Surveillance

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Surveillance is published as the Ministry for Primary Industries' authoritative source of information on the ongoing biosecurity surveillance activity and the health status of New Zealand's animal and plant populations in both terrestrial and aquatic environments. It reports information of interest both locally and internationally and complements New Zealand's international reporting.

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Editorial

Plants and Environment Investigations – what do you need to know?

Surveillance has traditionally maintained a strong focus on exotic pests and diseases by providing quarterly reports on investigations of suspect exotic animal diseases, and marine and freshwater pests and diseases. More recently this focus has been extended by adding quarterly reports of investigations of pests and diseases associated with plants and the natural environment. These reports help provide information on New Zealand's biological invasion pressure and evidence of disease status for trade purposes. These investigations are an important part of the biosecurity process and they often complement surveillance programmes when an early detection has been made.

An investigation takes place after the Ministry for Primary Industries (MPI) receives notification of a potential pest or disease. MPI assesses the potential risk or harm and may undertake urgent action, depending on the severity of the potential impacts of the pest or disease concerned. Within MPI there are two teams that investigate these notifications. The Animal and Marine Team investigates risks associated with diseases and parasites of animals and aquatic organisms, and the Plant and Environment Team is responsible for risks associated with diseases and pests of plants, and terrestrial and aquatic weeds.

The pest and disease hotline (0800 809 966) helps New Zealanders to meet their obligations under sections 44 and 46 of the Biosecurity Act 1993, which obliges every person to inform MPI if they suspect the presence of an organism not normally seen or detected in New Zealand. The hotline is operated 24/7 by a call centre whose staff inform the relevant investigation team via an electronic paging system. There were 1750 notifications to the Plant and Environment team in the 2015 calendar year and on average there are about 1320 notifications per year.

Once a notification is received by an investigator, the biosecurity risk is assessed by evaluating the credibility of the information to determine whether a biosecurity risk is present and whether it involves an "unwanted" or new organism. If the notification does not relate to a current biosecurity risk, no further investigative action is undertaken.

About 570 investigations were carried out by the Plant and Environment team during 2015 and on average there are about 525 investigations each year. If an exotic species cannot be ruled out, a sample or specimen is obtained and sent to MPI's diagnostic laboratories for identification. Other activities that may be undertaken during the investigation phase include urgent measures to control and contain the new or unwanted organism, while still reserving future biosecurity response options. Failure to implement such actions may compromise the success of the investigation or eventual response. Urgent measures usually take the form of organism management or movement controls on materials and goods that could pose a biosecurity risk.

Most investigations involve various life stages of insects, from eggs and larvae to adults, associated with imported inanimate goods and food products. There are also cases that involve illegal or non-compliant plant and seed imports, suspect new diseases and insects that could pose a threat to amenity trees or crops.

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MPI reaffirms New Zealand is free from equine influenza

MPI responds to the suggestion of NZ horses being the source of the Malaysian equine influenza outbreak

Background

On 6 September 2015, a report was posted on ProMED-mail, a website run by the International Society for Infectious Diseases, regarding the current outbreak of equine influenza (EI, caused by equine influenza type A viruses) in Malaysia. In the posting (ProMED, 2015), The Malaysian Department of Veterinary Services suggested that the source of the infection could have been four horses imported from New Zealand via Singapore.

New Zealand has never had a case of EI, and it was important for the Ministry for Primary Industries (MPI) to clear up any potential confusion in the international community and provide extra assurance of New Zealand's EI freedom to our trading partners and the OIE (World Organisation for Animal Health). Accordingly, MPI investigated these horses and the properties from which they were exported.

The investigation

Properties of origin

The horses were traced back to four properties in the Waikato, which were visited within three days of the ProMED posting, by MPI Incursion Investigation veterinarians assisted by the regular veterinarians for these properties. Horses present were examined for signs of respiratory illness. Those that had previously been in contact with the exported horse from each property were identified and blood samples were taken from them for testing. The health of the horses on these properties during the preceding few months was discussed. All properties were free of any current signs of respiratory illness, and none had had any recent illnesses of concern.

Testing

Blood samples from the in-contact horses were tested at MPI's Animal Health Laboratory. All horses were negative for antibodies to EI. Had there been any exposure to EI when the exported horses were on the properties, these in-contact horses would have generated antibodies as an immune response to infection. The exported horses had been sampled in pre-export isolation in New Zealand, and tested negative for EI on nasopharyngeal swabs as part of their export requirements. The blood samples had been retained and were tested for antibodies to the virus as part of the investigation, and they were all negative.

Disease and quarantine timelines

The travel and quarantine periods of the exported horses were reviewed in relation to the incubation and infectious periods for EI. The four horses had all been subject to a 14-day pre-export isolation period in a dedicated horse export quarantine facility, with disease testing and close monitoring for any signs of ill-health.

