria transmission dynamics in New Zealand. Dan and Chris are currently adapting a malaria-forest bird epidemiological model, originally developed in Hawaii, to fit the New Zealand situation, incorporating both *C. quinquefasciatus* and *C. pervigilans*. This work has potential for indicating management options beyond avian malaria, to include other mosquito-borne diseases (e.g. West Nile, Ross River, dengue) that could eventually make their way into New Zealand.

This work has been funded by Landcare Research and the University of Otago, Department of Zoology.

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Chris Niebuhr

Chris Niebuhr

Clare Cross

a) The St. Arnaud range above Lake Rotoiti in Nelson Lakes National Park where birds and mosquitoes are being sampled.
b) A banded bellbird caught in a mist-net.
c) A carbon dioxide trap used to collect host-seeking mosquitoes.

Investigating the cause of *disease in kākāpō*

Diseases of endangered wildlife pose critical threats to their survival. However, often very little is known about the causes and even the history of wildlife diseases. This is exactly the situation for critically endangered kākāpō suffering from exudative cloacitis.

The kākāpō is a flightless night parrot endemic to New Zealand. Considered to be almost extinct by the mid-1970s due to the impact of introduced mammalian predators, intensive efforts to recover the species on predator-free offshore islands are now underway. The Kākāpō Recovery program (http://kakaporecovery.org.nz/) is considered to be one of the most intensive recovery programs worldwide and includes exhaustive monitoring, supplementary feeding, artificial incubation and hand-rearing, regular health checks and predator control.

Since 2002, cloacitis (inflammation of the cloaca) has been confirmed in nine individuals, and suspected in a further two, with infected tissue frequently becoming ulcerated and exudative with associated heavy bacterial growth. With such symptoms potentially resulting in either infertility or mortality, and a current total population of just 126 birds, there is a critical need to understand the cause(s) of cloacitis in kākāpō to guide both treatment and preventive management.

To help find the cause of the disease, Daniel White and colleagues at Landcare Research teamed up with Richard Hall and colleagues at Environmental Science and Research Ltd (ESR) to do *de novo* metagenomics on samples from kākāpō. *De novo* metagenomics is the linchpin of the newly emerging field of pathogen discovery. It works by generating millions of DNA or cDNA sequence 'reads' from biomedical samples, such as faeces or cloacal swabs, followed by aligning all these reads against sequence databases to identify which taxa are present (*Fig. 1*). This work relies heavily on Landcare Research's pre-existing collaboration with the National eScience Infrastructure (NeSI) for computing power (https://www.nesi.org.nz/). As such, *de novo* metagenomics performed on faeces has the potential to generate an unbiased representation of all organisms present in the digestive tract of infected birds, importantly including viruses.

Comparing the taxa in an infected bird (named Rakiura) both before and after treatment with antibiotics, with those in a pool of eight healthy individuals (*Fig. 2*), has yielded identification of a possible cause of the disease. In contrast to the healthy kākāpō tested, a bacteriophage TL-2011b, or something closely related, was found in *Rakiura*. Bacteriophages are essentially viruses that infect bacteria, and the host *E. coli* strain of this phage is known to be associated with serious foodborne disease in humans. Veterinary studies were not able to identify this *E. coli* strain as a potential cause of cloacitis in kākāpō because it couldn't be distinguished from other *E. coli* strains that are components of healthy kākāpō gut microflora.

This exciting result needed confirmation and the team has now developed a diagnostic PCR assay for the TL-2011b-like

bacteriophage, which has provided strong support for its presence in *Rakiura*. The next step is to try and replicate the finding in another kākāpō. This relies on getting a faecal sample from another infected bird, which thankfully are in short supply. However, in March another bird started to show signs of the disease and, working closely with the New Zealand Centre for Conservation Medicine and the Department of Conservation, samples have been taken and are currently at the Ecogene Laboratory at Landcare Research, Tamaki. The team is now processing samples to try to replicate a positive result with the newly developed PCR assay. The presence of this phage in a second cloacitis case would strengthen evidence for its cause, making it possible to put in place better tailored treatment for the disease, and importantly preventive measures to reduce its incidence.

This work is funded by the New Zealand Ministry for Business, Innovation and Employment and the Department of Conservation.

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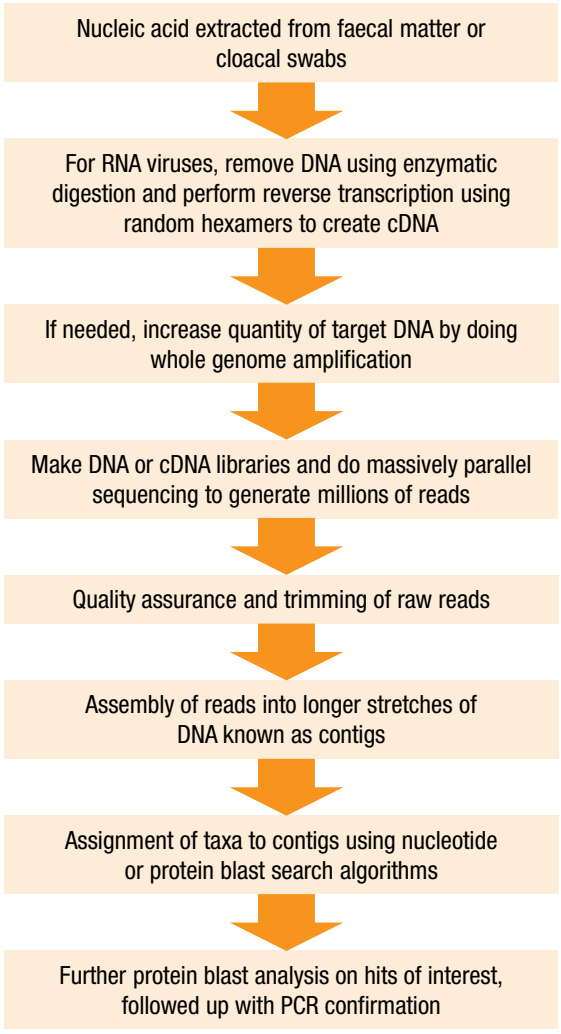


