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Editor: Michael Bradstock
Technical Editors: Jonathan Watts, Lora Peacock

Correspondence and requests to receive *Surveillance* should be addressed to:
Editor
Surveillance
Ministry for Primary Industries
PO Box 2526
Wellington, New Zealand
email: surveillance@mpi.govt.nz

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EDITORIAL

THE ROLE OF PHEL IN SUPPORTING MPI OUTCOMES

The Plant Health and Environment Laboratory (PHEL) provides diagnostic testing and technical expertise for exotic (regulated) pests and diseases affecting plants and the environment. It also provides diagnostics and advice on exotic and invasive plants that may affect the terrestrial and aquatic environment. These activities contribute to MPI's outcomes: harmful organisms are prevented from establishing; the harm caused by established pests and weeds is managed and New Zealanders are informed participants in the biosecurity system. PHEL, as part of the Investigation and Diagnostic Centres and Response, works closely with the Surveillance and Incursion Investigation, Response, Major Incident Management, Long Term Pest Management, Animal Health Laboratory and Laboratory Information Management and Laboratory Support groups to achieve these outcomes.

To protect New Zealand's primary industry sector and environment, it is vital to identify exotic organisms quickly and distinguish them from those that are already present. PHEL employs more than 40 scientific staff at two sites (Auckland and Christchurch) where it identifies a broad range of pests and diseases including insects, mites, fungi, bacteria, phytoplasmas, viruses, viroids and weeds. The tests used to detect new organisms range from morphological examination, biological indicators, serological testing and real-time polymerase chain reaction to cutting-edge technologies such as loop-mediated isothermal amplification and next-generation sequencing. Digital image capture and remote diagnostics provide much easier access to overseas expertise if required. The laboratory's specialist facilities include containment laboratories, Level 3 quarantine glasshouses and tissue culture facilities, equipment for molecular and serological procedures, electron and light microscopes, and reference collections of viruses and arthropods. Fungal and bacterial collections are maintained as part of the Landcare Research International Collection of Micro-organisms from Plants (ICMP).

The credibility of test results is critical since significant biosecurity decisions, such as the initiation of incursion responses or the treatment, destruction or re-shipment of consignments at the border or in quarantine, are based on our identifications. One of the ways of ensuring credible results in a laboratory environment is to demonstrate their consistent quality. Since July 2007, PHEL has been accredited to ISO 17025 *General requirements for the competence of testing and calibration laboratories* (www.ianz.govt.nz), the gold standard for laboratory quality systems.

PHEL is responsible for providing diagnostic services for a variety of surveillance programmes, including surveillance of high-risk establishment sites (e.g., around ports) and other targeted surveillance for fruit flies, animal disease vectors and honeybee pests. There is also a passive surveillance programme through which the general public, growers and regional councils submit samples of suspected exotic pests and diseases. The laboratory conducts more than 10 000 diagnostic tests each year to support these programmes. These activities provide important information on New Zealand's plant health and pest-free status to reassure our trading partners and ensure continued market access for exports.

The following example clearly demonstrates the importance of having an internationally accredited national reference laboratory. In 2011 a consignment of New Zealand avocadoes was rejected by US Department of Agriculture (USDA) inspectors suspecting the presence of avocado scab disease. A specimen was identified as *Sphaceloma perseae* (avocado scab) by a US diagnostic laboratory. There were serious implications for continued market access (annual avocado exports are worth $80 million) if avocado scab was confirmed in New Zealand. MPI negotiated with the USDA to obtain some of the affected sample for testing in the MPI laboratory. PHEL performed the tests using morphological techniques and a molecular protocol specific to avocado scab disease. The diagnostic was completed within two days of receiving samples and the disease was not detected. The fungus on the avocado skin was actually *Colletotrichum acutatum*, a common fungal pathogen found on wide range of plants. Both the US and Australia accepted PHEL's test results, the avocado shipment held in the US was released and trade was resumed. The outcome was that PHEL protected New Zealand's avocado scab disease-free status. There are many examples like this to demonstrate PHEL's role in facilitating trade activities.

PHEL provides technical advice and diagnostics in support of exotic pest and disease investigations. Each year the laboratory performs more than 1500 diagnostic tests. It is important that this work is carried out quickly, efficiently and to a high standard, so that incursions of exotic pests and diseases can be rapidly identified or ruled out.

The laboratory tests imported and exported plants to ensure their health, and diagnoses pests and diseases intercepted on plants and plant products (e.g., fresh fruit) at the border or in quarantine (e.g., ornamental and crop plants). Each year the laboratory identifies around 6000 arthropods (insect, mites, etc.) and 1000 plant pathogens (bacteria, fungi, phytoplasmas, viruses and viroids). PHEL has a specific role in facilitating the importation of germplasm and provides post-entry quarantine (PEQ) services (glasshouse space and/or testing) for new varieties. Since 2006, PHEL's PEQ service has facilitated the importation of blackberry, blackcurrant, blueberry, citrus, grape, hazelnut, kiwifruit, kumara, potato, raspberry, strawberry and walnut. For some industries, e.g., kumara, this was the first

time in the last 10 years that new germplasm could be brought into the country. An export testing service is also available to support phytosanitary certification of plants and plant products.

Achieving excellence in diagnostics requires investment not only in technology but also in people and relationships – within New Zealand and overseas. The laboratory maintains an extensive network of national and international links to facilitate its work. PHEL experts participate in multi-national plant disease working groups such as the International Plant Protection Convention, Australian Sub-Committee on Plant Health Diagnostics and the diagnostics tools collaboration programme of the QUADS countries (Australia, Canada, New Zealand and the US). PHEL also undertakes projects to extend the laboratory's capability and capacity, e.g., to broaden the range of pests and diseases that can be detected or to improve the sensitivity and specificity of existing tests. These activities underpin MPI's capability to respond to incursions, undertake surveillance and provide border testing.

There are plenty of challenges for PHEL and our partners. Growing volumes of tourism and trade from increasingly diverse places are bringing more potential pests and diseases to our doorstep. Minute organisms are much harder to detect and identify at the border than minute traces of other risk goods such as illegal drugs. We depend on all New Zealanders – travellers, growers, importers and exporters and those involved in diagnostic work – to report promptly any suspicious organism or symptoms they encounter.

In a perfect world, every incursion by a pest or disease would be detected, analysed and reported. While that level of perfection may never be reached, we can be confident that that we have robust diagnostic systems in place to detect and identify exotic organisms.

Lalith Kumarasinghe
Manager, Plant Health and Environment Laboratory
Ministry for Primary Industries
Lalith.Kumarasinghe@mpi.govt.nz

VACCINATION AS A RESPONSE TOOL FOR EQUINE INFLUENZA

This paper draws on experience with the 2007–08 incursion of equine influenza (EI) in Australia and summarises key findings from results of Australian and New Zealand simulation modelling, as well as other data from the literature. This will help inform decision making regarding a response policy for vaccination of horses for EI in the event of an incursion into New Zealand.

INTRODUCTION

In the event of an incursion of EI virus into the horse population of New Zealand the use of vaccination as a response tool will be an option for decision makers to consider. Strategic vaccination was used as a tool to help eradicate EI during the 2007–08 incursion into Australia. There was limited time available for Australian authorities to critically analyse the most effective vaccination regime used in the response, so the strategies they used were untested and may have had limited impact on control (Cowled *et al.,* 2009; Garner *et al.,* 2011).

Queensland and New South Wales used different control strategies, yet the time taken to eradicate the disease was not greatly different between the two states. Analysis of the effectiveness of vaccination was complicated by the strategies changing over time. The New South Wales approach was mainly focused on vaccination of horses around infected premises (suppressive vaccination), in contrast to Queensland's approach, which was mainly focused on creating protective buffer zones (Perkins *et al.,* 2011). Regardless of its benefit as an eradication tool, vaccination was a key to allowing horse movements necessary for some equine industries to resume operation on a limited basis (Garner *et al.,* 2011). The current Australian response plan for EI includes the use of vaccination as a response tool (Anonymous, 2011).

Australian data helps determine the merits of different vaccination strategies. Simulation modelling is one method of examining the effect of a range of control scenarios on specific outcomes, such as the number of infected horse premises. Strategies that have the most significant effect on these outcomes can then be incorporated into disease control policy. Ideally policy is formulated prior to a response as the maximum payoff is likely to result if response strategies are carried out in a timely manner (Cogger *et al.,* 2011).

SIMULATION MODELLING

AUSTRALIA MODELLING

The Australian outbreak of EI lasted four months, with about 140 000 horses vaccinated as part of control measures (Garner *et al.,* 2011). Given that the number of cases of EI was falling by the time vaccination was initiated, its benefit to containment and eradication during this outbreak was questionable (Cowled *et al.,* 2009; Garner *et al.,* 2011). Other vaccination strategies might have been more effective, and these were examined by modelling different Australian scenarios.

Modelling showed that movement restrictions and biosecurity measures were highly effective in controlling the outbreak. All early vaccination strategies (beginning seven days into a control programme) where vaccination of horses from high-density horse areas was prioritised, reduced the size of the outbreak. In this scenario there was a 60 percent reduction in the number of infected properties and an 8–9 percent reduction in the size of the area affected. Where resources for carrying out vaccination were limited, a 1 km suppressive ring vaccination strategy was most effective (Garner *et al.,* 2011).

Unfortunately these results cannot be extrapolated to an outbreak scenario in New Zealand as the structure of the horse population, the density of horse properties, local geography and other factors such as climate are very different.

NEW ZEALAND

A stochastic simulation model using InterSpread Plus (Sanson, 1993) was used to model outbreak scenarios of EI under New Zealand conditions. The model was parameterised using demographic and movement data collected by Rosanowski (2011) and from other data gained from the experience of the Australian EI outbreak. Initial findings from the model did not suggest that vaccination offered any greater benefit than a national standstill of horse movements (Cogger *et al.,* 2011). However, when modelling was carried out with vaccination targeting regions where there was a high density of horses (e.g., Auckland and the Waikato), the results agreed with the Australian findings.

It was determined that vaccination plus movement restriction was more effective than movement restriction only. A suppressive vaccination strategy (ring vaccination in a 3 km zone around infected properties) generally was the most effective strategy tested, with regard to minimising the number of infected properties, the number of vaccinated properties (the lowest number being the most optimal in terms of minimising resources and costs associated with vaccination) and the duration of the outbreak (Rosanowski, 2011).

Comparisons between movement control only and the use of suppressive vaccination (at a 3 km radius) combined with movement control showed that the median duration of the outbreak was 51 percent less when vaccination was carried out (88 vs. 178 days). In addition, there was a 75 percent reduction in the number of infected properties (793 vs. 3136). The median number of properties where vaccination was carried out was 2726.

During the first 10 days of the Australian outbreak, most local spread was within 5 km of infected properties, with the maximum spread occurring out to a distance of 15.3 km. Over the entire outbreak 83 percent of infected properties were within 15 km of those

premises infected through network spread in the first 10 days (Dhand *et al.*, 2013). In light of these findings, analysis was carried out using the New Zealand InterSpread Plus model to examine the effect of incorporating these results into local spread parameters. Results indicated there was some advantage in extending the radius of suppressive vaccination from 3 km to 5 km. In this scenario the number of infected properties was reduced by about 10 percent and the duration of the outbreak reduced by about two weeks. However, 20 percent more horses had to be vaccinated.

Sensitivity analysis showed that if the local spread parameters were increased by 25 percent from baseline levels, the number of properties that had to be vaccinated could increase by a further 13 percent and the number of infected properties by 34 percent (Rosanowski, 2013). Hence, in response planning, disease control authorities need to take account of uncertainty by ensuring that there are more than sufficient resources available for any given scenario.

Development of an agreed vaccination policy and an established relationship with vaccine companies would most likely be among the conditions necessary to carry out early vaccination. Delays in the start of vaccination could still occur, firstly, because of the need to type the vaccination strain (molecular sequencing can take up to four days) and to ensure that any vaccination used provides adequate protection against the field strain; and secondly, because it takes time to obtain the vaccine and develop operational procedures. Subsequently, expert opinion would be sought on the suitability of available vaccines. Initial sourcing of vaccine and negotiations to purchase vaccine could be started immediately, with a final decision made once the suitability of vaccine for use against the field strain was confirmed. It seems unlikely that even under the best circumstances vaccination could start within less than seven to 10 days. Despite this, Rosanowski (2011) determined that starting vaccination at seven days vs. 14 days had no effect on the duration of the epidemic, though it did reduce the median number of infected properties by 23 percent.

CONCLUSIONS

Movement standstill and other biosecurity practices are the key responses for controlling or eradicating equine influenza (Firestone et al., 2013; Garner et al., 2011). Modelling is a tool to help understand the value of other response policies, including vaccination. Modelling using InterSpread Plus showed that vaccination after seven days or sooner did not greatly improve the efficiency of EI control. Suppressive vaccination out to a radius of 3 km in regions where there is a high-density horse population (e.g., Auckland and the Waikato) will provide more effective control than national movement standstill alone. Where vaccination resources are unlimited, increasing the radius of suppressive vaccination out to 5 km will reduce eradication time and the number of infected properties, but for this scenario to work, horses on 20 percent more properties would have to be vaccinated. Sensitivity analysis shows that if the local spread parameters were increased by 25 percent from baseline levels, the number of properties where vaccination is required could increase by a further 13 percent and the number of infected properties could increase by 34 percent. Hence, in response planning, disease control authorities need to take account of uncertainty by ensuring that there are more than sufficient resources available for any given scenario. Suppressive vaccination as a response tool does not preclude additional targeted vaccination of a subgroup of animals within a sector for the purpose of business protection and long-term business continuity. Targeted vaccination would not compromise either eradication or proof of eradication.

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Andy McFadden
Incursion Investigator
Surveillance and Incursion Investigation (Animals and Marine)
Ministry for Primary Industries
Andrew.Mcfadden@mpi.govt.nz

Sarah Rosanowski
Postdoctoral Researcher
Veterinary Epidemiology, Economics and Public Health,
Hawkshead, UK
srosanowski@rvc.ac.uk

Trish Pearce
Equine Health Association
pearce.patricia@gmail.com

QUARTERLY REVIEW OF DIAGNOSTIC CASES: JULY TO SEPTEMBER 2014

GRIBBLES VETERINARY PATHOLOGY

BOVINE

A sample of fixed skeletal muscle was submitted from a well-grown beef heifer home-killed in the Wairarapa region. The muscle contained several pale green streaks measuring up to 5 cm long. Microscopic examination revealed an intense infiltrate of eosinophils associated with myofibre degeneration and myocytolysis. A diagnosis of **eosinophilic myositis** was given. This condition is thought to be an inflammatory response to ruptured sarcocysts.

Ten late-term abortions occurred over a few weeks in a Taranaki dairy herd of 250 cows. One aborted fetus and a sample of placenta were submitted for laboratory analysis. Gross findings included marked expansion of the intercotyledonary membrane by gelatinous material, and patchy infiltration of the lungs. Key histological changes were vasculitis and interstitial inflammation in the placenta, marked lymphoplasmacytic interstitial pneumonia and lymphoplasmacytic conjunctivitis. A diagnosis of likely ***Ureaplasma diversum*** **abortion** was made despite a negative culture result from fetal stomach contents (the organism is highly fastidious.) *U. diversum* is a commensal of the upper respiratory tract or lower reproductive tract, found in up to 14 percent of cattle (Thornton and Wake, 1997). It causes a distinctive granular vulvitis in some cows and may cause late-term abortion, stillbirth, or weak calves. Retained placenta is common. Being highly infectious, it may be transmitted by natural mating, artificial insemination, through urine, and to calves during parturition. Once in a herd, it is difficult to eliminate.

On a Taranaki dairy farm veterinary attention was sought for a Friesian cow with an apparent uterine prolapse after delivering a healthy calf. The attending veterinarian found that the suspected prolapse was actually a mass of abnormal tissue associated with the placenta. Gross pathological assessment showed a bisected pleomorphic tissue structure measuring about 28 x 25 x 12 cm. Identifiable within the mass was a pair of spongy red organs 8 cm long, suggestive of lungs, at the base of which was a ring of cartilage within glistening red tissue (heart base). There were several thin-walled sac-like structures reminiscent of gastrointestinal tract, among which was a 4 x 3 x 3 cm dark red vascular parenchymatous organ (liver). Other organs suspected to have been present were spleen, diaphragm, omentum and gall bladder. There were no recognisable cranial, axial, or appendicular bones. The mass was given a morphological diagnosis of **acardiac acephalic fetal monster.** These are generally thought to occur as the result of a twin pregnancy. The co-twin is usually normally developed. Because there are no discernible CNS elements or heart chambers, it may be hypothesised that the insult occurs during the first month of gestation. Theories as to how the abnormal twin develops include anastomosis of the umbilical vessels and interference with the blood flow from the placenta to the twin.

Two aborted calves from different farms in Canterbury had histological lesions of myocarditis without lesions of myositis or encephalitis, meaning that *Neospora* infection was unlikely to be the cause. Both fetuses were positive for BVD virus by PCR tests on heart blood. Myocarditis is a recognised lesion in some fetuses with **BVD infection,** which may have been the cause.