They travelled to Malaysia via Singapore, where they were isolated from other horses during a two-hour transit period. Seventeen other NZ horses travelled to Singapore on the same flight; these had been vaccinated against EI and undergone similar isolation and testing in New Zealand as the Malaysia-bound horses. MPI contacted authorities in Singapore and established that the 17 Singapore-bound horses had not come down with any respiratory illness after arrival, and nor had any of the horses they were subsequently in contact with.

Upon arrival in Malaysia, the four horses of concern were quarantined for 14 days with no signs of infection. Horses with EI typically shed the virus for 7–10 days (Animal Health Australia, 2011) and during this time they are clinically sick.

In summary therefore, the horses concerned had a total of 30 days' quarantine and travel before being released into Malaysia, during which they showed no sickness, and testing confirmed they were not infected before travelling, so it is not plausible that they could have carried an infection from New Zealand to Malaysia.

The report posted by the Malaysian authorities described the horses as having influenza-like nasal discharges at some unspecified time after release from post-arrival quarantine. If true, this would mean that the horses were acutely sick with EI, and could only have become infected during the previous 5 days (Animal Health Australia, 2011) – i.e., in Malaysia, either during quarantine or soon after release.

Investigation findings

Putting the above findings together, the most logical conclusion is that these horses, which were naïve to an infection not present in New Zealand and not vaccinated against it, were stressed after air travel and quarantine, and then mixed with large numbers of horses post-quarantine, so they were among the first to be stricken with EI at the beginning of the Malaysian outbreak.

Assurances

New Zealand has a robust exotic disease surveillance system, and even before this targeted investigation we were able to be very confident that EI was not present in the country. No reports of any suspicious respiratory illness in horses had been received by the MPI Surveillance and Incursion Investigation team within the previous 3 months, and such a contagious and serious illness would not have escaped attention.

Upon learning of the ProMED posting, MPI's Manager of Import and Export Animals, Regulation and Assurance Branch, notified our trading partners in New Zealand's largest horse importing countries (Singapore, Australia and Hong Kong) that the information in the posting was incorrect and that New Zealand was still free of EI. They were advised that we were undertaking an investigation and would inform them of the outcome.

Additionally, when the investigation concluded that there was a negligible likelihood of EI in the exported horses while they were in New Zealand, MPI's Director of Animal and Animal Products released a statement to ProMED-mail with New Zealand's reply to the original posting (Promed, 2015b). This included the assurance that New Zealand does not have EI, some details of the investigation, and information on NZ's strict import

controls to prevent the entry of this and other diseases.

MPI has confirmed that the suggestion that New Zealand could have been the source of EI infection for the outbreak in Malaysia was unfounded. New Zealand remains free from EI.

References

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Quarterly report of diagnostic cases: July to September 2015

Gribbles Veterinary Pathology

Bovine

Four Northland dairy cows from a herd of 200 aborted at about six months' gestation. Blood samples collected for *Neospora caninum* immunofluorescent antibody testing (IFAT) testing were elevated, with a titre of 1:1 000 in one cow (> 1:600 considered positive), and negative titres in two others. A collection of fetal tissues submitted from the fourth cow showed severe placentitis and placental necrosis in association with fungal hyphae, suggesting that **mycotic placentitis** was the cause of abortion in these cows.

Thirteen of 47 one-to-two-week-old crossbred calves from South Auckland died over a two-week period. They had recently been purchased at a sale. Three were examined and found to be pyrexic, with diarrhoea and haemochezia. Faecal culture yielded *Salmonella* Bovismorbificans from each calf and some were also positive for rotavirus and/or *Cryptosporidium parvum* by ELISA, confirming a likely diagnosis of combined **salmonellosis, cryptosporidiosis and/or rotaviral infection**.

A calf-rearing unit in the Bay of Plenty received two groups of calves over a week. Two subsequently died and others developed diarrhoea. Post-mortem examination of one calf revealed lesions of enteritis. Histopathology of various tissues identified necrotising and neutrophilic abomasitis with infiltrating fungi, and necrotising and neutrophilic enterocolitis with attaching-and-effacing *Escherichia coli* and *Cryptosporidium* spp. organisms. The latter were also found by faecal ELISA, confirming a diagnosis of combined **enteropathogenic *E. coli*, cryptosporidiosis** and (probably opportunistic) **fungal abomasitis**.

A six-year-old Jersey cow from the Auckland region was bright but had diarrhoea and weight loss. A Ziehl-Neelsen-stained smear of faeces contained clumps of acid-fast bacteria resembling ***Mycobacterium paratuberculosis***, and a faecal Johne's ELISA test was positive, confirming a diagnosis of **Johne's disease**.