Fig. 1 *De novo metagenomics.*

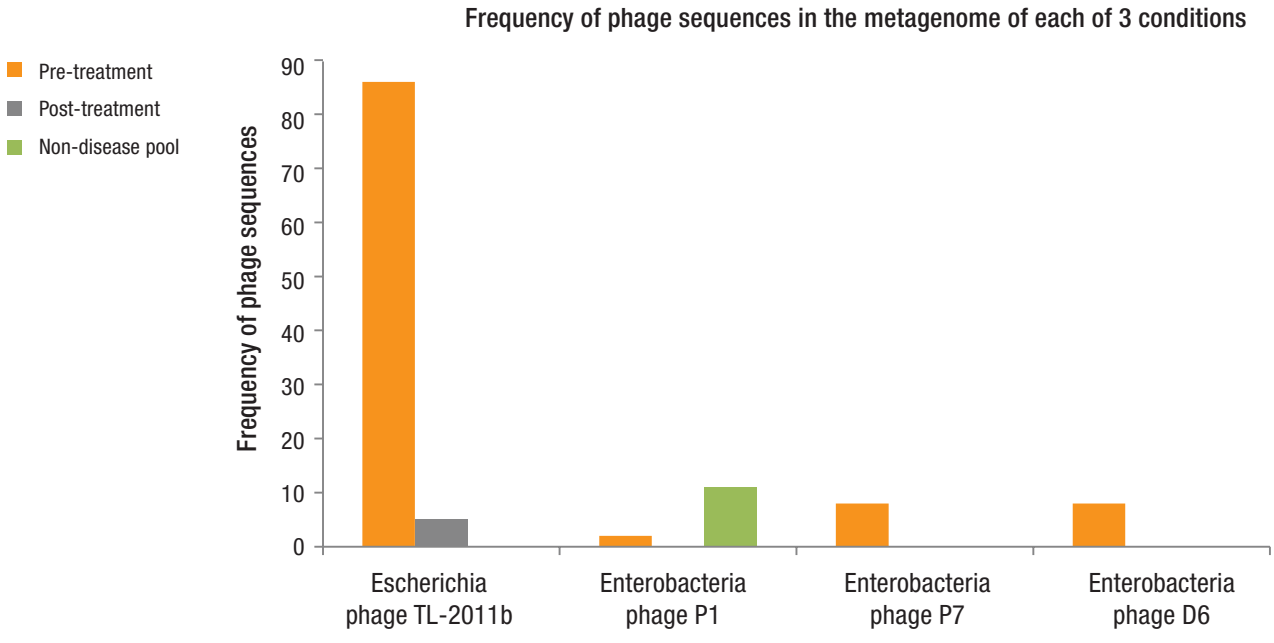
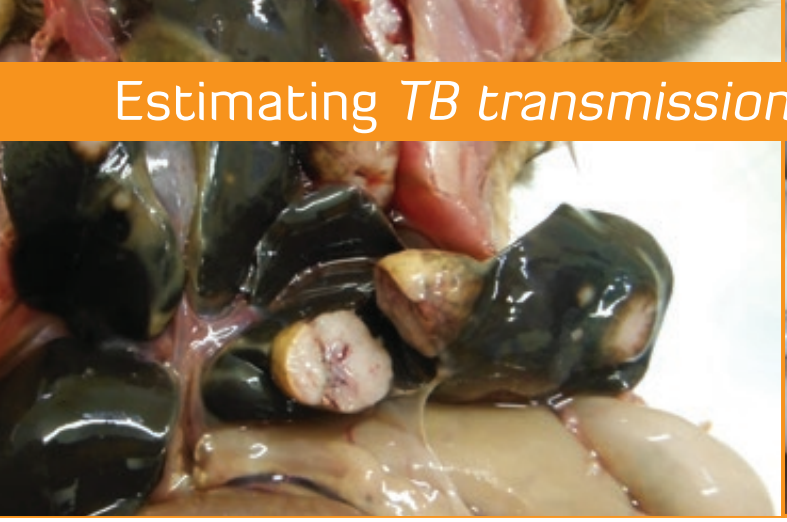


Fig. 2 *A summary of the number of viral phage cDNA sequences present in pre-treated* Rakiura, *post-treated* Rakiura *and a pool of healthy birds.*

Estimating *TB transmission rates* among possums in the wild



TB lesions in the liver of a possum; infecting a possum with TB (photos: Carlos Rouco).