A three-week-old calf on a South Canterbury farm suddenly developed acute convulsions and died. It was one of a group of about 400 calves that had been raised in good conditions, in a shed where no problems had been experienced the previous year. No lesions were detected on histological examination of the brain. **Lead toxicity** was initially considered unlikely but a Ziehl-Neelsen stain of the kidney revealed many intra-nuclear acid-fast inclusions in cortical tubular epithelial cells, indicating exposure to lead. Fresh kidney was then tested and the lead level found to be 84 mg/kg (toxic level > 5 mg/kg). A painted gate was eventually traced as the likely source of the lead.

A mid-Canterbury dairy farmer was concerned that many of his calves had multiple small slightly raised lesions on the nose and in the mouth. The calves were vigorous and healthy and the veterinary practitioner was not concerned but the farmer wanted the matter investigated further. A biopsy of a typical lesion in one calf showed multifocal areas of keratinocyte swelling with variable-sized amphophilic inclusions in many of the swollen cells. The lesions were typical of **bovine papular stomatitis virus** infection**.**

An adult Friesian dairy cow from Northland was sick two weeks after calving. She had watery diarrhoea, was tachycardic and hypothermic. Others in the herd were not milking well. Faecal culture yielded ***Salmonella*** **Typhimurium,** typed as **phage type 56** variant by a reference laboratory, suggesting a diagnosis of **salmonellosis**.

A middle-aged Jersey cow from the Bay of Plenty was scouring. A faecal smear for acid-fast organisms was negative, but a serum Johne's ELISA test was positive, suggesting a diagnosis of **Johne's disease**.

A three-year-old Friesian dairy cow from Northland was circling, not eating and showed no response to treatment. A brain submitted for transmissible spongioform encephalopathies surveillance showed areas of necrotising and suppurative meningoencephalitis centred on the brain stem, with a few Gram-positive bacilli characteristic of **listeriosis**.

A group of housed 2-10-day-old Northland calves had severe scouring. A faecal sample from a four-day-old calf was positive for rotavirus antigen, indicating likely **rotaviral diarrhoea**.

Two weaner beef calves from Northland were scouring. Faecal examination yielded growth of ***Salmonella*** **Typhimurium,** typed as **phage type 9** by a reference laboratory. Also present were moderate numbers of coccidial oocysts and high numbers of strongyle eggs (up to 2500/g), consistent with combined **parasitism and salmonellosis** or *Salmonella* carriage.

A yearling Friesian heifer from Northland was lethargic and thin with watery, green-brown diarrhoea. There was a history of recent feed shortage in the mob. There was hypoalbuminaemia (14 g/L; reference range 26–35) but serum pepsinogen was normal and the heifer was negative for serum BVD antigen. Faecal culture yielded ***Yersinia pseudotuberculosis***, consistent with a diagnosis of **yersiniosis**.

An adult Friesian dairy cow from the Waikato was anorexic, agalactic and pyrexic (39.1°C), with pale pink mucous membranes. Significant biochemical changes included increased GGT (121 IU/L; reference range 7–40), GLDH (132 IU/L; reference range 0–45) and AST (615 IU/L; reference range 62–206), suggestive of liver damage (and possibly muscle damage) and cholestasis. Haematology showed marked anaemia, with HCT 0.11 L/L (reference range 0.24–0.4), RBC 1.16 x 10^{12}/L (reference range 5–7.7), Hb 35 g/L (reference range 85–130), macrocytosis (MCV 93 fL; reference range 38–56), absolute reticulocytosis (114.84 x 10^9/L; reference range 0–1) and immature red cells (67 per 100 leukocytes; normal 0). About 100 ***Theileria*** organisms per 1000 red blood cells were identified, consistent with ***Theileria*-associated bovine anaemia**. The hepatic damage was associated with tissue hypoxia.

A one-week-old beef calf from Northland died after scouring for two days, despite electrolyte treatment and milk by stomach tube. Faecal antigen ELISA was positive for ***Cryptosporidium* spp**., indicating a diagnosis of **cryptosporidiosis**.

A two-year-old first-calving Friesian heifer on a Wairarapa dairy farm died unexpectedly. At postmortem melena was noted and there was a focal segment of jejunum filled with blood and fluid associated with marked reddening of the mucosa. Histopathology confirmed coagulative necrosis in this area, associated with clusters of large bacterial rods. Culture yielded a heavy growth of ***Clostridium perfringens***, suggesting acute clostridial bacteria proliferation in the intestine was a factor in the death of the heifer. Direct causation of the **haemorrhagic bowel syndrome** diagnosed in this case was not entirely clear but some studies have also implicated mycotoxic fungi and shigatoxic *E. coli*, suggesting a multifactorial disease complex. Risk factors include high protein and carbohydrate in the diet, rumen acidosis, decreased intestinal motility and cold winter conditions.

A mature eight-year-old dairy cow from Taranaki developed a mass around the gingiva of the most caudal upper molar, extending into the soft palate and maxilla. Histopathological examination of the tissue revealed an **acanthomatous epulis**. This tumour is locally infiltrative and destructive and commonly recurs at the site of excision. Very wide surgical excision is required to remove the tumour and this was unlikely at this site in an older cow.

On a Hawke's Bay farm, four beef steers died from a mob of 45 cattle trucked from the South Island five days previously. Tissue samples were collected, tested and examined but the only significant finding was a markedly elevated liver selenium concentration of 39 290 nmol/kg (normal range 850–15 000), consistent with **selenium toxicity**.

Ongoing abortions in a crossbred dairy herd on the Hauraki Plains prompted serological testing of five aborted two-year-old cows. Two had immunofluorescent antibody titres of > 1:2000 to ***Neospora caninum***, confirming recent activation of infection. Two others had titres of 1:200 and 1:600, confirming previous exposure.

A two-week-old Friesian calf on a South Waikato dairy farm had severe diarrhoea. At the time of sampling it was the only one affected in the calf shed. ***Salmonella* Ruiru** was cultured from a faecal sample, confirming a diagnosis of **salmonellosis**. ***Cryptosporidium parvum*** and **rotavirus** infections were also detected by ELISA testing.

Nine dairy heifers from a mob of 106 were found dead the morning after they had been shifted to new grazing. The grazed area was adjacent to a swamp containing **swamp grass** (*Poa aquatica*) and Sudan grass (*Sorghum bicolor*). Both these species had been grazed. Testing of rumen contents found evidence of cyanide, confirming **cyanide toxicity** from eating swamp grass.

In the Waikato, nine cows died from a 350-cow dairy herd and five were examined recumbent, trembling and convulsing. Testing of the pasture eaten revealed a high nitrate concentration of 3 percent (> 1 percent is elevated), consistent with **nitrate toxicity**.

A 17-day-old calf from a dam that had been vaccinated some years ago with a now-withdrawn vaccine against bovine viral diarrhoea virus, was severely leukopenic (white blood cell count 1.5 x 10^9/L; reference range 4–12 x 10^9), thrombocytopenic (platelets < 10 x 10^9/L; reference range 200–600 x 10^9) and anaemic (Hb 39 g/L; reference range 80–140 and PCV 0.11 L/L; reference range 0.24–0.46). Histopathological examination of the bone marrow revealed a severely hypoplastic marrow and confirmed this to be the result of **bovine neonatal pancytopenia**.

In another case, a two-week-old female calf of unspecified breed in the Taranaki region was clinically normal one night but had a swollen head early the next morning. It was given an injection of ketaprofen. By evening it was dyspnoeic, with pale mucus membranes, petechial haemorrhages and a swollen area where it had been previously injected. Haematology showed the calf was severely leucopenic (total white blood cell count 0.1 x 10^9/L; reference range 4–12 x 10^9), thrombocytopenic (platelets < 10 x 10^9/L; reference range 200–00 x 10^9) and anaemic (haemoglobin 32 g/L; reference range 80–140 and PCV 0.10 L/L; reference range 0.24–0.46). There was a history of herd vaccination with a BVD vaccine, indicating **bovine neonatal pancytopenia**.

Blood was received from a herd of cows in the Taranaki region with a history of haematuria four to 20 days after calving. Serum phosphate concentrations ranged from very low (0.18 and 0.26 mmol/L) to normal and within the reference range of 1.41–2.95). This was therefore a case of **post-parturient haemoglobinuria**.

***Salmonella* Brandenburg** was identified as the cause of six outbreaks of calf diarrhoea and two small outbreaks of abortions on Otago and Southland dairy farms this spring. In one outbreak during July, 2 percent of mixed-age cows in a herd aborted over a 10-day period. The affected cows did not appear off-colour and the dead calves were not retained – the feature of *Salmonella* Brandenburg abortion outbreaks seen in other years.

Four dairy cows from a mob of 100 wintering on fodder beet on a paddock

leased from a Southland gun club were found dead. Another cow showed severe nervous signs of salivation, ataxia, circling and blindness. Whole blood lead concentration from this cow was 0.42 mg/L (toxic level > 0.35), consistent with acute **lead toxicity**. These cows had been grazing the crop for two weeks. The same paddock had been grazed over the previous two years without problems. The first year the paddock had been sown in grass and the second year kale was grown. Examination of the remaining plants and the soil on this paddock showed large numbers of lead pellets, both in the soil and embedded in the fodder beet stems and leaves. High concentrations of lead were found in the soil and the plant tops (518 and 184 mg/kg DM respectively). The cows were shifted off the contaminated paddock as soon as the first cases were seen. A day later, two more had died and a number were showing nervous signs. Four of the affected cows were tested and their blood lead concentrations were just below toxic levels, at 0.21–0.28 mg/L. The blood lead in another cow found recumbent *in extremis* was 0.40 mg/L. Two affected cows were necropsied and their reticulums were found to be packed with lead pellets.

Kidney from one of these cows had a lead concentration of 27 mg/kg (toxic level > 5). Tissues from a fetal calf taken from one of these cows also had elevated lead concentrations, confirming a diagnosis of **lead toxicity**.

About 25 of the 100 exposed cows died or were killed *in extremis* over a few days. Most of the remaining cows were eventually euthanased on the farm owing to their elevated blood lead concentrations.

A mob of 37 calves aged two to three months on a Southland dairy farm were transported to a runoff that contained an old shed with a dirt floor. A large amount of old machinery was shifted to one side and fenced off so the calves could use the shed for shelter. A small amount of straw was placed over the floor. Within 24 hours one calf was found dead and another was recumbent with nervous signs. A blood sample from this calf had a high lead concentration (0.87 mg/L; toxic level > 0.35). The remaining calves were all tested for lead and 18 had toxic blood levels, the highest being 1.4 mg/L. A few calves remaining on the home farm were also tested for lead, with negative results, confirming that the source of the lead was at the runoff. Although not tested, the source was likely to be the dirt floor of the shed, which was contaminated with engine oil, a known source of lead. ***Salmonella* Typhimurium phage type 1** was isolated from faecal samples taken from two cows with pyrexia and severe diarrhoea containing fragments of mucosa. In this small outbreak on a 900-cow dairy herd in Southland two cows died and six more were affected. The cause was thought to be a combination of feeding mouldy palm kernel and an oral copper supplement that may have caused gastrointestinal irritation.

On Southland dairy farms in late autumn and early winter there were a few single cases of **hepatic photosensitivity** in adult dairy cows. In mid-June there was a small outbreak where six of 220 cows on a swede crop were affected by photosensitivity caused by liver damage. Over the next three months a large number of similar outbreaks occurred, peaking in September. The highest number of cows reported as dying on one farm was 30. One large veterinary practice estimated that 300 cows died on farms they serviced. Most affected cows showed a photosensitivity but some (possibly those that did not have depigmented areas) showed severe metabolic signs and recumbency and often died shortly after treatment with metabolic solutions. In one case three cows became depressed, dehydrated and recumbent. Blood samples showed a severe azotaemia, ketosis and elevated liver enzymes. In one cow the bilirubin was 230 µmol/L (reference range 0–13), GGT 2847 IU/L (reference range 6–37) and GLDH 1502 IU/L (normal < 59).

Necropsy of affected cows found that the livers were large and yellow. Histopathological examination of these livers revealed marked loss of the smaller bile ducts and increased numbers of lymphocytes, collagen and fibroblasts in the portal areas; but the hepatocytes, apart from a mild retention of bile pigment, appeared unaffected.

Investigation of the outbreaks to date suggests an association with grazing herbicide-tolerant swedes, but these were also being grazed on other farms where the cows were unaffected. At the time of writing a survey is underway to collect further information on the outbreaks.

Six dairy heifers out of a recently purchased line of 130 heifers on a Southland farm developed spontaneous humeral fractures and another developed a fractured pelvis at calving. Serum and liver copper concentrations from one were 4.0 µmol/L (adequate > 8.0) and 28 µmol/kg (adequate > 95) respectively, indicating severe **copper deficiency.**

In a similar outbreak on another Southland dairy farm, four pre-calving heifers also developed spontaneous humeral fractures. All were noticed limping up to five days before the fractures developed. Liver copper levels from two affected heifers were also very low, at 21 and 24 µmol/kg (adequate > 95), again confirming severe **copper deficiency.**

Moraxella bovis was isolated from three of four ocular swabs from dairy calves with severe conjunctivitis, which usually began with a markedly watery eye at one day of age and developed to a more purulent discharge over a period of two to three weeks. A PCR for infectious bovine rhinotracheitis on dry pooled ocular swabs from five affected calves was negative.

OVINE

A Hawke's Bay farmer reported trickling deaths in nine-month-old Romney lambs grazing pasture under a pivot irrigator. There was surface water on the tracks. In all, 20 deaths and 60 sick lambs were reported out of 3500 at risk. Clinical features were lethargy, anaemia and red urine. Histology on an affected lamb showed hepatic centrilobular dissociation, degeneration and necrosis, and there was renal tubular haemoglobin deposition. These changes were considered to confirm the clinical suspicion of **leptospirosis.**

A Central Hawke's Bay sheep farm had 10 ewe deaths and six sick ewes over a five-day period. The ewes were being mob-stocked and numbered 2400 in total. Post-mortem findings included enlarged gall bladder, enlarged mesenteric lymph nodes and intensely congested caecal wall. Histology revealed a suppurative and erosive abomasitis accompanied by fine bacterial rods. ***Salmonella* Hindmarsh** was cultured from the caecal contents, confirming an outbreak of **salmonellosis.**

Lesions of **chronic vasculitis** were seen in multiple organs in a rising-one-year-

old lamb from North Canterbury. Similar lesions had been seen in two lambs on a South Canterbury property in 2013 and were investigated at the time by MPI. The cause remains unknown but could be the sheep **herpesvirus** that causes malignant catarrhal fever in cattle and deer. However, proving this is difficult as the virus is widespread in lambs.

A Banks Peninsula farm had 230 ewes and two had aborted. Both lambs were examined and had a placentitis, with one having massive numbers of Gram-negative coccobacillary bacteria in the areas of inflammation. A pure growth of ***Mannheimia haemolytica*** was recovered from the stomach contents of both lambs. The identity of the isolates was confirmed using MALDI technology. ***M. haemolytica*** **abortion** is uncommon in sheep.

Thirty five-week-old lambs from Northland died with oral frothing within 48 hours of docking, marking and vaccination. A 5-in-1 clostridial vaccine containing selenium had been accidentally used. At first, phosphatic fertiliser toxicity was considered possible because the lambs were in a recently fertilised paddock, but there were no significant histological lesions and liver selenium in one lamb was 173 800 nmol/g (reference range 450–9999), indicating that **selenium toxicity** was responsible.

Two Romney X Finn lambs from the Waikato were unable to stand on their hind legs after birth. Grossly the cerebrum was markedly cavitated, with only a thin rim of cortical tissue visible. Histologically there was cerebral white matter rarefaction or cavitation, and there were areas of grey matter gliosis or neuronal necrosis, with normal mid- and hind-brain structures. Samples of spinal cord showed scattered digestion chambers in lateral and ventral white matter tracts. Liver copper was 52 umol/kg (reference range 95–2999), suggesting that this was a case of hydrocephalus or hydranencephaly caused by **congenital copper deficiency (swayback)**.

Ewes began aborting three weeks before lambing on a Wairarapa sheep farm. Three dead lambs were collected and submitted for testing from a mob of 200 ewes. ***Campylobacter fetus fetus*** was cultured from the stomach contents of all three, confirming a diagnosis of **campylobacter abortion**. The flock had not been vaccinated against this.

Four ewes in a flock of 35 died on a kiwifruit orchard in the Bay of Plenty. Grass under the kiwifruit vines had recently been sprayed with copper oxalate. On examination of the dead ewes there was jaundice of the carcass and the ears and liver were swollen. The kidneys appeared dark and metallic. Histopathology confirmed haemoglobinuric nephrosis and massive hepatic necrosis. Liver copper concentration in this ewe was increased, at 3760 µmol/kg (reference range 95–2999). The kidney copper concentration in another dead ewe was 308 µmol/kg (toxic level > 160). These findings are consistent with **copper toxicity**.