A two-year-old beef cow from Northland was small and poorly gown. A BVD-antigen ELISA test detected a high viral load, confirming that this animal was persistently infected with **bovine viral diarrhoea virus**.

An adult dairy cow from Northland had mastitis in a back right quarter that was not responding to treatments including cloxacillin, procaine penicillin, tylosin and ketoprofen. Milk culture yielded a pure growth of *Trueperella (Arcanobacterium) pyogenes,* which was sensitive to all antibiotics tested. ***Trueperella* mastitis** may not be antibiotic-responsive if the affected quarter is severely damaged or abscessed.

Three outbreaks of **abortion caused by *Salmonella* Brandenburg** in heifers, and several occurrences in cows, were seen on farms in mid- and South Canterbury. The heifers often presented as dystocias with rotten calves, and were a potential hazard to farmhands assisting them to calve. The heifers were not sick, and being late-term they came into milk well. On one farm 12 heifers were calving each day and three or four of them required assistance. In the heifer outbreaks *S.* Brandenburg was cultured from fetal tissues. The cases in cows mainly presented as non-specific abortions and were diagnosed on culture of fetal tissues or uterine discharge.

A few cases of **Bovine neonatal pancytopenia** were diagnosed on two farms: one in North Otago and another in South Canterbury. In the former example, the farmer had seen suspect cases some years previously when calves developed spontaneous haemorrhage from orifices. Bone marrow was examined and there was sparse or no haematopoietic tissue. Other histological findings included bacterial infections in the liver, with a complete lack of neutrophil response to the infection.

***Mortierella* abortion** was diagnosed histologically in cows from one farm in mid-Canterbury. One of the cows subsequently died of fungal pneumonia and large numbers of thin hyphae typical of *Mortierella wolfii* were seen in the lung. In the other case, fungal hyphae were present in the inflamed placenta. This infection is uncommon in Canterbury but this case occurred after feeding out mouldy silage.

Low-phosphate-induced post-parturient haemoglobinuria (PPH) was interpreted to be the cause of a severe, often haemolytic anaemia on three Canterbury dairy farms. Twelve cases occurred on one farm grazing 950 cows, and eight cases in another with 850 cows (figures for the third farm were unknown.) The cows were pale and weak, and death followed quickly. Laboratory examination revealed a marked regenerative anaemia, with high bilirubin and normal serum protein levels. The cows were not grazing kale. Chronic copper poisoning, *Theileria orientalis* Ikeda infection, and PPH were considered. When tested, liver and kidney copper levels were not high, there were no Heinz bodies, and the cows were negative on PCR for *T. orientalis* Ikeda infection. Histologically they had changes typical of periacinar hypoxic necrosis in the liver; and unusually, many fibrin thrombi were present in hepatic sinusoids within and near to the necrotic parenchyma. The cows' serum phosphorus levels were reduced. Supplemental dicalcium phosphate was administered and eventually the problem ceased.

In Southland, ***Salmonella* Brandenburg** was the cause of diarrhoea and death in one-week-old calves on four dairy farms. ***S.* Typhimurium** caused calf deaths on one farm and ***S.* Hindmarsh** calf deaths on another.

About 10 yearling dairy heifers from a mob of 150 wintering on a Southland beef farm died over the winter. One was found recumbent, in poor condition, dehydrated and with diarrhoea. Histological sections of the distal intestine and colon revealed a heavy burden of **coccidia** and a faecal egg count had large numbers of coccidial oocysts.

There was an outbreak of sudden deaths in a mob of 49 one-to-two-month-old

dairy calves on a Southland dairy farm. Initially eight calves were found dead and another 21 were off-colour and pyretic, with green diarrhoea. Necropsy of a euthanased calf revealed only a large number of 1–2mm haemorrhagic ulcerations through the abomasal mucosa. An EDTA blood sample from this calf contained 1.6 mg/L of arsenic (normal range < 0.5), confirming **acute arsenic toxicity**. A search of the paddock in which the calves were kept revealed a pile of partially burnt tantalised timber, which was the likely source of the arsenic.

Severe conjunctivitis developed in one of four mobs of yearling heifers on a Southland dairy farm. ***Moraxella bovoculi*** was cultured from conjunctival swabs from two heifers and ***M. bovis*** from a third. A likely contributing factor in this infection was that the affected mob was pastured in a very windy area by the sea, so they had been exposed to fine particulate material blown off the beach, causing the irritation. Other mobs in more sheltered paddocks on the farm were unaffected.

A number of heifers on a Southland dairy farm were found recumbent at calving and on examination were found to have humeral fractures. Liver copper concentrations were very low (14–53 µmol/kg; adequate level > 95), confirming **copper deficiency**.