The primary wildlife reservoir of bovine tuberculosis (TB) in New Zealand is the possum, with transmission of infection from possums to livestock regarded as the largest barrier to eradicating TB from livestock. Managing infectious diseases such as TB requires a comprehensive understanding of how the disease is transmitted from one individual to another and the rate at which it does so. However, estimating transmission rates for wild animals is particularly difficult. As a consequence, such rates are frequently estimated indirectly, e.g. as the rate required for an observed level of infection. When disease dynamics are unclear, the measurement of transmission rates from experimentally infected individuals to other susceptible individuals can provide direct estimates to confirm or question indirectly obtained values.

To provide such a test for TB in possums, Carlos Rouco and colleagues from Landcare Research, Massey University and AgResearch have been running 'transmission trials' with possums in the Landcare Research Orongorongo Valley research area in the Rimutaka Forest Park. The study sought to determine the number of individuals contracting TB from experimentally infected individuals sharing their home range, and to calculate transmission rates directly from these data. Since the possum population in the study area is to be controlled (poisoned) in the very near future (and any infected possums destroyed), the team was able to introduce a strain of TB for the experimental ('secondary') infections that differed from local natural infections.

Trapping grids of 100 cage traps set at 40 m spacings (c. 13 ha) were trapped monthly to identify possum home ranges. In the first trial, four adult possums were experimentally infected on each of four such grids (two in winter (Grids C & D) and two in spring (Grids A & B) 2012; *Fig. a*). In the second trial, eight adult possums were experimentally infected on each of two further grids in spring 2013 (*Fig.b)*. Six months after each experimental infection, all possums trapped on each grid were euthanased and examined for visible signs of TB and their key lymph nodes pooled for bacteriological culture to distinguish experimental from background strains of TB.

TB transmission rates from all euthanased possums were calculated for each trapping grid from the number of secondary infections detected divided by the total number of home range overlaps between experimentally challenged possums and all other possums. In the first (2012) trial there were 80–142 such overlaps on the four trapping grids, with three secondary cases of TB being detected (*Fig.a*). Based on this data, the possum-to-possum TB transmission rates were estimated as 0.000, 0.008, 0.004 and 0.000 on the four grids. Zero to one secondary cases therefore occurred in every 125 overlaps every 125 overlaps in home range between experimentally infected and other possums on these grids. In the second (2013) trial there were 166–182 overlaps in home range with eight secondary cases detected (*Fig. b*), resulting in estimated transmission rates of 0.013 and 0.003 (and approximately one to four secondary cases occurring for every 300 overlaps).

Of these six estimated transmission rates, only two were close to the level required for TB to persist in the Orongorongo Valley possum population. Based on the local possum densities on each grid, the transmission rate of 0.008 recorded in the first trial would have resulted in similar numbers of secondarily infected to experimentally infected individuals, while the rate of 0.013 in the second trial would have resulted in approximately 50% more secondarily infected possums. On the other four grids, there was insufficient transmission for the experimentally infected individuals to replace themselves.

These results have two key implications for the team's understanding of TB dynamics in possum populations. Firstly, transmission is highly variable across both space and time. Hence, understanding the conditions that lead to high rates of transmission could potentially allow the better targeting of control efforts for TB management. Secondly, the generally low rate of transmission observed suggests that 6 months may have been insufficient to document all of the secondary cases that would have occurred. Indeed, infected possums have been documented surviving for many years, periodically relapsing with and then resolving clinical disease. Such dynamics are common for TB infection in other species (including humans), and their consideration may be important for understanding how TB persists in possum populations.

This work is part of a larger project jointly funded by TBfree New Zealand and the Royal Society of New Zealand Marsden Fund.