Three out of 114 hoggets in Hawke's Bay died suddenly. They had been grazing an orchard sprayed with copper eight weeks previously. There was no water trough or other water source and the sheep were drinking from puddles. Post-mortem examination revealed gunmetal-coloured kidneys, pale liver and jaundice. Kidney copper levels in one sheep were 1666 µmol/kg (toxic level > 160), confirming **chronic copper toxicity**.

On a Central Otago farm two ewes from a flock pastured near some yards were found collapsed and shivering. They were unresponsive to treatment and eventually died. Blood samples taken from both ewes before death showed very high sodium concentrations of 174 and 192 mmol/L (reference range 140–148), consistent with **salt toxicity**. An opened bag of salt was later found in the yards, where the ewes had access to it.

A flock of 1200 two-tooth Merino ewes on a Central Otago farm were being fed baleage. The last lot of baleage being fed was noted to be spoiled. The ewes were then shifted to another paddock, which had a reasonable amount of grass. Two hundred ewes aborted and six died over a 2–3-week period starting about six days after baleage feeding was stopped. ***Listeria ivanovii*** was isolated from the stomach contents of two of the aborted lambs, confirming a diagnosis of **listeriosis**. There was probably sheep-to-sheep transmission as they were heavily stocked and Merinos tend to mob up.

Salmonella **Brandenburg** was the predominant cause of abortions in ewes in Otago and Southland this season, with 50 outbreaks diagnosed at the laboratory from early July to late September. Other causes of abortion diagnosed over that period included ***Campylobacter fetus fetus*** (6 outbreaks), ***Campylobacter jejuni*** (3), ***Listeria monocytogenes*** (2), ***Listeria ivanovii*** (1), ***Toxoplasma gondii*** (3) and **border disease** (5).

CAPRINE

About a dozen dairy goats in the Waikato died over a period of two days. Grain overload was initially suspected as the cause, but more goats were deteriorating clinically despite a change in feed and magnesium oxide supplementation. Significant biochemistry and haematology changes included hypocalcaemia (as low as 1.44 mmol/L; reference range 2–2.7), hypomagnesaemia (0.59 mmol/L; reference range 0.76–1.2), hyperfibrinogenaemia (up to 10.5 g/L; reference range 1–4) and neutrophilia with a left shift (bands up to 0.8 x 10^9/L; reference range 0–0.11 x 10^9). Moderate numbers of faecal coccidial oocysts were found. Histopathology showed multifocal suppurative enterocolitis and lymphadenitis with colonies of Gram-negative bacilli, considered classical for **yersiniosis**.

Four goats died on a Waikato dairy goat farm milking 500 goats. A new lactation was underway and there had been ongoing mineral supplementation and a new source of pelleted feed. The affected goats first presented with depression, anorexia, pale mucous membranes and jaundice. Anaemia was confirmed on haematology as the haematocrit was 0.17 L/L (reference range 0.27–0.42) and there was also marked liver damage as GLDH was elevated, with one affected goat registered a concentration of 7078 IU/L (reference range 0–31). Histopathology on a dead goat revealed massive hepatic necrosis and nephrosis. Liver copper concentration of one animal was 3330 µmol/kg (reference range 99–2999) and kidney copper concentration was 218 µmol/kg (toxic level > 160), confirming a diagnosis of **copper toxicity**. Copper was removed from multiple sources including the feed and water, and molybdenum was added to the pellets. Monitoring of liver

copper continued until the concentration decreased, then the molybdenum was withdrawn.

LLAMOID

An adult alpaca on a Canterbury farm was depressed and had pale mucous membranes. Its haemoglobin was 52 g/L (reference range 106–178). A faecal egg count was 3150 eggs/gram and the larvae cultured from the faeces were 100 percent *Haemonchus*. Several cases of haemonchosis in alpacas have occurred in Canterbury in the last few years and this too was confirmed to be a case of ***Haemonchus* infestation**.

On a small breeding alpaca stud in Southland three adult alpaca appeared to be losing weight and were anaemic. They had been recently brought in from a farm in Canterbury. Blood samples showed a severe non-responsive anaemia in two animals and low total protein concentration in all three. These findings suggested they had developed a blood-loss anaemia, most likely losing blood into the gastrointestinal tract. Faecal egg counts from the three varied from 150 to 650 eggs/gram. These samples were pooled and cultured for third-stage strongyle larvae. ***Haemonchus* sp.** was the only strongyle identified, confirming that this nematode was the most likely cause of the chronic blood loss. This species is rarely found in Otago and Southland and was probably imported from Canterbury with these alpacas.

CANINE

Four of a litter of six 4-week-old Bulldog pups from Hawke's Bay died after brief periods of illness. Gross post-mortem examination of one pup revealed pulmonary oedema, petechial haemorrhages on the kidney, and gas-filled intestines. Histology confirmed the classic lesions of **canine herpesvirus type 1**, including necrotising bronchopneumonia, multifocal necrotising hepatitis, fibrinosuppurative splenitis and necrotising and haemorrhagic interstitial nephritis. Pneumocytes, hepatocytes and occasional renal and splenic endothelial cells contained eosinophilic intranuclear inclusion bodies.

All two-month-old puppies in a litter of crossbred dogs from Auckland had diarrhoea. They seemed to improve after a course of antibiotics, but one had persistent intermittent diarrhoea with haematochezia. On faecal testing there was a growth of ***Campylobacter jejuni*** and also a faecal antigen ELISA for ***Giardia*** spp. was positive. There were moderate numbers of coccidial oocysts, suggestive of **mixed intestinal parasitism** and possible **camplyobacteriosis**.

A nine-year-old Huntaway bitch in the Whanganui region was presented for veterinary attention after sudden onset of lethargy, anorexia and pyrexia. She was mildly azotaemic, with serum creatinine of 164 µmol/L (reference range 40–109) and urea of 22 mmol/L (reference range 2.5–9.0). She was seronegative to *Leptospira interrogans* serovars Pomona and Copenhageni and *L. borgpetersenii* serovar Hardjo. Treatment with antibiotics was started. Four days later the azotaemia was more severe, with serum creatinine elevated to 300 µmol/L and urea to 40.6 mmol/L. Serum samples tested negative at this time for leptospires by PCR. Microscopic agglutination titres for *L.* Pomona were negative but there was an antibody titre of 1:200 to *L.* Hardjo and 1:50 to *L.* Copenhageni. Seven days after the first blood sample, the azotaemia was decreasing as creatinine had fallen to 115 µmol/L and urea 14.7 mmol/L. The *L.* Pomona titre was now 1:800, *L.* Hardjo 1:400 and *L.* Copenhageni 1:200. Ten days after initial presentation (following intensive fluid therapy and antibiotic treatment) the dog was clinically normal and her titre to *L.* Pomona had risen to 1:51 200, while the *L.* Hardjo titre remained at 1:400 and *L.* Copenhageni at 1:200. This was therefore a ***Leptospira interrogans* serovar Pomona** infection. It has been noted that sometimes, early in infection, titres to non-infecting serovars may be higher than for the actual infecting serovar (paradoxical response) (Sykes *et al.*, 2011). This case demonstrates that, depending on the stage of infection, multiple tests and screening over time are needed to diagnose leptospirosis.

CERVINE

A deer farmer from the lower central North Island reported seven sudden deaths among a group of 500 rising-one-year-old male weaners. The deaths occurred over a period of two weeks. Large colonies of fine bacterial rods were seen in histological sections of the ileum. These colonies were associated with inflammation. Culture of the faeces produced a heavy growth of ***Yersinia pseudotuberculosis***, confirming a clinical suspicion of **yersiniosis.** In addition, liver copper levels were found to be depleted (71 µmol/kg; reference range 100–2000). Liver selenium levels were normal and no strongyle eggs were detected on faecal egg count.

FELINE

A four-month-old Burmese kitten from Auckland rapidly developed diarrhoea with mucous, anorexia and third eyelid prolapse. A faecal sample was negative for *Campylobacter* and *Salmonella* spp. on culture, but was positive by ELISA for *Giardia* spp. antigen and positive by PCR for *Tritrichomonas foetus*, suggesting combined **giardiasis and tritrichomoniasis**.

PORCINE

Three 14-week-old piglets from the same litter in a Rotorua backyard pig-raising operation died with chronic ill-thrift and diarrhoea. At post-mortem there was marked thickening of the distal small intestine and colon, with haemorrhage and necrosis of the spiral colon. Histopathology of the intestine found necrotising enteritis and colitis with botryoid inclusion bodies in macrophages of the Peyer's patches. Silver stains identified helical bacteria in the ulcerated lesions on the intestine surface, consistent with ***Brachyspira* spp**. infection complicated by immunosuppression induced by **porcine circovirus type 2**.

EQUINE

A South Canterbury property had five horses abort in three days. Grossly, the aborted foals had oedematous lungs. Histological examination revealed intranuclear inclusions in bronchiolar epithelial cells, thymic epithelial cells and hepatocytes, typical of the lesions seen in **equine herpesvirus abortion.**

Blood was received from a seven-year-old Thoroughbred mare in the Gisborne region. This horse was recumbent and did not want to get up, although eventually the owners got her to her feet. Clinically she showed few signs, although she was lethargic and wanted to rest her head and hold it down all the time. There was very little pasture in the paddock, which was dominated by weeds including oxalis and mallow, and the mare was eating as much

mallow as grass. The horses were being supplemented with hay. Two horses had been euthanased during the previous week: one for atypical colic-like signs and the other for tetanus-like signs.

This horse had a stress leucogram and marked increases in creatinine kinase (CK) at 40 410 IU/L (reference range 169–628) and aspartate transaminase (AST) at 2523 IU/L (reference range 219–473), indicating severe myopathy or myonecrosis. Globulin was elevated, at 44 g/L (reference range 20–39) and bilirubin was mildly increased at 58 µmol/L (reference range 17–41), which was likely due to a functional cholestasis. Two weeks later, after the horse was moved to grazing without weeds, the abnormal clinical signs disappeared and the CK returned to normal. Serum AST concentrations had decreased to 1465 IU/L. Serum ST concentration rises after serum CK and takes longer to decrease.

Sycamore seed poisoning was one differential, but the practitioner did not think this was likely. It was also suggested to look for sycamore seeds in the hay supplement, but none were seen.

There are reports of mallow toxicity caused by small-flowered mallow (*Malva parviflora*): (www.herbiguide.com.au/Descriptions/hg_Smallflowered_Mallow.htm, www.weeds.mangrovemountain.net/.../Malva%20parviflora%20-%20Ma). Mallow is reported to have toxic concentrations of nitrate, but a test on this horse's serum was negative. Mallow is toxic to horses, sheep, cattle and poultry. Clinical signs of toxicity include muscle weakness and staggering gait, trembling muscles when forced to exercise, and increased heart and respiratory rates. Other signs include staggers, stiff action of back legs, back arched, head stretched forward, knuckling-over of front legs and sitting with the head turned into the body. The animals relapse and recover in cycles and most recover when removed from access to the plant. Given the history and the fact that the veterinarian saw this horse eating mallow, mallow toxicity was very likely.

NON-POULTRY AVIAN

A seven-year-old female Sun Conure parrot (*Aratinga solstitialis*) in a mixed aviary in Hamilton was found dead with no history of illness. Necropsy and histopathology revealed a significant burden of ***Capillaria* spp.** in the intestinal tract, accompanied by multiple granulomas in the small intestinal lamina propria. Zeihl-Neelson staining showed abundant acid-fast bacteria in the granulomas. PCR analysis on formalin-fixed paraffin-embedded intestinal tissue detected ***Mycobacterium avium* ssp. *avium*,** confirming a diagnosis of **avian mycobacteriosis.**

Recently purchased canaries in a Southland aviary started dying during a period of very cold weather. Over a three-week period 17 birds died. The affected birds were either found dead on the bottom of the aviary or found sick, dying shortly afterwards. Necropsy of two dead birds revealed swollen livers, from which a heavy pure growth of ***Yersinia pseudotuberculosis*** was isolated.

NEW ZEALAND VETERINARY PATHOLOGY

BOVINE

The owner of a group of 15 two-year-old cattle in the Kaipara area fed the animals about 50 kg of kumara each. Some of the tubers were observed to be mouldy. Three days later one animal died and eight more exhibited dyspnoea with an elevated temperature. Histology revealed a marked diffuse interstitial pneumonia with prominent type II pneumocyte hyperplasia. **Mouldy sweet potato toxicity** was diagnosed. The toxic principle in mouldy sweet potatoes is 4-ipomeanol, produced when *Fusarium* spp. colonise the tubers.

A cow in the Kaipara area produced a dead calf and the next day developed mastitis in at least two quarters, accompanied by clinical signs of endotoxaemia. Milk culture produced a heavy growth of ***Pseudomonas aeruginosa***, which was resistant to penicillins, cephalosporins, tetracyclines and macrolide antibiotics. Previously the farm had experienced an outbreak of *Pseudomonas* mastitis following dry-cow treatment with cephalonium.

In the Rodney district, nine calves died out of a mob of 80 aged two to three weeks, with a further 10 exhibiting diarrhoea and depression. Histology revealed the presence of a moderate enteritis. ***Salmonella* Typhimurium** was isolated from the mesenteric lymph node of one animal.

A one-year-old heifer grazing new grass in the Waikato appeared weak and then went down, with brownish-coloured mucus membranes. The heifer responded to treatment with new methylene blue. The serum nitrate level was 250 mg/L (toxic level > 25), consistent with **nitrate toxicity**.

Two one-year-old calves in the western Bay of Plenty developed round lesions on the feet and lower limbs. The skin developed flakes and scabs, with haemorrhage when the scab was picked off. Fungal culture of the lesions yielded ***Trichophyton rubrum***.

A group of one-year-old calves in the Franklin district exhibited weight loss, ill-thrift and scour, with pneumonia in some animals. Faecal samples from three animals were submitted for culture and ***Yersinia pseudotuberculosis*** was isolated from two of these samples. **Yersiniosis** was diagnosed.

One animal out of a group of 100 ten-month-old calves in Taupo died suddenly. A severe haemorrhagic enteritis was visible at postmortem. Histology revealed an acute haemorrhagic enteritis with intranuclear inclusion bodies typical of **bovine adenovirus** infection.

In a similar case in Rotorua, a yearling died suddenly. Histology was submitted and examination revealed a severe acute haemorrhagic enteritis and abomasitis, with intranuclear inclusion bodies typical of **bovine adenovirus** infection.

A mature cow in Otorohanga that had calved two weeks previously, presented with fever, scour, dehydration and poor body condition. Faecal culture revealed the presence of ***Salmonella* Typhimurium**.

Tissues from a 10-year-old cow that had been slaughtered at home in Whangarei were submitted to a veterinarian because there were multiple large cysts present through the carcass, grossly resembling parasitic cysts. Histological examination of these structures revealed that they were spindle cell neoplasms of soft tissue, and **bovine neurofibromatosis** was diagnosed. This is an asymptomatic disease typically noted at slaughter in aged animals.

In Opotiki, 10 out of a group of 30 Jersey calves less than four weeks of age developed diarrhoea. Six died. The calves were kept out in a paddock and appeared

to be scouring. Antigen ELISA testing on faeces was positive for **rotavirus**. This type of presentation was common this season. In some cases **cryptosporidium** was also present.

Two old dairy cows in in the Thames/Coromandel region presented with red urine, jaundice and brown mucous membranes. Haematology revealed a marked haemolytic anaemia but no evidence of *Theileria* spp. Both animals had a marked hypophosphataemia. **Haemolytic anaemia caused by severe hypophosphatemia** was diagnosed.

In Taupo, faeces from five calves under four weeks of age with marked diarrhoea was submitted for testing. One of the calves had a positive faecal antigen ELISA for **coronavirus**. Testing for rotavirus, cryptosporidia and *Salmonella* was negative on all of the other samples tested. **Coronaviral diarrhoea** was diagnosed.

Also in Taupo, faeces from seven calves under four weeks of age with marked diarrhoea, fever, and dehydration were submitted for culture and faecal testing. ***Salmonella* Bovismorbificans** was isolated from five of the animals and three were also positive for rotavirus on antigen ELISA testing. **Enteric Salmonellosis** complicated by **rotavirus infection** was diagnosed.

A property in Hamilton had problems with calves scouring shortly after birth. An antigen ELISA for K99 performed on the faeces of one calf revealed the presence of **enterotoxigenic *Escherichia coli*** (with K99 pilus antigen).

Another property in Taupo had severe diarrhoea and deaths in animals less than a week old. Five calves died over one weekend. Culture from these animals revealed ***Salmonella* Bovismorbificans**. Several animals were also antigen-positive for **rotavirus** in the faeces, and several also had **cryptosporidium** visible in the faeces.

A Waikato property submitted samples from three five-day-old calves with milky-yellow scours. ***Salmonella* Kottbus** was isolated as the cause of the diarrhoea. Testing for **rotavirus** (antigen ELISA) and **cryptosporidium** (microscopy with modified acid-fast stain) showed that these agents were also present.