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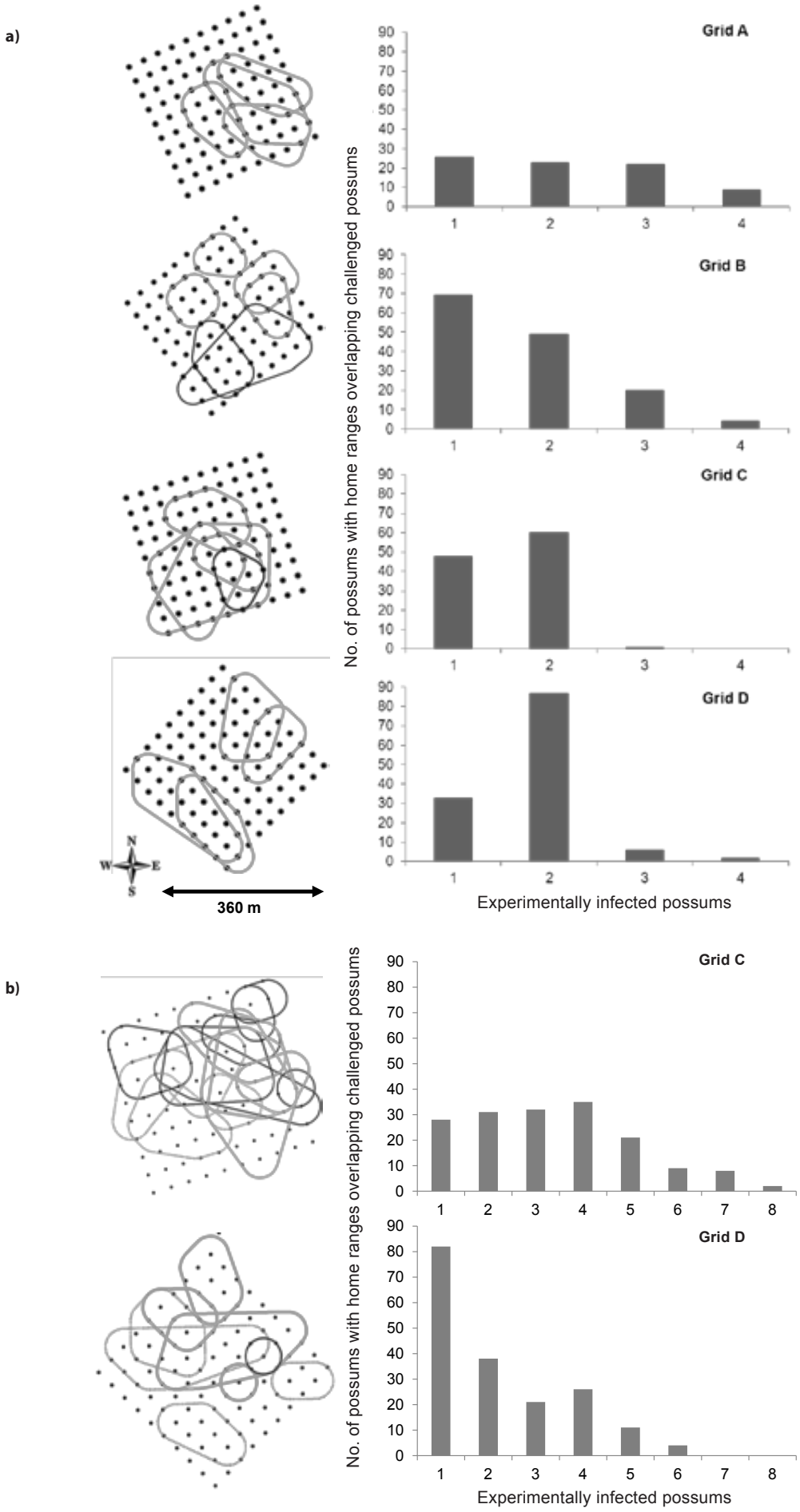

Fig. *Minimum convex polygon (MCP) home ranges of the possums experimentally infected (challenged) with TB (in grey), and the non-challenged possums with culture-confirmed secondary TB infection (in black) for each of the study grids during (a) the first trial (2012) and (b) second trial (2013), respectively. Graphs show the number of possums whose home ranges overlap those of the four or eight challenged possums on each grid.*

Could a naturally occurring disease be used *to help control rats?*

Courtesy of Nga Manu Images

Rather than causing direct harm, many diseases can have more subtle effects on their hosts. For instance, parasite infection in red grouse in Europe is well known to make the birds more vulnerable to predation due to them being in poorer condition. This phenomenon is believed to be quite common in nature, and has been termed the 'healthy herd' hypothesis because the overall population is kept healthier by the removal of the more heavily diseased individuals. More extreme than this are cases where parasites actually cause behavioural changes in their hosts that lead to their increased predation. Such parasite manipulation of host behaviour occurs when the parasite needs to be transmitted to its definitive host to complete its lifecycle. The classic example of such manipulation is where the trematode parasite *Dicrocoelium dendriticum*, which must be transmitted by ingestion from an ant to a sheep, causes infected ants to climb to the tip of blades of grass and stay there waiting for a grazing sheep.

Another compelling example of parasite manipulation of host behaviour is how rats become less neophobic (literally, a fear of anything new) when infected with the protozoan parasite *Toxoplasma gondii*. This is believed to be an 'intentional' effect of the parasite, since it relies on transmission to its definitive hosts (felids, most notably domestic cats) to complete its lifecycle. Solid experimental evidence has demonstrated how this reduction in neophobia leads to increased predation of rats by cats. This observation led Dan Tompkins (Landcare Research) and Clare Veltman (Department of Conservation) to wonder if, in addition to increasing predation by cats, such an effect could potentially improve kill-trapping efficacy of ship rats in the wild (*photo*). Wild rats are among the most innately neophobic mammals known, reacting to novel stimuli (such as traps) with extreme caution and often total avoidance, making them particularly hard to trap. However, laboratory-based experiments have demonstrated that the '*Toxoplasma* effect' increases their trapability in cage traps.

To explore the potential of this approach, Dan and Clare conducted a mathematical modelling exercise to see whether the improvements in trapping efficacy that *Toxoplasma* could theoretically make, could make it worth testing as an 'adjunct' to standard rat kill-trapping. Although trapping is frequently the preferred approach to control ship rats, as it avoids the potential poisoning of valued non-target species, it is generally less successful than poisoning, and its labour-intensive nature can make the cost prohibitive unless community initiatives are involved. Improvements to the efficiency of rat trapping could thus enable a big step towards our current aspirations for a 'Predator Free New Zealand'.

The modelling process combined existing data in three steps. First, the prevalence of *Toxoplasma* that may be established in rats was identified from the parasitological literature. This showed that *Toxoplasma* prevalence of up to 70% has been documented in wild ship rat populations in other countries. Second, the per night probability that an uninfected ship rat will interact with a trap located within its home range was calculated from the New Zealand pest literature, and extrapolated to the predicted population control efficacy of different trapping events. This identified an average capture probability of 3% per night per trap, and converted into a range of trapping events to achieve 90% control

of a ship rat population from an estimated 4 nights trapping with 16 kill-traps per hectare up to an estimated 19 nights trapping with 4 kill-traps per hectare. Third, the likely influence of *Toxoplasma* on the probability of trap success was drawn from the host manipulation literature, and used to predict how prevalence of *Toxoplasma* infection up to 70% may influence the efficacy of these simulated trapping events. This showed that at 70% prevalence, *Toxoplasma* reduced the predicted required length of trapping down to just 2 nights trapping with 16 kill-traps per hectare or 12 nights trapping with 4 kill-traps per hectare.

This exercise shows that *Toxoplasma* infection has the potential to increase the efficacy of trapping to control wild populations of ship rats for the purpose of biodiversity protection in New Zealand. This might help improve levels of rat control for more resource-limited operations, such as those conducted by community groups. In addition, since cats can drive *Toxoplasma* persistence, this suggests that cats may be best left until last in multi-predator trapping programmes, to facilitate rat trapping. However, as with any modelling study, these findings are based on a set of assumptions that need testing to generate confidence in predictions. Such testing would involve behavioural studies of infected ship rats and their interactions with kill-traps. Should these studies confirm Dan and Clare's assumptions, the next step would be to conduct investigations into whether rat populations could be seeded with *Toxoplasma* infection in a cost-effective and safe manner. The use of such an adjunct to improve the efficacy of rat control is not as far-fetched as it may seem – Singapore already manages rat populations for food security by seeding with bait infected with protozoan parasites.

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The role of multiple wildlife hosts on *TB persistence*

Mycobacterium bovis, the causative agent of bovine tuberculosis (TB), has a very broad host range, which in New Zealand is dominated by cattle, brushtail possums, red deer, ferrets and feral pigs. The possum has long been considered the primary wildlife maintenance host in which the disease can persist independently. Therefore, wildlife management programmes aimed at reducing TB transmission to domestic cattle and deer in New Zealand have focussed on lethal control of possums. However, there is still the occasional report of TB being found in deer, pigs, or ferrets long after the imposition of intensive possum control, sparking concerns around the combined role of other wildlife species in maintaining *M. bovis* and thus disease across landscapes, and the potential for spillback transmission of *M. bovis* to recovering possum populations.

To investigate these concerns, Mandy Barron and colleagues constructed a multi-host TB model incorporating population dynamics for three host species with both intra- and inter-species *M. bovis* transmission. The models were parameterised for two case studies of current concern for TB management in New Zealand, namely chronic TB persistence in (i) a possum-deer-pig complex in extensive forest such as the Hauhungaroa Ranges and (ii) in a possum-pig-ferret complex in semi-arid shrub and grasslands, such as on Molesworth Station *(Fig.)*. TB persistence in the face of 'best practice' possum control was evaluated from model simulations and from the contribution of the different hosts to TB persistence by removing each host species in turn from the simulations. Demographic parameter values for the different host species were readily obtained from the literature. However, disease parameter values, in particular transmission rates between host species, were largely unknown, and were derived from contact and scavenging rates or by using the ratio of home range sizes as a proxy for contact rates. Because of this uncertainty, a sensitivity analysis was done to explore how different parameter values affected modelled TB persistence.

The forest case study showed that inter-specific transmission could influence TB dynamics in possums, but this was predicted to have little effect on the success of best-practice TB control. The presence of deer had very little influence on TB dynamics in possums, supporting their long inferred status as a largely inconsequential spillover host for the disease. Also, the long-run consequences of spillback transmission to possums are minimal other than extending the duration of the possum control required. Pigs were predicted to have the most influence on possum TB prevalence, largely as a consequence of their disease amplification role. Because they are relatively short-lived, pigs did not have any great effect in extending the spillback risk to possums. Sensitivity analyses indicated these interpretations were robust to uncertainty in model parameter values.