Several heifers on a dairy property in Invercargill developed acute lameness with multiple humoral fractures. Liver from one of the animals that had been euthanased was submitted for copper analysis. Copper levels were < 50 umol/kg (reference range 95–2000), indicating that copper stores were severely depleted. While this type of presentation may be associated with **copper deficiency**, affected heifers often also have a history of more generalised undernutrition in the previous year, which could have affected the density of developing bones.

A group of 50 heifers in south Waikato were treated with selenium by injection into the rump. Forty animals developed severe swelling with crepitus over the injection site. Culture of fluid aspirated from the injection site of one of the animals revealed ***Clostridium perfringens***. The animals were given a booster of 5-in-1 clostridial vaccine in an effort to prevent mortalities.

Five young animals died among a group of 100 in the Waikato (age not stated) after exhibiting blindness and nervous signs. BVD testing was negative. Histological examination revealed cerebrocortical oedema with scattered haemorrhages within the neuropil. Serum lead testing revealed a value of 2 mg/L (toxic level 0.35), consistent with **lead poisoning**.

A group of 350 dairy calves included 100 affected by severe conjunctivitis and keratitis. Twenty animals died (though deaths may not have been directly attributable to the conjunctivitis). ***Moraxella bovis*** was isolated from the affected calves.

A neonatal calf exhibited marked hydranencephaly and cerebellar hypoplasia on gross post-mortem examination. BVD antigen ELISA and PCR for BVD performed on spleen tissue was positive, indicating that the calf was likely persistently infected with **BVD virus**. BVD antibody testing on the heart blood was also positive, but this may have been due to maternal antibody after colostrum ingestion.

A 26-day-old calf exhibited severe neurological signs with circling, tongue extrusion and abnormal behaviour followed by death. Necropsy revealed a large soft mass in the brain dorsal to the thalamus. Histopathology identified the presence of a mass identified as a probable primitive **neuroecto-dermal tumour**.

CAMELID

A seven-year-old alpaca from the Franklin district presented with a foot abcess lateral to the foot pad. Culture of the abscess revealed a mixed growth of ***Trueperella pyogenes*** and ***Serratia marcesans***.

EQUINE

A two-year-old cob in Invercargill had severe watery diarrhoea and was inappetant. Faeces were submitted for culture, faecal egg count and larval cyathostome counts. Culture revealed the presence of ***Campylobacter jejuni***. Three larval cyathostomes were noted. Haematology was also performed and revealed a marked neutrophilia and a significant increase in fibrinogen. Larval **cyathostomiasis** was considered to be the likely cause of the enteritis, possibly complicated by *Campylobacter jejuni*.

An eight-year-old mare aborted on a Waikato property that had a history of equine herpesviral encephalomyelitis. The fetus had multifocal necrosis in the liver, lung and spleen with intranuclear inclusion bodies consistent with **equine herpesvirus infection**.

A yearling gelding in South Auckland suffered chronic diarrhoea and weight loss. Serum amyloid A testing and haematology were within normal limits, but larval cyathostomes were present in the faeces. **Larval cyathostomiasis** was diagnosed.

CAPRINE

Three six-week-old goat kids died suddenly on a property in the Waikato. Necropsy revealed that all had an **acute fibrinous bronchopneumonia**. Some of the animals also had evidence of peritonitis and sepsis. ***Pasteurella multocida*** is the most common cause of this type of lesion in goats.

A property with 500 goats in South Auckland experienced an outbreak of pneumonia. Ten animals died and 20 more appeared sick. Gross necropsy revealed the presence of an acute fibrinous bronchopneumonia. Culture of the affected lung grew ***Mannheimia haemolytica*** and ***Escherichia coli***.

A property with 70 dairy goats had two animals die after a period of recumbency one week after kidding. The animals appeared weak and comatose. Histology revealed a **lymphohistiocytic meningoencephalitis**

with microabcesses typical of listeriosis. There was also evidence of **bronchopneumonia** with secondary **septicaemia**.

OVINE

Ten out of 400 two-tooth sheep in the Waitaki district aborted. Fetuses of four animals were necropsied and culture of abomasal contents of all these animals yielded a growth of ***Campylobacter fetus* ssp. *fetus***.

PORCINE

One eight-week-old piglet out of a litter of nine in Whanganui was submitted for necropsy. The litter had had a high mortality rate, with four piglets having died during the previous month. Histology on the lung revealed a marked bronchopneumonia with evidence of infection by ***Metastrongylus apri***, but there was an absence of concurrent bacterial pneumonia. The lungworm infection may not have been the cause of death in this case.

FISH

A captive blue maomao (*Scorpis violacea*) from an aquarium in Auckland was submitted for necropsy and histological examination. Histology revealed numerous small granulomas affecting the heart, spleen, kidney and abdominal fat. The liver had moderate fatty change. Acid-fast staining of the granulomas revealed that they contained numerous short acid-fast bacilli. **Disseminated mycobacteriosis** was diagnosed. Mycobacteriosis in marine species is most frequently caused by *Mycobacterium marinum*, *M. fortuitum* or *M. cheloniae*.

POULTRY

A flock of broiler breeder chickens in the Auckland region experienced three days of high mortalities. Mouth ulcers and tongue abnormalities were observed on post-mortem examination, accompanied by hepatomegaly and internal haemorrhage. Histology revealed an acute ulcerative heterophilic laryngotracheitis, with intranuclear inclusion bodies and the formation of syncytial cells, consistent with infectious laryngotracheitis virus infection. The ulcerated areas of the oral cavity and the tongue were colonised by large colonies of mixed bacteria. One animal also had a multifocal necrotising hepatitis with intralesional bacteria. **Infectious laryngotracheitis virus** infection was diagnosed, accompanied by complicating ulcerative stomatitis or glossitis with superimposed bacterial infection. Some animals also had evidence of **bacterial septicaemia**.

Another flock of 38 day-old chickens in the Auckland region presented with suspect **infectious laryngotracheitis**. Histology showed viral lesions similar to those described above, confirming the presence of the disease.

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QUARTERLY REPORT OF INVESTIGATIONS OF SUSPECTED EXOTIC DISEASES

Exotic disease investigations are managed and reported by MPI Investigation and Diagnostic Centre (IDC) and Response, Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from July to September 2014.

EXOTIC VESICULAR DISEASES RULED OUT

A veterinarian called the MPI exotic pest and disease hotline after examining a dry cow with pyrexia and a large erosive lesion (about 3 cm) on the hard palate. An initial investigating veterinarian (IIV) visited the property, inspected the herd and clinically examined the affected cow. The cow had gradually worsened over the previous 7–10 days with loss of condition and was now pyrexic, frequently teeth-grinding and a staring coat. Additional mouth lesions included smaller (< 1 cm) round ulcerated areas further rostral on the hard palate. The remainder of the mouth and tongue were clinically normal, as were the feet, udder and vulva. Exotic vesicular disease was excluded on clinical and epidemiological grounds. Serum biochemistry was unremarkable, antigen and antibody ELISA tests for bovine virus diarrhoea gave negative results and a molecular assay for malignant catarrhal fever was also negative. No presumptive aetiology for the condition was identified. The cow received routine antibiotic therapy, recovered uneventfully and the investigation was stood down.

A veterinarian called the MPI exotic pest and disease hotline after examining a mob of 7–10-month-old Hereford steers and heifers in which some were pyrexic with erythema and crusting involving the nasal planum and lips. An IIV was despatched to the property and inspected the herd and clinically examined any affected cattle. The IIV reported that in addition to the lip and nasal lesions, three animals had erosive lesions affecting the rostral portion of the tongue. There were no other mouth lesions in these animals. Two heifers also had periorbital and vulval oedema, mild icteric mucous membranes and a mild dermatitis affecting the pastern area of some feet. Exotic vesicular disease was excluded on clinical and epidemiological grounds. Blood samples were collected to help establish an endemic diagnosis. Haematology of affected animals indicated an inflammatory leucogram, and serum biochemistry identified raised levels of bilirubin, serum glutamate dehydrogenase, aspartate transaminase and gamma-glutamyl transferase, indicative of moderate hepatocellular damage with cholestasis. Given the inflammatory leucograms, leptospirosis was excluded after negative titres for *Leptospira* Hardjo, Bovis and Copenhageni in the microscopic agglutination test. A presumptive diagnosis of hepatogenous (secondary) photosensitivity was made and the investigation was stood down.

ANTHRAX RULED OUT

A veterinarian called MPI's exotic pest and disease hotline to report sudden death in heifers. This presentation is consistent with the exotic disease anthrax. The heifers were part of a mob of about 150 on dairy grazing in South Canterbury. Within three days two heifers died acutely and two others aborted. The two dead heifers were reported to have frank blood coming from the nostrils and vulva, with further bleeding around the eyes. Testing to rule out anthrax was undertaken prior to more extensive sampling of the heifers or aborted fetuses. Blood from both dead heifers was sent to MPI's Animal Health Laboratory, where tissues were processed in the Physical Containment 3 laboratory. Multiple stained blood smears were negative for bacterial rods resembling *Bacillus anthracis*. Following the exclusion of anthrax, endemic differential diagnoses were considered, including clostridial infection, salmonellosis and other bacteraemia, and toxicosis (e.g., nitrate poisoning). Clostridial disease was thought to be possible, since the animals had been vaccinated twice in 2012 against five strains of clostridia but not vaccinated since. Aqueous humour tested by the veterinarian was negative for nitrates, making nitrate toxicosis less likely. Serum from the aborting heifers was negative for antibodies to *Leptospira* spp., bovine viral diarrhoea virus and *Neospora caninum*. Placenta examined by histopathology had lesions consistent with necrotising placentitis, with intralesional Gram-negative coccobacilli. Culture of fetal stomach contents grew *Salmonella* Brandenburg and a heavy growth of *Corynebacterium* sp. The *Corynebacterium* was thought to be a contaminant and it was considered that the placentitis was caused by *Salmonella* Brandenburg.

Salmonellosis in cattle can cause both abortion and sudden death, and is most common in the winter months, when stock are crowded. In other countries, cattle salmonellosis is often caused by *Salmonella* Dublin, which is exotic to New Zealand. *Salmonella* Typhimurium is considered to be the most common cause of cattle salmonellosis in New Zealand. However, in the past two decades *Salmonella* Brandenburg has emerged as an important cause of death and diarrhoea in both sheep and cattle in the South Island (Clark *et al*., 2004). The cause of death and abortion in this case was considered to be *Salmonella* Brandenburg infection and the investigation was stood down.

EXOTIC *LEPTOSPIRA* SEROVARS EXCLUDED

An MPI scientist notified the Incursion Investigation Team after a serum test required for export of bovine semen returned a positive titre for *Leptospira* Canicola. The bull in question had no history of travel outside of New Zealand and had been vaccinated frequently for leptospirosis. The original microscopic agglutination test was only for *L.* Canicola and returned a low positive. Subsequently, serum was tested for several other *Leptospira* serovars and was highest equal for *L.* Grippotyphosa, *L.* Mozdok and *L.* Pomona (all 1:800). The pattern was thought to be consistent with high-intensity vaccination for several *Leptospira* serovars.

L. Grippotyphosa and *L.* Mozdok are not present in New Zealand, but Grippotyphosa is commonly used in vaccines, and Mozdok is known to antigenically seroreact with Pomona. The exotic *L.* Canicola was excluded and the investigation was stood down.

ANAPLASMOSIS RULED OUT

An MPI scientist informed the Incursion Investigation Team of an alpaca that had tested positive in a cELISA for bovine anaplasmosis. The alpaca was an eight-month-old female that had been tested along with 11 other young alpaca as part of routine pre-export testing. All animals had been born on the same farm in New Zealand and there were no significant health events or concerns with this animal or relating to the farm. The remainder of the consignment had tested negative in the *Anaplasma* ELISA. Repeat serum and whole blood samples were collected and submitted to the AHL for further testing. The repeat serum sample was negative in the *Anaplasma* cELISA and the whole blood sample gave a negative result in the *Anaplasma* sp. PCR. Exotic disease was excluded and the investigation was stood down.

EIA/EVA RULED OUT

A veterinary pathologist contacted MPI about an 11-year-old Clydesdale gelding horse with possible haemolytic anaemia, marked systemic inflammation and loss of condition for two weeks. Exotic disease rule-outs included equine viral arteritis (EVA) and equine infectious anaemia (EIA). The horse also had markedly elevated muscle leakage enzymes (CK 44 665 IU/L; normal range 63–469). Endemic differentials included autoimmune and paraneoplastic conditions. Serum samples were negative for EVA by virus neutralisation test and for EIA by agar-gel immunodiffusion. Exotic diseases were ruled out and the investigation was stood down.

BRUCELLOSIS EXCLUDED

An MPI Animal Health Laboratory scientist informed the duty Incursion Investigator of an alpaca that had tested positive in a cELISA for *Brucella* sp. The alpaca was a seven-month-old female that had been tested along with 11 other young alpacas as part of routine pre-export testing. All animals had been born on the same farm in New Zealand and there were no significant health events or concerns. The remainder of the consignment had tested negative in the *Brucella* ELISA. Repeat serum, whole blood and a faecal sample were collected and submitted to the AHL for further testing. The cELISA was positive at the repeat sampling. A Western Blot assay for *Brucella abortus*, *B. mellitensis* and *Yersinia* gave a negative result for *Brucella* but developed a band consistent with a positive result for *Yersinia enterocolitica*. The whole-blood sample was negative by PCR for *Brucella* species and *Y. enterocolitica* serotype 0:9 was cultured from the faeces. These tests enabled us to conclude that *Yersinia* was the likely cause of the serum reactors identified in the cELISA assay. *Y. enterocolitica* Serotype 0:9 shares common antigens with *Brucella* and is well recognised as a cause of false positive cross-reactions in serological tests for *Brucella*. Exotic disease was excluded and the investigation was stood down.

CANINE DISTEMPER RULED OUT

A veterinarian called the MPI exotic pest and disease hotline to report a sick two-year-old Rottweiler dog with a history of having received only part of its primary vaccination course. The dog presented with clinical signs consistent with a potential aetiology of distemper, including anorexia, pyrexia, bilateral nasal discharge and conjunctivitis. The dog had also been vomiting. Blood and nasal swab samples were collected and submitted to the MPI AHL. A virus neutralisation test carried out on the initial serum sample was positive for antibodies to canine distemper virus. PCR tests on whole blood and the nasal swab sample were negative for distemper virus. Unfortunately a convalescent serum sample was not collected as the dog recovered uneventfully over the following few days and the owners did not return to the veterinary clinic. In light of the dog's rapid recovery and the negative molecular findings for distemper, the involvement of distemper virus was excluded and the investigation was stood down.

AVIAN PNEUMOVIRUS RULED OUT

An MPI scientist contacted the Incursion Investigation Team to report elevated avian pneumovirus ELISA titres in Cobb laying hens undergoing routine egg pre-export testing. Preliminary testing of 90 serum samples had been performed, with 33 returning a positive result. The pre-export test used was a common commercial test which had previously given false positive results (Bingham, 2007a, 2007b). In this case, positive serum was retested for three serovars of avian pneumovirus at National Veterinary Services Laboratories (NVSL) in Ames, Iowa and returned a single positive result. This positive sample was retested by NVSL using a confirmatory ("gold standard") IFAT, with negative results. The investigation has been closed. Pre-export testing protocols are being reviewed to address the issue of apparent poor specificity in the current test.

AVIAN INFLUENZA AND NEWCASTLE DISEASE EXCLUDED

A veterinarian called the MPI exotic pest and disease hotline to report three female Indian runner ducks that were lethargic, with limb weakness and diarrhoea. Two of the ducks also had periorbital oedema and a clear ocular discharge. The ducks were from a smallholder property where one, a drake, remained unaffected. The worst-affected duck was euthanased and submitted to MPI's Animal Health Laboratory (Wallaceville) for postmortem and laboratory testing for exclusion of exotic diseases. Subsequently a further duck was euthanased on humane grounds and underwent post-mortem examination. Both ducks were in thin body condition and one had substantial dark green staining surrounding the vent. Gross postmortem findings were otherwise unremarkable. Histological findings included marked dysplasia and degeneration of the koilin layer of the gizzard in one bird, and large amounts of haemosiderin within the Kupffer cells of the liver in both birds. Sections of lung, trachea, heart, kidney, oesophagus, proventriculus, pancreas and brain showed no obvious abnormalities. Haemorrhage and inflammatory lesions in key target organs for exotic viral diseases (proventriculus, caecal tonsils and intestinal Peyer's patches) were absent. Faecal and visceral tissue samples from each bird were negative for the causative agent of Newcastle disease (Avian paramyxovirus type 1) and avian influenza (influenza A) by

TaqMan RT-PCR. General bacterial culture identified no significant bacterial colonies and was negative for *Pasteurella* spp. A PCR for botulinum toxin gave negative results. Histological lesions and laboratory findings were not consistent with an infectious or toxic aetiology, and pointed towards the possible involvement of feed or husbandry-related factors. The drake remained unaffected throughout the episode and the remaining affected bird recovered after antibiotic therapy and supportive care. The investigation was stood down after excluding exotic diseases.