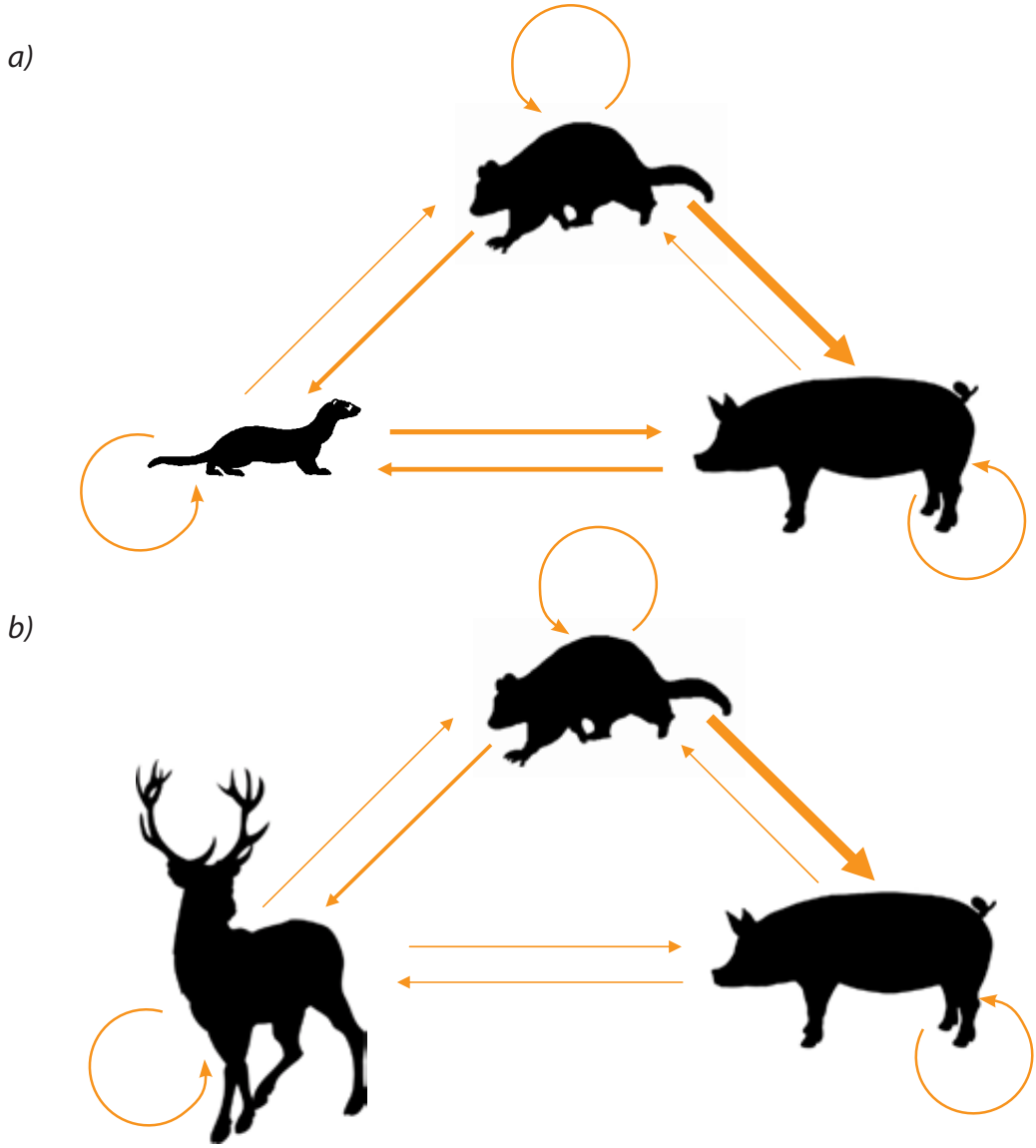

Fig. *Schematic diagram of multiple-host complexes and TB transmission pathways for (a) grassland habitat (ferrets, possums and pigs) and (b) forested habitat (deer, possums and pigs). The width of the arrows indicates the magnitude of the force of infection when hosts are at equilibrium density.*

The grassland case study showed TB could not persist in pig or ferret populations alone, but that the simultaneous presence of moderate density ferret and pig populations enabled TB to persist even when the density of possums was zero. This resulted in spillback transmission to possum populations after they had recovered from simulated control and ultimately, TB eradication failure. The ability of ferrets and pigs to maintain TB within their collective populations was contingent upon sufficient pig-to-ferret and ferret-to-pig transmission, which was assumed to occur via reciprocal scavenging. However, TB persistence outcomes were most sensitive to these inter-specific transmission rates and unfortunately these rates are the ones the team has least information about. Simulation of population control of either pigs or ferrets to reduce their combined abundance below the threshold for disease persistence (<5 per km^2) showed that, in conjunction with possum control, TB could be eradicated from an area.

This study was the first attempt to characterise multi-host TB dynamics for New Zealand wildlife and many of the disease parameter value estimates used were coarse approximations. However, the sensitivity analysis has shown which multi-host species complexes disease modellers and disease managers need to be concerned about (possums-pigs-ferrets) and which they don't (possums-deer-pigs), as well as which parameters make the most difference to TB persistence predictions. Thus, future work should focus on the empirical estimation of ferret and pig intra- and inter-specific transmission rates to determine if multi-host dynamics could be jeopardising TB eradication programmes in semi-arid shrub and grassland habitats.

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Feather loss in *Adélie penguins*

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Adélie penguin at Cape Bird, Ross Island showing feather loss on its back.

Landcare Research (and previously the Department of Scientific and Industrial Research) has been studying Adélie penguin colonies on Ross Island in Antarctica since the 1980s. Population sizes are currently healthy; however in the austral summer of 2011–12, an abnormal feather loss condition was observed for the first time at the Cape Bird and Cape Crozier colonies, affecting approximately 1 in 1000 birds (*photo*). Such feather loss has been observed previously on rare occasions in African penguin chicks in Africa, Adélie penguins in East Antarctica, king penguins on Possession Island, Magellanic penguin chicks in South America, and rockhopper penguins on the Falkland Islands, with malnutrition, lice infestation and ticks being identified as possible but unproven causes (aetiology).

With one key role of feathers being to provide birds with insulation, abnormal feather loss is likely to threaten survival of affected penguins. Hence, understanding its cause(s) is important, particularly since the paucity of prior records of abnormal feather loss in penguins suggests it could be an emerging disease. With this in mind, Melanie Massaro (United States Antarctic Program) (USAP) and colleagues collected biomedical samples from 30 adult Adélie penguins exhibiting abnormal feather loss and 30 adults without obvious signs of feather loss in January 2012. Penguins were caught using long-handled nets as they returned from foraging at sea and before they reached their nesting sites. The birds were examined for abnormalities, such as wounds and external parasites, before blood samples and cloacal swabs were collected. Feather samples from Adélie penguins at Cape Bird were also taken from the border of areas of feather loss on affected birds by University of Otago PhD student Wray Grimaldi. The collected material was returned to New Zealand and, under the supervision of Dan Tompkins (Landcare Research), subjected to a range of tests.

Affected birds had bare patches of skin on parts of the body otherwise normally feathered. However, the underlying skin appeared normal and no external parasites were observed. Likewise, the feathers collected looked normal when examined by scanning electron microscopy, and no ectoparasites or fungal elements were observed. An examination of thin blood smears on slides failed to show any blood parasites, but birds showing feather loss had higher proportions of lymphocytes and basophils and lower proportions of heterophils than birds showing no loss of feathers. These differences in white blood cell counts could suggest a viral aetiology - viral infection is one cause of raised counts of lymphocytes while raised counts of basophils have been associated with skin disorders in mice and humans.

To further investigate a potentially viral aetiology, pooled cloacal swabs from both birds with feather loss and those without it were subjected to metagenomic viral pathogen discovery (see article by Dan White in this issue for brief details of this technique). The sequence data produced showed evidence of three putative (supposed) new enteric penguin viruses, showing similarity to rotaviruses, turkey hepatitis viruses and astroviruses, respectively. Of the three penguin viruses, only the putative astrovirus was detected solely in the sample pool from birds exhibiting feather loss. (The putative rotavirus was detected in birds exhibiting feather loss and in those unaffected (to a much lesser extent), while the putative turkey hepatitis virus was only detected in the sample pool from birds without signs of feather loss.) Astroviruses and rotaviruses have been isolated from poultry, both in healthy and sick flocks, though no reports associate either of these viruses with feather loss. Follow-up work is recommended to investigate whether the novel astrovirus detected in Adélie penguins is causing the observed feather loss condition.

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Evolution of rabbit haemorrhagic disease virus *in New Zealand*


John Hunt

Since its illegal introduction in 1997, rabbit haemorrhagic disease (RHD) virus has spread throughout New Zealand and greatly reduced rabbit populations in many regions. However, rabbit numbers in some areas are now recovering as an increasing proportion of rabbits have developed protective antibodies against the disease. This allows them to survive any subsequent exposure to the RHD virus. Rabbits can acquire the protective antibodies following exposure to the RHD virus early in life or possibly from infection by a closely-related but benign form of rabbit calicivirus that was present in New Zealand rabbits before the RHD virus was introduced. Janine Duckworth and the Rabbit Biocontrol Initiative team have been working to identify ways to maintain and improve the ability of the RHD virus to kill rabbits. Two key questions being addressed by the team are whether the RHD virus has changed since its release, such that different strains now exist in New Zealand, and, if so, do the strains differ in their ability to kill rabbits.

With assistance from local farmers, rabbit control contractors and land users, Janine and the Rabbit Biocontrol Initiative have collected 24 wild New Zealand field strains of RHD virus (*Fig. 1*) and genetically tested them. Such analysis shows that RHD virus has evolved (*Fig. 2*), is now genetically distinct from the original virus (Czech V351 strain) released in New Zealand, and is also different to wild strains of RHD found in Australia.

Eight of the wild New Zealand RHD strains were selected for testing in domestic rabbits to see if genetic changes in the virus resulted in changes in its pathogenicity. The team's aim was to identify New Zealand RHD strains that killed quickly and humanely. Results indicate that all the strains of RHD virus tested were lethal to rabbits (*Table*) and the overall kill rate was 97%. All but two of the New Zealand strains were similar in pathogenicity to the originally released Czech V351 strain. One exception was isolate 64 Otago Alexandra, which took significantly longer to kill (125.6 ± 1.7 hours from exposure to death), with a longer period of fever (57.5 ± 7.2 hours), indicating poor efficacy and animal welfare impacts. For those reasons the 64 Otago Alexandra strain was not selected for further evaluation. In contrast, strain 95 Mackenzie Basin caused death significantly faster (39.9 ± 1.7 hours) than the Czech V351 strain (74.3 ± 16.0 hours), (*Table*) and animal welfare impacts were acceptable. This indicates that strain 95 Mackenzie Basin is a highly pathogenic strain of RHD virus and worthy of further investigation. The strain is currently being assessed for its ability to kill wild rabbits, including those with antibodies against benign rabbit calicivirus.

Concurrently, molecular techniques were used to confirm the presence and identification of benign rabbit caliciviruses present in New Zealand. Serum antibody levels measured by Leila Nicholson (PhD student) showed that benign rabbit calicivirus is widely spread throughout the country, and recently Leila isolated and identified the first New Zealand strain of benign rabbit calicivirus. Janine and her team are now determining the extent to which prior exposure to benign rabbit calicivirus protects rabbits against subsequent RHD virus challenge, and quantifying its potential to impact on the effectiveness of RHD biocontrol.