MYCOBACTERIUM AVIUM SSP. *HOMINISSUIS* CONFIRMED

An avian veterinarian phoned MPI to report the isolation of *Mycobacterium avium* ssp. *hominissuis* in a single lorikeet from a public aviary in the central North Island. An autopsy showed multiple intracoelomic caseous nodules and histopathology showed that acid-fast bacteria were present in the lesions. These changes are characteristic of avian tuberculosis, which is typically caused by *M. avium* ssp. *avium*. The subsequent isolation of *M. avium* ssp. *hominissuis* was unusual as it has only very rarely been reported in birds. The identification was confirmed by sequencing of the hsp65 gene by ESR.

An investigation was undertaken to ascertain the status of *M. avium* ssp. *hominissuis* in New Zealand, and to help liaise between the clinical veterinarian and the local Medical Officer of Health for the purpose of evaluating the public health risk. *M. avium* ssp. *hominissuis* is primarily a pathogen of pigs and humans, and is thought to be acquired from the soil. The agent has been reported previously in New Zealand from a deer sample (Turenne *et al.*, 2008). It appears to be rare in birds as we could find only one report in the literature (Shitaye *et al.*, 2009).

The local Medical Officer of Health was notified of the small but potential human health risk to aviary workers and was put in touch with the notifying veterinarian for follow-up. A history of the aviary indicated that the birds were of mixed origins, with native and non-native birds kept separately. Two other birds had died recently although tuberculotic lesions were not present in those birds. After it was determined that the pathogen was endemic, and once public health concerns had been addressed, the investigation was stood down.

EXOTIC TICKS INVESTIGATED

The MPI Live Animal Imports Team called the MPI exotic pest and disease hotline to report that two dogs shipped from Singapore were infested with large numbers of *Rhipicephalus sanguinius* (brown dog ticks), which are exotic to New Zealand. There was concern that personal effects of the family concerned might bring exotic ticks into New Zealand. An interview with the owner established that the large-breed dogs had been kept outside in a fenced yard in Singapore, with no access indoors. No dog bedding, collars, leads or toys had been shipped. Household effects in transit included mainly toys, clothing and kitchen utensils, but no furniture or bedding. While in quarantine, the ticks were removed, the dogs were treated for ticks, tested for infectious tick-borne disease such as *Babesia canis*, and all cage bedding was marked for destruction. The risk of exotic ticks being transported into New Zealand on the household items was judged to be low and the investigation was stood down.

An MPI animal imports veterinarian contacted the Incursion Investigation Team to report illness in a nine-year-old intact male Labrador dog imported from Singapore the previous week. During the quarantine period the dog became acutely ill, with clinical signs including tachycardia, palor, dyspnoea and hypothermia. Both dogs in the household had harboured multiple ticks when imported and tick-borne illness such as babesiosis was considered possible. Acaricide treatment was initiated to ensure the dogs would comply with import health standards by the time they exited quarantine. The ticks were identified as *Rhipicephalus sanguineus* (brown dog ticks), which are important vectors of disease. Blood samples were taken for serology and antigen detection of multiple tick-borne agents, including *Babesia gibsoni*, *Theileria* spp., *Borrelia burgdorferii* (the agent of Lyme disease), *Anaplasma* spp. and *Ehrlichia* spp. The dog was also moderately anaemic (PCV 0.25; reference range 0.37–0.55). Unfortunately the dog deteriorated and died within two days of illness onset. A post-mortem examination showed evidence of chronic right-sided heart failure, including a globoid heart with a markedly dilated right ventricle, and systemic changes indicative of passive congestion, including hepatomegaly, ascites and pleural effusion. Histology was supportive of the gross findings, although myocardial histology was unremarkable and no clues to the cause of the dilation were evident. Laboratory tests were negative for all tick-borne agents, including serology (IFAT) for *Babesia canis*, *B. gibsoni* and *Ehrlichia canis*, and PCR (antigen detection) for *Anaplasma phagocytophilum*, *Babesia* spp., *Borrelia* spp. and *Ehrlichia* spp. The cause of death was chronic congestive heart failure with terminal circulatory collapse. The anaemia was considered most likely due to exsanguination via ticks, with a possible anaemia of chronic disease component. Congestive heart failure is often idiopathic in large-breed dogs. In this case the condition evidently developed undetected while the dog was still in Singapore and may or may not have been exacerbated by the stress of travel. The other dog remained clinically healthy. Exotic disease was ruled out and the investigation was closed.

A pathology laboratory phoned the MPI exotic pest and disease hotline to report that they had received a medical sample comprising a tick embedded in human skin. The tick had been removed surgically from a man just returned from Samoa. The tick was identified by an MPI entomologist as an ixodid tick from the *Amblyomma* genus, and was an exotic species but it could not be identified to the species level. *Amblyomma* ticks are exotic to New Zealand. The patient was made aware of the possibility of exotic tick-borne disease such as Lyme disease and the local Medical Officer of Health was notified. The investigation was stood down.

A human health laboratory scientist phoned the MPI exotic pest and disease hotline to report receiving a tick specimen believed to be from overseas. The tick had been removed from a 69-year-old man recently returned from Queensland, Australia. The patient had noticed a tag-like structure near his armpit following a trip to the Sunshine Coast. He had attempted to remove it himself, but failed. Upon his return to New Zealand 10 days later he saw his physician, who removed the tick. Molecular identification by the MPI Plant

Health and Environment Laboratory indicated the tick was *Ixodes holocyclus*, the Australian paralysis tick. The tick was negative on PCR for the agent of Lyme disease (*Borrelia burgdorferii*). The Australian paralysis tick is thought to transmit several other human diseases which were not tested, including Rickettsial spotted fever (caused by *Rickettsia australis*); a Lyme-disease-like illness whose causative agent is not known; and rarely, the bacterium *Coxiella burnetti* (the agent of Q fever). The physician and Medical Officer of Health were informed and the physician reported that the patient was well. This represents the interception of an exotic tick, where the risk was ameliorated by the death of the tick. The investigation was closed.

MONKEY BITE INVESTIGATED

An infectious diseases physician rang the MPI exotic pest and disease hotline to report suspected herpes B infection in a person bitten by a monkey. The bite victim was a young woman in good health who had been bitten two days prior by an apparently healthy capuchin monkey *(Cebus cabucinus)* in an MPI-approved Transitional Facility. The bite site came to the attention of the physician when it became swollen, blistered and numb. These can all be signs of infection with herpes B, although the timing was earlier than would typically be expected. The capuchin monkey was one of a group of four, and was initially thought to have been exposed to a pig-tailed macaque *(Macaca nemestrina)* housed at the same facility. Macaques carry herpes B (aka *Cercopithecine Herpesvirus*, *Macacine Herpesvirus*), which is subclinical in that species but potentially fatal in humans. Capuchin monkeys are usually susceptible to the disease and show severe clinical signs, but have rarely been reported to carry the virus asymptomatically (Coulibaly *et al.*, 2004). During the investigation the facility manager was able to confirm that there had been no contact whatsoever between the macaque and capuchin monkeys, including during their time at a previous facility. Testing of the capuchin monkey for herpes B was considered but not carried out owing to factors such as the anaesthetic risk for the animal and the negligible impact of any test result on the bite victim's treatment protocol. The bite wound infection resolved after antibiotic treatment and the patient was placed on prophylactic doses of acyclovir antiviral therapy. At last report the patient was clinically healthy. Herpes B is almost ubiquitously found in healthy macaques, where it remains latent until reactivated during times of stress. This is almost identical to the behaviour of the human herpes simplex viruses 1 and 2 (HSV1 and 2), which are the causes of human cold sores. Because herpes B is common in macaques, it is extremely likely that the virus is present in New Zealand. Import Health Standards for macaques do not cover these endemic viruses and it seems likely that herpes B has never been tested for in New Zealand's macaque population. As the disease risk in the bite victim was minimal, and since Herpes B is not a biosecurity risk for New Zealand's native or agricultural animals, this investigation was closed.

TRACHEAL MITE AND EFB EXCLUDED

A hobbyist beekeeper phoned the MPI exotic pest and disease hotline to report a single hive that had died out within the past month. A further hive on the property appeared to still be healthy. An AsureQuality Apiary Advisory Officer (AAO) inspected the hives and collected appropriate samples. Both hives had been under treatment for varroa for the previous seven weeks. The dead hive had what appeared to be a white fungal growth affecting much of the brood. The appearance was not typical of chalkbrood, an endemic syndrome caused by *Ascosphaera apis*, a spore-forming fungus. On inspection, the other hive was strong, although there was evidence of parasitic mite syndrome and indications of high viral loads as some emergent bees had deformed wings that were likely the result of infection by deformed wing virus. The AAO collected samples for submission to the MPI Animal Health Laboratory for exclusion of exotic disease. Tracheal mite dissection and external mite washings were negative for exotic mites. There were a few phorid larvae and adult rover beetles in the sample, as expected in any collapsing hive. Molecular testing for European foul brood (EFB) and *Nosema ceranae* were both negative. Fungal culture identified various non-pathogenic *Penicillium* fungi including *P. corylophilum* and *P. olsonii*. Exotic disease was excluded and the investigation was stood down.

RISK GOODS INVESTIGATED

After visiting an Asian supermarket in Christchurch, an MPI biosecurity inspector called MPI's exotic pest and disease hotline to report duck eggs of Australian origin, pork rind chips of Philippine origin and suspected Korean frozen pork dumplings for sale. A biosecurity inspector revisited the premises to determine the origin and importation route of these products. Three importers' premises supplying the supermarket were subsequently visited and the manufacturers' declarations and importation paperwork assessed. Further investigation revealed that the pork dumplings were in fact made in Christchurch but packaged to mimic a genuine Korean product. The other two products were legally imported and complied with all relevant import health standards.

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Paul Bingham
Team Manager
Surveillance and Incursion Investigation (Animals and Marine)
Ministry for Primary Industries
paul.bingham@mpi.govt.nz

QUARTERLY REPORT OF BIOSECURITY RESPONSES

The Response Group was managing 37 high-priority responses and two low-priority responses (i.e., where full responses were not initiated) at the end of the July–September 2014 reporting period. During that time the group initiated five new responses, closed two responses and stood down one low-priority response. (**Figure 1)** shows the number of responses managed during the 12 months up to the end of the current reporting period and (**Figure 2)** shows a breakdown by sector most affected by the organism or risk goods.

The Ministry for Primary Industries (MPI) Response Group sits within the Operations Branch and is responsible for managing the biosecurity risk posed by exotic and emerging pests and diseases detected within New Zealand. Responses are initiated to organisms or risk goods that may affect New Zealand's primary industries or the marine, freshwater or terrestrial environments.

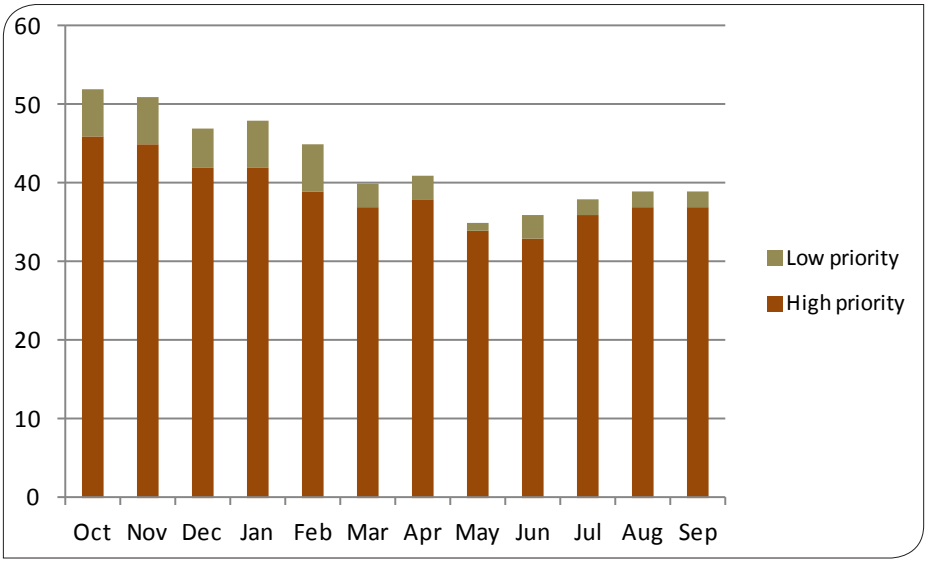

Figure 1: Biosecurity responses for 12 months from October 2013 to September 2014

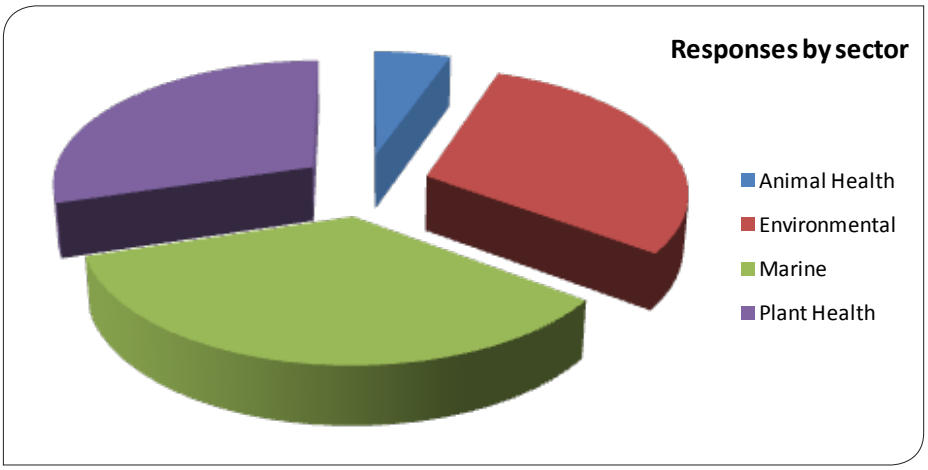

Figure 2: Sector breakdown for responses, July–September 2014

The Response Group also manages the eradication programmes for nine national interest pests that have been in New Zealand for several years (**Table 1**, page 20).

NEW RESPONSES

The following significant responses were initiated during the reporting period.

PERKINSUS OLSENI IN GREEN-LIPPED MUSSELS

In September 2014 the protozoan parasite *Perkinsus olseni* was isolated from a green-lipped mussel (*Perna canaliculus*) taken from an aquaculture facility in the South Island. This was the first time that *P. olseni* had been detected and identified in green-lipped mussels in the South Island.

As *P. olseni* is a Notifiable Organism listed by the World Organisation for Animal Health (OIE), the OIE has been notified because the occurrence was in a new host species.

Investigations suggest that *P. olseni* is present in the mussel populations at very low levels and is causing no significant mortalities or observable ill-effects. MPI is also working with the aquaculture facility to improve the biosecurity standards and prevent the spread of the disease to other susceptible species.

SUBTERRANEAN TERMITES AT OMAHA

Australian subterranean termites were located in a garage in Omaha, Northland, on 5 September 2014. The specimens were identified as

TABLE 1: NATIONAL INTEREST PESTS

RESPONSE NAME	SCIENTIFIC NAME	SECTOR	TYPE OF ORGANISM
Cape tulip	*Moraea flaccida*	Environment	Plant
Hydrilla	*Hydrilla verticillata*	Environment	Plant
Johnson grass	*Sorghum halepense*	Environment	Plant
Manchurian wild rice	*Zizania latifolia*	Environment	Plant
Phragmites	*Phragmites australis*	Environment	Plant
Pyp grass	*Ehrharta villosa*	Environment	Plant
Salvinia	*Salvinia molesta*	Environment	Plant
Water hyacinth	*Eichhornia crassipes*	Environment	Plant
White bryony	*Bryonia cretica* sp. *dioica*	Environment	Plant

Coptotermes acinaciformis. The initial inspection and installation of bait stations were completed in September and a delimiting survey was completed in early November; six infested properties have been identified. The baiting and monitoring programme will run for several years until eradication is assured.

MPI is currently managing eradication programmes at seven different sites for this species of termite, and has successfully eradicated it from three other sites.

Frances Velvin
Senior Adviser
Plants and Environment Response
Ministry for Primary Industries
Frances.Velvin@mpi.govt.nz

INTERCEPTIONS OF REPTILES AND AMPHIBIANS

Lizards and amphibians are highly mobile animals that can easily hitchhike with cargo and people. So unsurprisingly 80 percent of all the interceptions Tony studied were lizards (geckos and skinks), and half of all the lizard interceptions were the common house gecko, *Hemidactylus frenatus*, which is native to the Asian and Indo-Pacific regions. However, interceptions have been made from all parts of the world except Europe (**Figure 1**).

From 2000, herpetologist Tony Whitaker (see obituary, page 22) was contracted by MPI to identify interceptions of lizards, snakes, frogs and other cold-blooded terrestrial animals, and to provide risk-assessment services. During that time he identified 1796 interceptions. An analysis of the data that Tony collected on these reveals some interesting information about exotic lizard and amphibian incursion pathways, and provides a fitting postscript to his life and work.