In the future, and subject to gaining appropriate approval, high-virulence strains of New Zealand-sourced RHD virus, and best practice methods for their use to minimise any effect of pre-existing benign rabbit caliciviruses on RHD impacts, may be made available to land managers to maximise the benefits of rabbit control and as tools supporting long-term control of wild rabbits in New Zealand.

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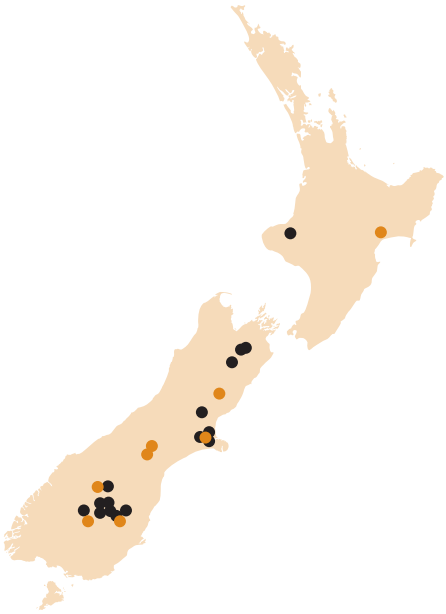

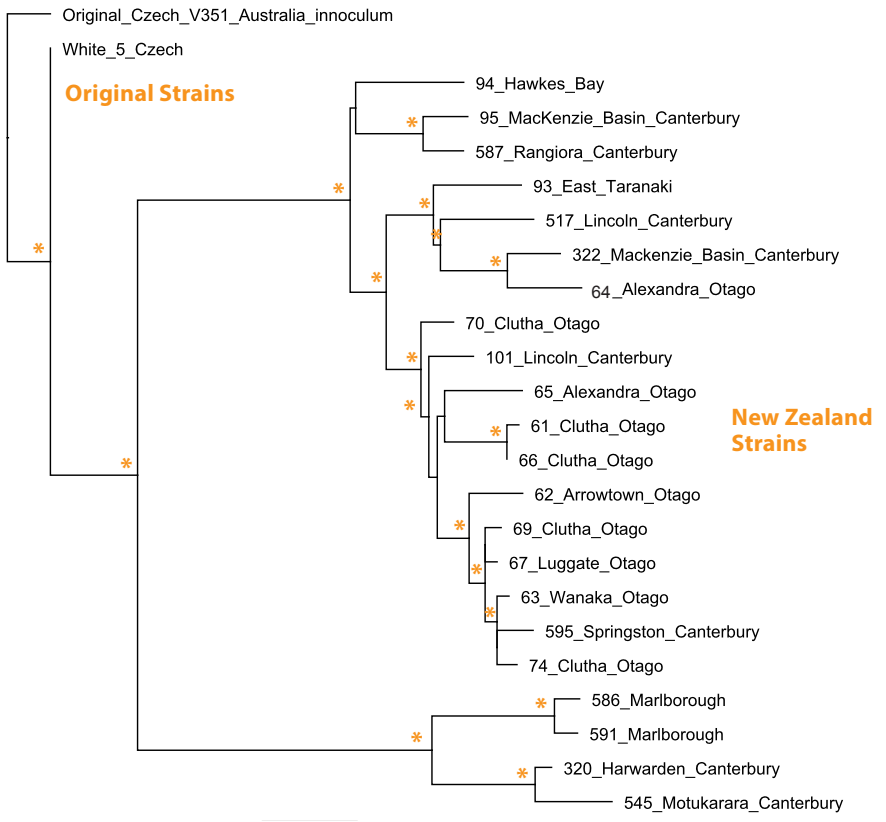

Fig. 1 *Map showing sites in New Zealand where wild strains of RHD virus were collected. Orange dots indicate strains tested for pathogenicity.*

Fig. 2 *New Zealand-sourced RHD virus strains clustered on phylogenetic analysis of gene sequences for the viral coat protein (VP60). Bootstrap probability values above 75% are indicated by asterisks at the nodes.*

Table. *Effect of RHD virus strain on kill rate, time from oral dosing (exposure) to death and time from onset of fever to death. Values within columns assigned different letters are significantly different (P<0.05).*

RHDV Strain	n	Kill rate (%)	Time to death (hours) mean	Time from onset of fever to death (hours) mean
63 Wanaka Otago	8	100%	49.4 ^{ab}	10.41 ^{a}
64 Alexandra Otago	5	80%	125.6 ^{c}	57.5 ^{b}
67 Luggate Otago	8	100%	52.3 ^{ab}	12.7 ^{a}
94 Hawkes Bay	8	100%	49.3 ^{ab}	16.3 ^{a}
95 Mackenzie Basin Canterbury	8	100%	39.9 ^{a}	14.1 ^{a}
320 Hawarden North Canterbury	8	100%	70.4 ^{b}	29.1 ^{a}
322 Mackenzie Basin Canterbury	8	100%	57.0 ^{ab}	12.8 ^{a}
517 Lincoln Canterbury	8	100%	53.1 ^{ab}	10.8 ^{a}
Czech V351	8	88%	74.3 ^{b}	28.6 ^{a}

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