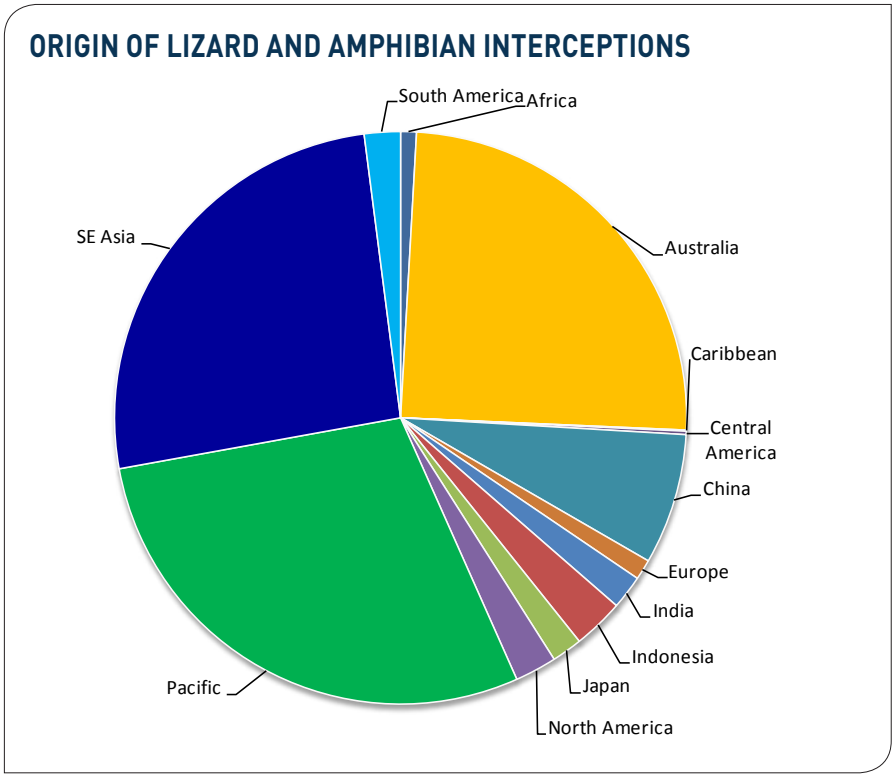

Figure 1: Origins of lizard and amphibian interceptions

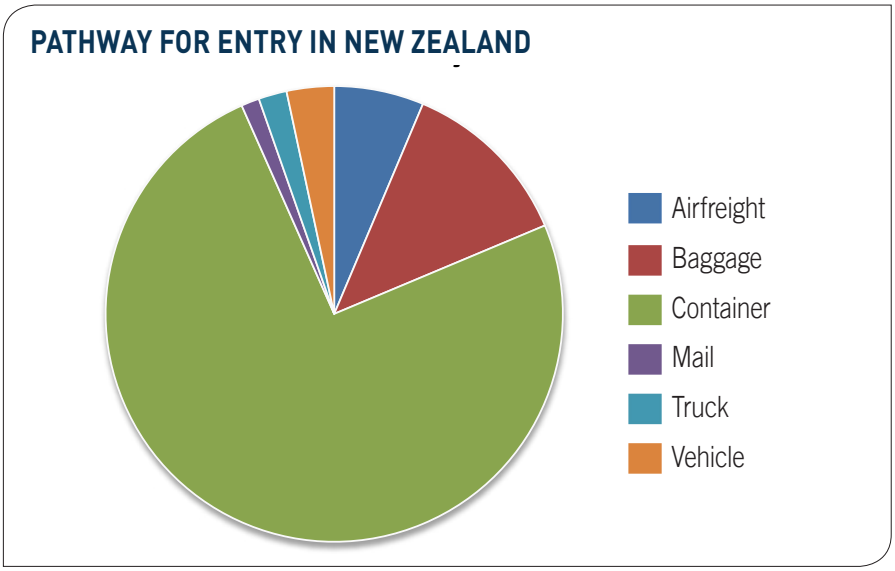

Figure 2: Pathways of entry to New Zealand

Amphibians featured in 13 percent of the interceptions – particularly *Litoria* (tree frogs), *Bufo* (cosmopolitan toads) and *Duttaphrynus* (Asian toads). Amphibians were often associated with vegetable and banana imports. The incidence of frogs associated with bananas can be attributed to the cultivation of large monocultural banana plantations in Central and South America. The tree frogs have adapted to this change, resting and feeding in the bracts and foliage of the plants. They were then accidently collected with the harvesting of bunches of bananas and exported around the world.

Tony's painstaking records show that the majority of interceptions are discovered in shipping container freight (58 percent), air freight (5 percent) and personal baggage (10 percent) (**Figure 2**). While there are records of species arriving from all over the world, the majority originated from the Pacific Islands (38 percent), Southeast Asia (33 percent) and Australia (20 percent). This reflects our common trading partners and popular holiday destinations. Frequently, travellers unwittingly return home with a "souvenir" in the form of a gecko or frog. Almost 20 percent of all post-border lizard or amphibian finds have been associated with personal baggage.

Tony's work was not only valuable for helping to keep unwanted reptiles and amphibians from establishing here. Novel pathogens and parasites travelling with these animals could also threaten our already endangered native lizards, frogs and tuatara. More than a quarter of the lizards intercepted had associated ectoparasites, and the fact that more than three-quarters of interceptions were alive

on arrival highlights the risk and emphasises the importance of people notifying MPI of any hitchhikers they may find on return from overseas.

The data that Tony collected over the last 13 years illustrates the pressure of trade and travel on our biosecurity systems and reinforces the importance of rigorous border clearance systems and biosecurity surveillance. While more than two thirds of the interceptions were made at the border by biosecurity staff, the rest were found post-border, often by the general public. This reinforces the vital role everyone can play in protecting New Zealand's economy, environment and health.

Lisa Maria
Incursion Investigator
Surveillance and Incursion Investigation (Animals and Marine)
Ministry for Primary Industries
lisa.maria@mpi.govt.nz

OBITUARY: TONY WHITAKER (1944–2014)

Recently, Tony Whitaker, a celebrated and highly respected New Zealand herpetologist, passed away. For more than half a century Tony explored the remotest corners of New Zealand and the Pacific, searching for native reptiles and amphibians. He discovered many new lizard species, including one named by a fellow herpetologist in his honour, Whitaker's skink (*Oligosoma whitakeri*).

Tony's contribution to New Zealand conservation and biosecurity was recognised in 2010 when he was appointed a Member of the New Zealand Order of Merit (MNZM) for services to herpetology. Tony was passionate about the animals he studied and always willing to share his time and knowledge. His passing will be a big loss to herpetology and conservation.

Tony Whitaker

Whitaker's skink (*Oligosoma whitakeri*)

QUARTERLY REPORT OF INVESTIGATIONS OF SUSPECTED EXOTIC MARINE AND FRESHWATER PESTS AND DISEASES

Exotic marine pest and aquatic disease investigations are managed and reported by MPI's Investigation and Diagnostic Centre and Response, Wallaceville. The following is a summary of investigations of suspected exotic marine diseases and pests during the period from July to September 2014.

COCKLE MORTALITY INVESTIGATED

A local marine scientist notified MPI of a mortality event occurring in New Zealand cockles (*Austrovenus stutchburyi*) in Whangateau estuary. A mass mortality had occurred previously in the same area in 2009, associated with a coccidian parasite and a *Mycobacterium* species (Bingham, 2009), resulting in about an 80 percent reduction in the population of cockles, and there was concern that this could develop into a similar catastrophic event. Samples were collected from Lews Bay at Whangateau and submitted to the MPI Animal Health Laboratory to rule out exotic diseases. Subsequently another local marine scientist reported cockles dying at Sandspit estuary, just south of Whangateau, and later at Orewa estuary, 25 km south of Sandspit. Cockles were collected and submitted from these two additional locations to see if the mortality had a similar cause. Histopathology conducted on shellfish from all three sites suggested that the cockles had recently spawned and were exhibiting a strong inflammatory response in the form of an influx of haemocytes to phagocytise residual gonadal tissue. Bacterial samples of cockles from Whangateau and Sandspit showed varying levels of mixed growth of environmental organisms, especially *Vibrio* bacteria, while samples from Orewa were infected with both *Vibrio* and *Rickettsia* spp. While the microorganisms found in the cockles in 2014 are different from those found in the 2009 event, the disease process may have been similar. Although no unusual environmental events were reported at the time, spawning combined with other stressors such as warmer than usual temperatures and/or spring tides leaving shellfish exposed for longer at low tide, may have increased the molluscs' susceptibility to opportunistic invasion by what are normally benign microorganisms. Initial observations of the remaining cockle population indicated that the impact was not as severe as in 2009. Exotic diseases were ruled out as cause of the mortality and the investigation was stood down.

OFFSHORE FOULING INVESTIGATED

After a vessel returned from maintenance in Singapore, a dive operator was contracted to perform routine cleaning of the hull. The contractor sent samples of a tubeworm found on the hull to the Marine Invasive Taxonomic Service (MITS). This worm turned out to be *Hydriodes elegans*, a common fouling species often found on vessels entering New Zealand from overseas and considered to be low risk. However, MITS contacted MPI expressing concern that the level of biofouling on this vessel might be of concern, especially given that there are known invasive species in Singapore. The Integrated Targeting and Operations Centre (ITOC) was contacted and the vessel owner was interviewed to find out more about the vessel's maintenance history, especially during its time in Singapore. Photos of the hull and sea chests were requested from the dive contractor. The Risk Analysis team was consulted and the conclusion was that the vessel owner had done everything possible to ensure minimal fouling on the vessel and it was deemed a negligible risk. The investigation was closed with no further action taken.

PERKINSUS IN GREENLIPPED MUSSELS CONFIRMED

During routine monitoring of shellfish health at a landbased aquaculture facility, a pathologist detected what he suspected was the protozoan parasite *Perkinsus* in a greenlipped mussel (*Perna canaliculus*). The pathology slide was referred to MPI's Animal Health Laboratory for second opinion and a *Perkinsus* species was verified by histopathology. *Perkinsus* cannot be typed to species level by histopathology. Both *P. olseni* and *P. marinus* are notifiable diseases and neither has been reported from greenlipped mussels before, so the MPI Surveillance & Incursion Investigation team was notified via the pest and disease hotline and an investigation was initiated. Polymerase chain reaction and molecular sequencing confirmed *P. olseni* and a biosecurity response was initiated. *Perkinsus* has not previously been found in greenlipped mussels. *P. olseni* and *P. marinus* are notifiable diseases and this occurrence in a new host species will require notification to the OIE.

P. olseni is an endemic protozoan parasite that was first confirmed from New Zealand in 2000. It is broadly distributed in four species of bivalves: New Zealand cockles, *Austrovenus stutchburyi* (Veneridae); *Macomona liliana* (Tellinidae); *Barbatia novaezealandiae* (Arcidae) and *Paphies australis* (Mesodesmatidae). Furthermore, in 2013 it was detected for the first time in paua, *Haliotis iris* (Haliotidae). *Perkinsus* had not been reported from shellfish populations south of Auckland. It has been assumed that, as has been observed overseas, *Perkinsus* does better in warmer waters in New Zealand. It has not been detected in the South Island, and no specific controls are in place to prevent its spread.

CARNOBACTERIUM PISCICOLA CONFIRMED

During routine export testing of salmon, the MPI Animal Health Laboratory made an incidental finding of *Carnobacterium piscicola,* an environmental bacterium, some strains of which have been reported to cause disease. *C. piscicola* has not previously been reported from live fish in New Zealand.

There have been prior reports of idiopathic speckled kidneys in New Zealand fish from the AHL. These affected kidneys had

similar histopathological characteristics to lesions occurring with bacterial kidney disease (BKD), an exotic disease in New Zealand caused by *Renibacterium salmoninarum,* an organism that is routinely tested for and excluded as part of the AHL's salmon export testing programme.

This investigation had two aims: to rule out potential contamination from non-fish sources and to test the hypothesis that *C. piscicola* was associated with these speckled kidneys. Sampling was conducted at the salmon processing factory from which *C. piscicola* had initially been isolated. The investigation concluded that *C. piscicola* was present as part of the normal non-pathogenic bacterial flora of fish in NZ. This finding was supported by the fact that no mortalities were reported in association with this case. There was no statistical evidence to suggest that that *C. piscicola* was associated with speckled kidneys. Since there was no biosecurity risk, this investigation was closed.

DISTAPLIA VIRIDIS ASCIDIAN CONFIRMED

On 29 July 2014, NIWA notified MPI that an ascidian specimen collected on 12 June during the winter round of the Marine High Risk Surveillance programme in Marsden Cove Marina, Whangarei, had been preliminarily identified as *Distaplia viridis*, a new to New Zealand species previously only recorded from Australia. Five small (~10 mm high) cushion-shaped colonies with short stalks were found attached to floating pontoons.

Three other species of *Distaplia* have been recorded from New Zealand (*D. knoxi* Brewin, 1954, *D. taylori* Brewin, 1950 and *D. marplesi* Brewin, 1952), but the Marsden Cove specimens had gonads enclosed in the gut loop and a stomach lacking folds, while the other species do not. Of the 22 species known from the southern hemisphere, only *D. florida* Kott, 1990 and *D. viridis* Kott, 1990 have these characters.

The specimens collected had almost all the morphological characters of *D. viridis* Kott, 1957, and based on colony morphology and zooid size were less likely to be *D. florida*. Additional sampling at the same location in September 2014 enabled the species to be confirmed as *D. viridis*. It is not known what prospects this species has of establishing in New Zealand, nor indeed whether it has already done so (Page, 2014). It is considered native to Australia and was originally described from Victor Harbour, South Australia (Kott, 1957). It is common in Port Phillip Bay, Victoria (Kott, 1990) but has been also recorded from Heron Island in Queensland. If this is an accurate reflection of its native distribution, then it suggests *D. viridis* has a wide temperature range. Larvae are thought to have limited powers of dispersal, so any long-distance spread probably needs to be facilitated by the movement of biofouled vessels or marine structures. It was the taxonomist's opinion that the impact of this species is likely to be relatively low (Page, 2014). Colonies form small mushrooms or flat-topped colonies and are relatively inconspicuous. The description of colonies in Kott (1990) gives no indication that they spread over large areas of substratum or competitively overgrow other species (Page, 2014). Results were communicated to the Northland Regional Council and the investigation was stood down.

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Paul Bingham
Team Manager
Surveillance and Incursion Investigation (Animals and Marine)
Ministry for Primary Industries
paul.bingham@mpi.govt.nz

BROWN CRAZY ANT IN NEW ZEALAND: A HISTORY OF RECENT INCURSIONS AND RELATIVE RISK

INTRODUCTION

Ants have a long history as nuisance pests in New Zealand. Periodic arrivals of non-native ant species since first European colonisation have typically been poorly documented. Ant species of exotic origin were often only found by accident and were usually well established by the time they were discovered.

Over the past 15 years there has been an increasing awareness of the biosecurity threat invasive ants pose to the economy, environment and human health in New Zealand. Following the discovery and successful incursion response to a nest of red imported fire ant (RIFA), *Solenopsis invicta*, found at Auckland International Airport in 2001, the Ministry of Agriculture and Forestry (now the Ministry for Primary Industries – MPI) undertook to develop a number of invasive ant management measures to reduce the threat.

One of these measures is the National Invasive Ant Surveillance (NIAS) programme, an annual targeted survey of international points of arrival for cargo (Peacock *et al*., 2014). The programme has been running since 2003.

The primary objective of NIAS is to detect newly established nests of invasive ant species at high-risk sites around New Zealand. This includes seaports and airports, as well as a range of other Approved Transitional Facilities (ATFs) most likely to be the pathways for exotic ant species brought in through international trade and travel.

The programme surveys sites using small GPS-tracked baited lures placed in systematic grids. This system provides a wealth of temporal and spatial data that shows the patterns of border invasions by exotic ants. In the 12 years since the NIAS programme began, the most frequently found species has been the brown crazy ant, *Paratrechina longicornis*. This species does not have the high profile of other invasive ants such as RIFA, but its incursion history provides an excellent illustration of the ongoing risk New Zealand faces from highly mobile invasive ant species known as "tramp ants".

BROWN CRAZY ANT

Brown crazy ant probably originates from the tropical climes of the Old World (Wilson & Taylor, 1967) such as Africa or Asia (Smith, 1965) but the current wide global distribution makes it difficult to determine the actual place of origin.

It is one of the most common tramp ants in the tropics and subtropics, and has probably achieved one of the widest distributions of them all. It has also become established in greenhouses and heated buildings in temperate regions, is frequently intercepted by border authorities and has been spread with trade for well over a century.

The brown crazy ant is highly adaptable and can live in both very dry and rather moist habitats. It is usually associated with disturbance. Foraging brown crazy ants are opportunists, feeding on live and dead insects, honeydew, fruits and many household foods (Smith, 1965), but are particularly fond of sweet foods. They often invade houses and heated buildings in tropical and temperate areas and their biological habits mean they are considered a household and environmental pest in many countries (Creighton, 1950; Lee, 2002).

Workers are small (2–3 mm) and brown in colour with extraordinarily long legs and antennae that are distinctive and defining field characters (**Figure 1**). Foraging ants are very fast moving, darting about in a jerky, haphazard fashion (hence "crazy" ant) (Smith, 1965). They commonly form wide but thinly populous trails up to half a metre wide over walls and floors, and can forage long distances – up to 25 metres from the nest.

Figure 1: Brown crazy ant (*Paratrechina longicornis*). Photo: Antweb.

The brown crazy ant is considered a high-risk ant in New Zealand owing to its pest status in other countries, its known pest characteristics, its adaptability, reproductive capacity and likely ability to establish in the New Zealand environment.

RECENT INCURSION HISTORY

Table 1 (page 26) shows that the brown crazy ant has been detected 65 times at New Zealand's borders during the NIAS programme. It can be seen that most incursions occur at northern ports and ATFs in Auckland and Tauranga, but the species has been found in several other locations nationwide, including as far south as the Port of Lyttelton (Christchurch) three times in the past 12 years. There have also been several incursions of this species detected by other means over the years at many of these locations and elsewhere.

The frequency of interception at Auckland and Tauranga is likely to be partly related to higher freight volumes and where imported goods arriving there originated. Nevertheless, the numbers of interceptions show that the species is regularly imported to New Zealand via contaminated goods.

In most (but not all) cases the response has resulted in the location of one or more established, active and productive nests of brown crazy ant. All incursions have been destroyed. All of the detections listed were in the warmer months of summer or early autumn (December to March) when the NIAS programme is undertaken. It is unclear whether the species would be able to survive over winter everywhere it has been found in New Zealand. Population attrition during winter is likely in most areas, particularly in the south. Notwithstanding this, anecdotal evidence from other sources has shown the species can survive and be

TABLE 1: LOCATION AND YEAR OF INCURSIONS OF BROWN CRAZY ANT (*PARATRECHINA LONGICORNIS*) DETECTED BY THE NIAS PROGRAMME

YEAR	AUCKLAND			TAURANGA		NAPIER		NEW PLYMOUTH		WELLINGTON		LYTTELTON		TOTAL
	Port	ATF	Airport	Port	ATF	Port	ATF	Port	ATF	Port	ATF	Port	ATF	
2002	2	1												3
2003	2	1		1	2									6
2004										1				1
2005					1									1
2006	3	1		1		1						1		7
2007	1	1	1								1			4
2008	4	1		2				1				1		9
2009	3			2										5
2010				1						1				2
2011	5	1		1			1							8
2012	2			2			1							5
2013	3			1	1		1	1						7
2014	5									1		1		7

active in Auckland, Tauranga and Napier during the colder months of the year.

Collectively, this information is firm evidence that brown crazy ant is capable of establishing in New Zealand. This, combined with the frequency of interceptions, underlines the importance of ongoing border surveillance for high-risk invasive ants.

Exotic ants are arriving in New Zealand every year, everywhere, and a decrease in vigilance would almost certainly result in the rapid establishment of an invasive species such as brown crazy ant.

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Paul Craddock
Flybusters/Antiants
paul@flybusters.co.nz

GETTING READY FOR MYRTLE RUST

PREPARING FOR THE INVASION

The damaging plant disease myrtle rust, caused by the fungus *Puccinia psidii,* is not yet known to occur in New Zealand. This disease seriously affects the growth of many Myrtaceae, including well-known garden plants such as eucalypts, feijoa, myrtles and bottlebrushes, and iconic New Zealand native species such as pohutukawa.

Myrtle rust attacks the actively growing leaves, shoot tips and young stems of susceptible plants, producing masses of powdery bright yellow or orange-yellow pustules on the leaves, tips and stems (**Figure 1**). These spore-filled lesions may go on to cause deformation of the leaves and shoots (**Figure 2**) and twig dieback. If the infection is sufficiently severe it may result in the death of highly susceptible species. Symptoms may also appear on developing flower stems (**Figure 3**), inflorescences and even fruit, affecting the reproductive efficacy of the host plant and putting natural plant populations at risk.

Figure 1: Myrtle rust caused by *Puccinia psidii* on the leaves of Geraldton wax (*Chamelaucium uncinatum*)

Figure 2: Myrtle rust on the leaves of willow myrtle (*Agonis flexuosa*)

The recently completed collaboration between Scion and MPI Plant Health and Environment Laboratory (PHEL) scientists on the development of a plant DNA barcoding database will pave the way for rapid and accurate identification of host plants during an incursion of myrtle rust (*Puccinia psidii*).

Jointly funded by the MPI Operational Research Programme and Scion, the project aimed to develop and validate a DNA barcode database of more than 100 species of plants in the myrtle family (*Myrtaceae*) that are known to occur in New Zealand. Priority was focused on species with known susceptibility to the devastating myrtle rust pathogen, and on species of cultural and economic significance.

This project is a key part of MPI's preparedness and ability to respond to damaging exotic pests and diseases in the event of an incursion.

Figure 3: Myrtle rust on the flowers of Geraldton wax

Myrtle rust poses a significant biosecurity threat to New Zealand's domestic and export industries and would be highly likely to have impacts on eucalypt forestry, production of manuka honey and feijoas, ornamental and amenity horticulture and nurseries. These industries are located in the North Island and a few isolated areas in the top of the South Island where the climate favours myrtle rust establishment. The disease also has environmental and cultural implications for native Myrtaceae, particularly iconic species such as pohutukawa (*Metrosideros excelsa*), and potentially other Metrosideros species, such as rata (*M. robusta* and *M. umbellata*) and critically threatened species, for example Bartlett's rata, *M. bartlettii.*

Since the detection of myrtle rust in eastern Australia in 2010 the known host range has significantly expanded and this pathogen now infects more than 100 species of myrtaceous plants (Carnegie & Lidbetter, 2012). This is unusual among rust fungi, which often have a restricted host range (Morin *et al.*, 2012).

While it is not known exactly when myrtle rust will arrive in New Zealand, it can be expected to arrive here eventually on the prevailing winds from Australia, or perhaps unintentionally as a contaminant of imports or with travellers. Myrtle rust spores are readily disseminated by wind and could travel on equipment and clothing. Both these modes of dispersal have the capacity to transport myrtle rust spores over a very long distance.

Once myrtle rust is found in New Zealand there will be little time to prepare the necessary resources to deal with the incursion, so it is essential to initiate a pre-emptive response. An important part of the response is the ability to quickly and accurately identify host plants affected with the rust. Timely botanical identifications will then enable more accurate surveillance in the affected regions. An incursion could occur in forests or in the natural vegetation of reserves and parkland, or even home gardens. Accurate surveillance of infected hosts will enable MPI to consider biosecurity measures in order to protect threatened plant species and vegetation communities and to reduce the risk of spread to new areas by workers and visitors.

MYRTACEAE DNA BARCODING DATABASE

There will be numerous challenges to the accurate field identification of plants with suspected myrtle rust. Recent estimates suggest that the family Myrtaceae includes more than 5600 species in 130–150 genera (Govaerts *et al*., 2008). While not all these species are present within New Zealand, there are some genera with a large number of closely related species, e.g., 700 species of *Eucalyptus,* with more than 250 in New Zealand – this genus alone poses a significant challenge to identify from traditional morphological characters. Besides requiring specialist botanical expertise, identification this way is time-consuming and may not be straightforward, depending on the presence or absence of helpful diagnostic features.

The correct botanical identification of Myrtaceae in particular often requires a combination of morphological characters that may not be available at certain times of the year or may require examination under a microscope. Distinguishing characters may include leaf morphology (both juvenile and mature), bark colour and type, and floral attributes such as colour, shape, the presence of buds, flower clusters, and the morphology of the capsule and fruit. During an incursion response, suspected myrtle rust host samples are unlikely to possess all these morphological characters at the same time.

In addition to *Eucalyptus*, there are many ornamentals in this family that are difficult to identify from morphological characters alone, so it would be challenging to identify a large number of species this way during an incursion response. Fortunately, however, there are potentially faster methods of identifying large numbers of plant specimens.

In particular, international practice favours the use of so-called DNA barcodes to facilitate the identification of both known and new species. This entails using sequences from short, standardised regions of broadly conserved gene regions and comparing an unknown sequence with a library of known sequences. No such reference library previously existed for the major species of Myrtaceae in New Zealand.

For this purpose, a DNA barcode database was developed of more than 100 species of Myrtaceae that are known to occur in New Zealand. Three or more replicate DNA sequences for each of the targeted species were obtained for the *matK* and *ITS* gene regions. In addition, the two largest genera (*Metrosideros* and *Eucalyptus*) plus *Angophora* and *Corymbia* (formerly *Eucalyptus*) were further analysed for the *ETS* gene region.

All newly obtained DNA sequences and relevant DNA sequences obtained from Genbank have been entered into a Geneious barcode reference database and all Scion-generated sequences are now deposited in Genbank, where they are internationally available to researchers. In addition, the Scion-generated sequences are all associated with voucher specimens.

Through this collaboration, the existing collection of Myrtaceae specimens in Scion's National Forestry Herbarium has been expanded with voucher specimens that were collected and used during the project. This has resulted in an improved resource to assist future identification of Myrtaceae based on morphology.

IMPLICATIONS FOR MYRTLE RUST DETECTION

Having the Myrtaceae DNA barcoding database will enable rapid identification of large numbers of plant species in the event of a myrtle rust incursion. This will help with:

- more accurate surveillance of the spread of the disease in nature reserves, state forests and urban areas; and
- better, more timely decision making and consideration of biosecurity measures in order to protect threatened plant species and communities and reduce the risk of spread to new areas.

To our knowledge this is the first barcoding database targeting a specific flowering plant family in New Zealand. The sequence data is not only a valuable reference collection for a myrtle rust response, but also a unique national botanical resource. The utility of this new database has been illustrated by its use to re-identify 30 misidentified collections sampled during the course of the project.

A myrtaceous plant sample collected in the field in New Zealand can now easily be identified by comparing its DNA sequences with those in the newly created barcoding reference library, or be compared morphologically with existing specimens in Scion's National Forestry Herbarium.

FURTHER PLANNED WORK

In addition to the DNA barcoding platform, MPI is currently tendering for external service providers to carry out host plant identification work using the DNA barcoding database in a myrtle rust response. The value of this work and the database will be demonstrated by MPI's ability to immediately put preparedness measures in place during a response.

Other future preparedness work will include a proposal to tender under the MPI Operational Research Programme for an external service provider to develop a Lucid™ interactive key to the family Myrtaceae. An interactive key would enable biosecurity officers from MPI and regional councils and citizen scientists to reliably and accurately identify Myrtaceae present in New Zealand. The interactive key, once released publicly and made available on smartphones and tablets, would enable rapid identification of suspected myrtle rust hosts.

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Brian Quinn
Scientist – Botany
Virology & PEQ, PHEL
Ministry for Primary Industries
Brian.Quinn@mpi.govt.nz

Matt H Buys
Curator, National Forestry Herbarium
New Zealand Forest Research Institute (Scion)
Matt.Buys@scionresearch.com

GIANT WILLOW APHID: A NEW APHID ON WILLOWS IN NEW ZEALAND

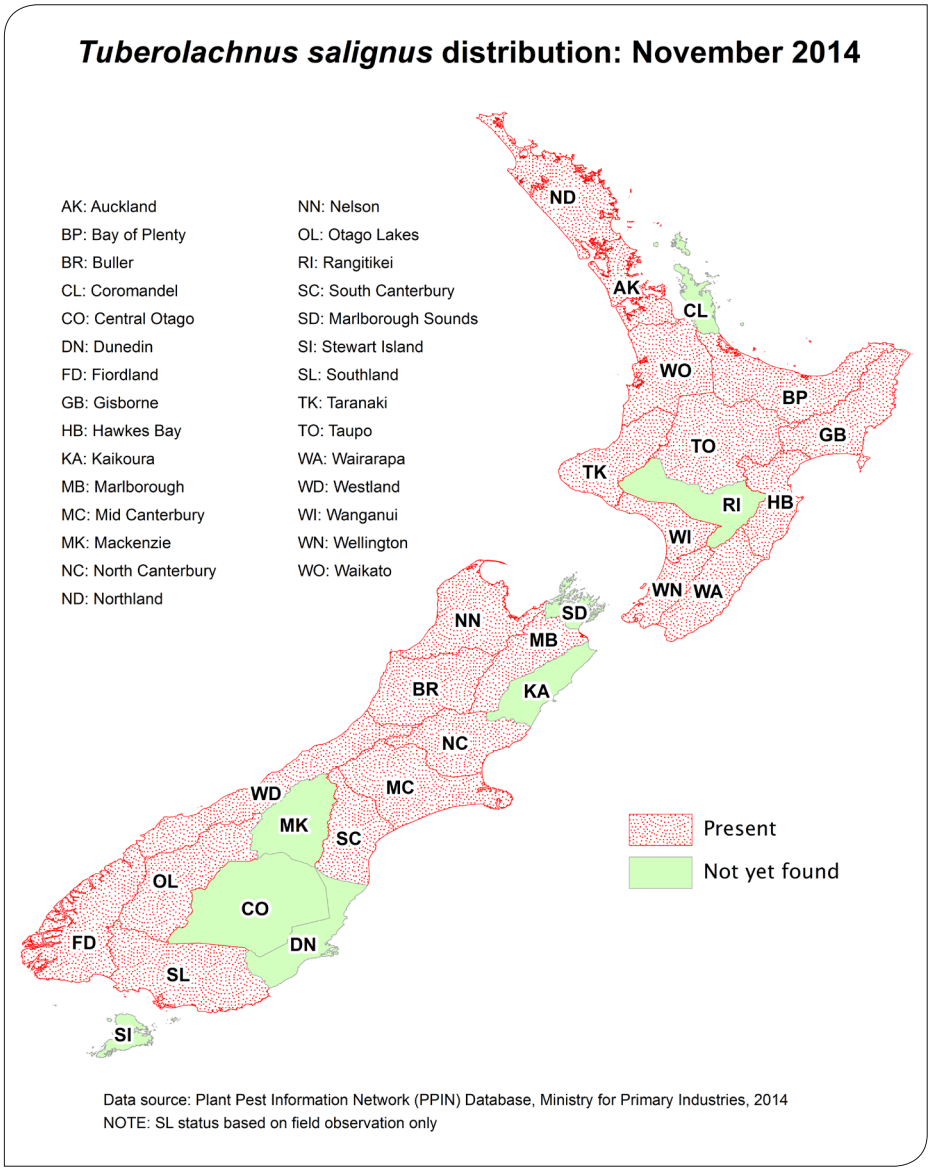

Figure 1: Present distribution of giant willow aphid in New Zealand

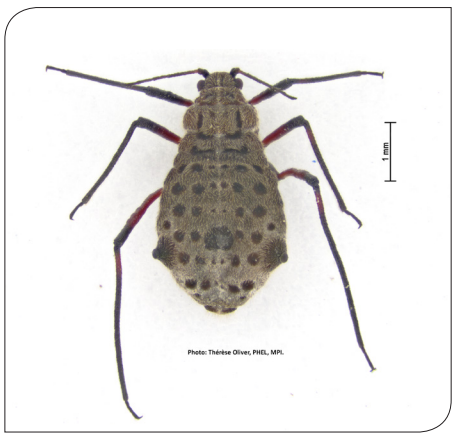

Figure 2: Giant willow aphid, *Tuberolachnus salignus*

Figure 3: Dense colonies of giant willow aphid enveloping a willow branch

Giant willow aphid (GWA), *Tuberolachnus salignus* (Hemiptera: Aphididae), is a new to New Zealand organism. GWA was first detected in Central Auckland on crack willow (*Salix fragilis*) in late December 2013. At the same time it was reported from Northland and picked up in the Ministry for Primary Industries (MPI) High Risk Site Surveillance Programme in southeast Auckland. Although its distribution is almost cosmopolitan wherever willows are grown, this is the first record in Australasia. Since its first detection in Auckland, *T. salignus* has been reported from both the North and South Islands (**Figure 1**). Prior to the New Zealand find, GWA was notably absent from Australia, but in early 2014 it was discovered in Tasmania, Australian Capital Territory and New South Wales.

GWA is one of the largest aphid species in the world. It is the largest of more than 120 species of aphids that feed on willows (*Salix* spp.), measuring up to 5.8 mm in length, and is brown to dark brown with several rows of black patches (**Figure 2**). GWA has a large, dark brown tubercle in the centre of the dorsum and two smaller tubercles just in front of the siphunculi, which are on dark cones. These are very distinctive characteristics. It lives on the stems and branches of willow, where it forms dense colonies (**Figure 3**). In Europe the life cycle takes two to three weeks when the temperature is 17.5–20°C. We can therefore expect multiple generations in a New Zealand growing season.

GWA reproduces solely by parthenogenesis and no males have ever been recorded. Aphids are known to be long-lived, with the winged forms (alates) in particular giving lengthy maternal care to their offspring. There are some unusual features about the seasonality of this organism. Whereas aphids in general tend to be less active in winter and more active in the warmer months, in the United Kingdom GWA has been recorded and collected from August (late summer) to early March (spring), with none being seen for the following five months. It is not known where, how, or in

what form these aphids spend the spring and early summer months. They seem to be very active in frost and heavy snow during the winter months of January and February. They are strongly aggregative and can build up to very large colonies in late summer.

Although we expected a similar trend in New Zealand (i.e., aphids present on the host from December to August), they were not observed or reported from late May to August 2014. In late August a few adults and nymphs were seen on new growth of *Salix sepulcralis*. Since there has been no ongoing surveillance, we cannot be certain of the presence or disappearance of *T. salignus* during winter in New Zealand conditions.

The hosts associated with GWA are various species of Salicaceae: willow (*Salix* spp.) and poplar (*Populus* spp.). New Zealand host records as of May 2014 include *Salix alba* var. *vitellina, S. babylonica, S. caprea, S. cinerea, S. fragilis*, *S. humboldtiana, S. matsudana, Salix* x *reichardtii* (hybrid), *Salix* x *sepulcralis* (hybrid), *S. viminalis* and *Populus nigra.*

GWA has been identified as a potential pest of hybrid willows grown for biomass production all around the world. It can markedly reduce willow tree growth, both above and below ground and reduces the survival of infested trees. The extent of damage in New Zealand is unknown but it could pose a threat to shelter belts, flood protection plantings and specimen trees in public parks and reserves. There is concern that the impact on the root mass of a willow may reduce its effectiveness for flood and erosion control.

GWA colonies can produce large amounts of honeydew, which attracts ants, flies and wasps. *Vespula* spp. and *Polistes* spp. wasp activity has been observed around honeydew deposits at some infestation sites in New Zealand. MPI has also received many public enquiries about wasp problems associated with GWA. Increased wasp activity around willows is a good indicator of the possible presence of GWA.

In Japan, *T. salignus* is parasitised by the braconid wasp *Pauesia salignae* (Hymenoptera: Braconidae). No parasitoid of the species has been recorded in Europe and to date no parasitoids have been observed in New Zealand, though predators such as *Adelia bipunctata* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae) have occasionally been found in association with GWA in New Zealand.

This aphid is a recent arrival and key areas requiring further study are its behaviour in a new environment, host preferences and management options.

Disna Gunawardana
Senior Scientist (Entomology), PHEL
Ministry for Primary Industries
Disna.Gunawardana@mpi.govt.nz

Alan Flynn
Manager (Entomology), PHEL
Ministry for Primary Industries
Alan.Flynn@mpi.govt.nz

Heather Pearson
Incursion Investigator
Surveillance and Incursion Investigation (Plants and Environment)
Ministry for Primary Industries
Heather.Pearson@mpi.govt.nz

Stephanie Sopow
Forest Entomologist
SCION (New Zealand Forest Research Institute)
Rotorua
Stephanie.Sopow@scionresearch.com

PLANTS AND ENVIRONMENT INVESTIGATION REPORT: JULY TO SEPTEMBER 2014

The Ministry for Primary Industries (MPI) Investigation and Diagnostic Centres and Response Directorate (IDC & R) is responsible for delivering the core functions of surveillance, incursion investigation, diagnostics and response by managing the surveillance and investigation of notifications of suspected exotic pests and diseases that may affect New Zealand's primary industries or aquatic and terrestrial environments.

GRAPEVINE VIRUS D CONFIRMED

A vitivirus, *Grapevine virus D* (GVD) was detected from *Vitis* sp. (chardonnay). This is the first notification of GVD in New Zealand. GVD is an unwanted organism (Biosecurity Act 1993). The grapevine specimens were collected by MPI from Lincoln University germplasm plantings. The GVD-infected plants at Lincoln originated from vines sourced from the Waikato region, which in turn originated from material imported from Europe in the late 1800s by Romeo Bragato. In addition to GVD, four other viruses were detected, all of which are considered present in New Zealand. These are: *Grapevine leaf roll associated virus 3* (GLRaV-3), *Grapevine rupestris stem pitting associated virus* (GRSPaV), *Grapevine rupestris vein-feathering virus* (GRVFV) and *Grapevine virus A* (GVA). The GVD-infected vine exhibited leafroll symptoms, but these were considered likely to result from the GLRaV-3 co-infection. Although GVD is reported to be associated with several grapevine disorders, the role it plays in causing disease symptoms remains unclear. Furthermore, the role vitiviruses play in grapevine diseases internationally is still largely unknown, with most studies limited to demonstrating an association of a particular vitivirus with a disease complex. GVD can be transmitted by mechanical inoculation and by members of several insect genera in a semi-persistent manner. Discussions between MPI and winegrape producer representatives concluded that GVD has most likely been present and undetected in New Zealand for many decades. Further testing remains an option to determine how widespread GVD is within New Zealand.

HIGH-RISK DISEASE OF CITRUS RULED OUT

A member of the public sent MPI images of a disease affecting imported mandarins bought from a local supermarket. The images were examined by an MPI plant pathologist, who was unable to rule out an exotic fungal disease, including citrus black spot, *Guignardia citricarpa,* a high-risk organism. A sample was received from the notifier and tests were carried out to establish the cause of the symptoms. Culturing and sequencing the fungi found three species that are already established in New Zealand, and tests were negative for *G. citricarpa*. The most likely causal agent was *Colletotrichum gloeosporioides*. Also present were *Pleospora herbarum* and *Alternaria alternata*.

SCORPION FOUND IN BAGGAGE

A scorpion was found in unaccompanied luggage that arrived the day after the notifier's flight from the Bahamas to New Zealand. Passengers with unaccompanied luggage are required to complete a quarantine "Irregularity Report" and when no biosecurity risk items such as food or camping gear are declared, the "low risk" luggage is subjected to X-ray screening. Scorpions can be difficult to detect by X-ray. This one was alive when found and molecular analysis determined it was a *Centruroides* sp. (Scorpiones: Buthidae). Morphological examination suggested it to be *C. guanensis*, which is known from the Bahamas (and also Cuba and Florida). Molecular sequence data for this species is unpublished and unavailable for comparison. It is not reported to be significantly venomous and habitat descriptions suggest it is an opportunistic species utilising any available shelter, which provides some explanation for its presence in luggage. The available evidence suggests this detection was limited to a solitary individual and the investigation was closed.

RISK SEEDS INTERCEPTED

Customs reported a person had purchased and received aquatic plant seeds from overseas using the on-line retailer AliExpress. The importer had germinated some of the seeds in preparation for transplant into a fish tank, but was told by an aquatic plant shop that it was illegal, as this method of importation by-passed New Zealand importation procedures. When contacted by MPI the recipient of the seeds agreed to place all seeds, plant media and containers in a plastic bag and deliver them to MPI. The parcel was received within an hour and the unauthorised goods placed into a bio-waste bin for destruction. The postage and packaging information had previously been destroyed. The aquatic plant retailer was phoned and thanked for his vigilance and the investigation was closed.

SUSPECT APHID INTERCEPTED

A Plant and Food Research scientist reported a suspect new to New Zealand aphid, *Myzus dycei,* collected from native ongaonga or tree nettle (*Urtica ferox*). Although *M. dycei* has not previously been recorded in New Zealand, a similar species, *M. ornatus*, has been reported from *U. ferox*. MPI entomologists re-examined microscope slides of *M. ornatus* from stored insect collections and found the original samples had been misidentified as *M. ornatus* at the time of the first record. The correct taxonomic description has now been confirmed as *M. dycei*, which has been known to be present in New Zealand since the 1970s.

VIRUS IN PEQ

A new to New Zealand virus was detected in *Hibiscus rosa-sinensis* plants that had been recently imported and were being held in a Level 2 post-entry quarantine facility. The virus, *Hibiscus latent Singapore virus* (HLSV), was found during routine testing. Subsequently the importers advised that plants growing in their nursery for 7+ years showed HLSV-like symptoms, and testing confirmed these plants were infected with HLSV. HLSV is a tobamovirus that was first described in 2002 in Singapore. It is typically symptomless under laboratory conditions, but typically minor field symptoms (including diffuse chlorotic rings and spots) are sometimes exhibited. The generally symptomless nature of HLSV makes it difficult to detect and the worldwide distribution is consequently unknown. HLSV is similar to another tobamovirus, *Hibiscus latent Fort Pierce virus*, recently recorded as present in New Zealand. Both are considered to pose negligible risk and are already established. HLSV is now listed as non-regulated by MPI. The investigation was closed.

WHITE-FOOTED HOUSE ANTS INTERCEPTED

In July, MPI received notification that a caravan imported into New Zealand six months previously from the USA via Australia, was now infested with ants. At the time of its arrival MPI border inspection had not found any ants in it. Specimens submitted to MPI were identified as the white-footed house ant, *Technomyrmex albipes* (Hymenoptera: Formicidae). Although this species has previously been intercepted on imported goods it is not known to be established in New Zealand. An incursion response was initiated and, with the assistance of contracted ant specialists FBA Consulting, toxic baiting was undertaken, both in the caravan and in the immediate vicinity of the campground where the caravan had been stored since arrival. Although the risk of ant colony dispersal to the surrounding area was considered low at this time, dispersal could potentially have occurred in late summer after the caravan arrived. Therefore, as an added precaution, ant surveillance with food bait lures is planned over a wider area of the campground during the 2014–15 summer, when warmer conditions will increase the likelihood of ant activity if colonies are present.

TRADESCANTIA VIRUS DETECTED

Notification of a viral disease spreading among plants in the Auckland Winter Gardens led to the collection of foliage samples from diseased *Tradescantia* sp., *Iris* sp. and *Hippeastrum amaryllis*. The tospovirus *Impatiens necrotic spot virus* and the potyvirus *Ornithogalum mosaic virus* were detected in the iris plants, while the potyvirus *Hippeastrum mosaic virus* and carlavirus *Nerine latent carlavirus* were diagnosed from *H. amaryllis*. These viruses are all previously recorded as present in New Zealand. However, several plants of *Tradescantia spathacea* exhibiting leaf malformation symptoms were found to contain a potyvirus not previously recorded. *Tradescantia mild mosaic virus* (TraMMV) was identified using electron microscopy and molecular (PCR and sequencing) techniques. TraMMV is reported from the USA, Italy and the former USSR, though its distribution is likely much wider. Its host range is limited to *Tradescantia* spp. and *Commelina diffusa*. TraMMV is transmitted by aphids, including the green peach aphid (*Myzus persicae*), which is abundant and widespread in New Zealand. However, the biosecurity risk of this virus is predicted to be very low. Several species of *Tradescantia* were subsequently purchased from Auckland retail stores (sold as houseplants), tested for TraMMV and found negative. *T. fluminensis* (wandering jew, a New Zealand weed of significance) plants from Christchurch and Auckland tested positive for TraMMV. These results suggest TraMMV is well established and widespread in New Zealand. The investigation was closed.

PINUS RADIATA FUNGUS STUDIED

The fungus *Sporothrix inflata* was identified from stained roots collected from *Pinus radiata* during surveillance. The first record of *S. inflata* in New Zealand was in July 2010. This case was identified by Scion, using molecular testing. Further diagnostic investigation by MPI revealed that sequence data provided by Scion was not a 100 percent match to *S. inflata*, and although collected isolates were related to *S. inflata*, they were in a different clade to the type specimen (CBS 239.68). Published phylogenetic studies on other *Sporothrix* spp. report this genus contains cryptic species, which are typically referred to as species complexes. In summary, based on the sequence analysis of the two *S. inflata* isolates collected, MPI concluded that they belonged to the *S. inflata* species complex. Importantly, no records were found to associate the *S. inflata* complex with plant disease. Although this complex has been isolated from *Quercus* and *Pinus sylvestris* roots in Europe, it is considered to be a group of endophytes or opportunistic fungi colonising already affected roots. The investigation was closed.

LADYBIRD ON FRESH PRODUCE

An intense blue-coloured beetle was reported on grapes imported from the USA. The insect was identified as the steelblue ladybird (*Halmus chalybeus*), a species already present in New Zealand. The notifier was informed and the investigation closed.

Mark Bullians
Team Manager
Surveillance and Incursion Investigation (Plants and Environment)
Ministry for Primary Industries
Mark.Bullians@mpi.govt.nz

PEST WATCH: 15 AUGUST 2014 – 4 NOVEMBER 2014

Biosecurity is about managing risks: protecting New Zealand from exotic pests and diseases that could harm our natural resources and primary industries. MPI's Investigation and Diagnostic Centres and Response (IDC & R) directorate devotes much of its time to ensuring that new organism records come to its attention, and to following up as appropriate.

This information was collected from 15 August to 4 November 2014. The plant information is held in the MPI Plant Pest Information Network (PPIN) database. Wherever possible, common names have been included. Records in this format were previously published in the now discontinued magazine *Biosecurity*.

To report suspect new pests and diseases to MPI phone 0800 80 99 66.

Validated new to New Zealand reports

Type	Organism	Host	Location	Submitted by	Comments
Fungus	*Agrocybe arvalis* No common name	Inanimate host	Dunedin	IDC & R (General Surveillance)	In Europe and North America this mushroom is a common saprophyte growing in mulch. It does not pose any biosecurity risk and is not associated with plants.
Insect	*Myzus dycei* Aphid	*Urtica ferox* Ongaonga; tree nettle	Mid Canterbury	Plant & Food Research (General Surveillance)	Identified in 2012. MPI has located previously misidentified material of *M. dycei*, indicating this aphid has been in New Zealand since at least 1966.
Insect	*Rhyzobius lophanthae* Scale-eating ladybird	Collected from *Phormium* sp. (flax)	Auckland	Plant & Food Research (General Surveillance)	An Australian ladybird species that has been released around the world as a scale predator. There are no confirmed records of its intentional release in New Zealand.
Virus	*Potyvirus Tradescantia mild mosaic virus* TraMMV	*Tradescantia* sp.	Auckland	IDC & R (General Surveillance)	This virus was named and characterised in 2006 from Italy. It has a narrow host range which includes *Commelina diffusa* and species in the genus *Tradescantia*.
Virus	*Vitivirus Grapevine virus D* GVD	*Vitis vinifera* Grape	Mid Canterbury	IDC & R (General Surveillance)	GVD, like other vitiviruses, is associated with rugose wood disease complex in grapevine. These diseases are normally latent in ungrafted *Vitis vinifera*, but develop in grafted vines.

If you have any enquiries regarding this information please contact surveillance@mpi.govt.nz.

Veterinary Diagnostic Laboratories

GRIBBLES VETERINARY PATHOLOGY

- AUCKLAND
  Courier: 37–41 Carbine Road, Mount Wellington, Auckland 1060
  Postal: PO Box 12049, Penrose, Auckland 1642
  Tel: 09 574 4701 Fax: 09 574 5304

- HAMILTON
  Courier: 57 Sunshine Ave, Hamilton 3240
  Postal: PO Box 195, Hamilton 3240
  Tel: 07 850 0777 Fax: 07 850 0770

- PALMERSTON NORTH
  Courier: 840 Tremaine Avenue, Palmerston North 4440
  Postal: PO Box 536, Palmerston North 4440
  Tel: 06 356 7100 Fax: 06 357 1904

- CHRISTCHURCH
  Courier: 7 Halkett Street, Christchurch 8140
  Postal: PO Box 3866, Christchurch 8140
  Tel: 03 379 9484 Fax: 03 379 9485

- DUNEDIN
  Courier: Invermay Research Centre, Block A, Puddle Alley, Mosgiel, Dunedin 9053
  Postal: PO Box 371, Dunedin 9053
  Tel: 03 489 4600 Fax: 03 489 8576

NEW ZEALAND VETERINARY PATHOLOGY

- AUCKLAND
  Courier: NZCCM, Gate 2, Auckland Zoo, Motions Road, Western Springs, Auckland 1022
  Postal: PO Box 44 422, Point Chevalier, Auckland 1246

- HAMILTON
  Courier: Cnr Anglesea and Knox Streets, Hamilton
  Postal: PO Box 944, Hamilton
  Tel: 07 839 1470 Fax: 07 839 1471

- PALMERSTON NORTH
  Courier: IVABS Building, 1st Floor, Massey University, Tennant Drive, Palmerston North
  Postal: PO Box 325, Palmerston North
  Tel: 06 353 3983 Fax: 06 353 3986

To report suspected exotic land, freshwater and marine pests, or exotic diseases in plants or animals, call:

0800 80 99 66

Investigation and Diagnostic Centre – Wallaceville
66 Ward Street
Upper Hutt
Tel: 04 526 5600

Investigation and Diagnostic Centre – Tamaki
231 Morrin Road
St Johns
Auckland
Tel: 09 909 3568

Investigation and Diagnostic Centre – Christchurch
14 Sir William Pickering Drive
Christchurch
Tel: 03 943 3209

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