A three-year-old Jersey bull was found dead in a paddock on a bull-breeding unit in the Waikato. The bull had been checked every day and no clinical signs of disease had been noted. No poisonous plants were within its reach: there was only grass, supplemented with good-quality hay. A gross post-mortem examination was unremarkable but when the brain was removed a **pituitary abscess** with extension of purulent material into the ventricles was seen. A pituitary abscess can lead to death from increased intracranial pressure, or may be associated with increased pressure on the hypothalamus, leading to bradycardia.

Cases of **salmonellosis** have been common in the spring. A calf from a calf-rearing unit in Hawke's Bay developed diarrhoea and ***Salmonella* Agona** was cultured from the faeces. On a property in the Wairarapa, an adult dairy cow was presented for examination because of sudden-onset diarrhoea with haemochezia, and ***S.* Typhimurium** phage type RDNC Aug 13 was isolated. On another Wairarapa property (a calf-rearing unit), calves were developing diarrhoea, and as well as finding rotavirus and cryptosporidia in the faeces, ***S.* Anatum** was cultured. On a dairy farm in the Manawatu a two-year-old Jersey cow developed diarrhoea and ***S.* Bovismorbificans** was isolated from its faeces. In a further case from Hawke's Bay, 50 seven-day-old Friesian calves had diarrhoea and four died from a mob of 100. Culture of faeces isolated ***S.* Typhimurium** phage type 42. Four milking cows from South Waikato developed diarrhoea with fibrinous casts in the faeces and were pyrexic; culture of faecal samples from each animal isolated ***S.* Bovismorbificans**. In another Waikato case, seven-day-old calves were developed diarrhoea and died; faecal culture from one affected calf isolated ***S.* Bovismorbificans.**

Two-week-old Jersey/Friesian crossbred calves on a north Waikato dairy farm were developing diarrhoea. All the affected calves were bright, alert and responsive although 19 of them were affected from the herd of 40. Faecal samples collected from three calves tested positive for **rotavirus** and ***Cryptosporidium parvum*** by ELISA. On another property in South Waikato all 20 calves in a calf-rearing unit had diarrhoea and 10 more had died. ***C. parvum*** was confirmed in three faecal samples by ELISA testing.

A 16-day-old Jersey/Friesian crossbred calf from the Rotorua district developed spontaneous haemorrhage. A complete blood count found decreases in the red blood cell count (3.75×10^{12}/L; reference range $4.9–10.9 \times 10^{12}$), haemoglobin (46 g/L; reference range 80–160), packed cell volume (0.14; reference range 0.17–0.47), total white blood cell count (2.3×10^{9}/L; reference range $2.6–14.6 \times 10^{9}$), neutrophils (nil; reference range $0.6–4.5 \times 10^{9}$/L), monocytes (nil; reference range $0.8–1.2 \times 10^{9}$/L) and platelets (10×10^{9}/L; reference range $200–600 \times 10^{9}$/L). Fibrinogen was elevated to 13 g/L (reference range 0–4). These findings confirmed pancytopaenia and hyperfibrinogenaemia consistent with **bovine neonatal pancytopaenia (BNP)** and acute inflammation. The dam of the affected calf had been vaccinated some years previously against bovine viral diarrhoea virus with a vaccine known to induce BNP in the offspring.

Three rising-one-year-old Angus heifers had died on an East Coast sheep and beef farm after the mob of 80 had been yarded for vaccination against clostridial diseases and for anthelmintic treatment. One heifer that the farmer had seen recumbent was dead by the next morning and a postmortem was carried out. Leaves were found mixed with the rumen contents and subsequently identified as tutu (*Coriaria arborea*), consistent with a diagnosis of **tutu poisoning**.

Twenty 6-month-old Friesian cross heifers had diarrhoea and three were found dead from a mob of 200 in Hawke's Bay. Post-mortem examination of one dead heifer, followed by histopathology, revealed parasite larvae in the abomasal mucosa, and there was eosinophilic enteritis. The faecal egg count from the dead heifer was 250 eggs per gram. These results confirmed **gastrointestinal parasitism** as the most likely cause of death. Bovine viral diarrhoea and yersiniosis were ruled out by the other tests.

A one-year-old Hereford steer from the Rangitikei district was examined because of mucopurulent ocular and nasal discharge, diarrhoea, ataxia and ulceration in the mouth. The rectal temperature was elevated to 40°C (normal range 38.3 ± 0.5). Ovine herpesvirus-2 was detected in a whole-blood sample, confirming a diagnosis of **malignant catarrhal fever**.

About 45 steers in a mob of a 140 ten-month-old Angus cattle from Hawke's Bay developed bilateral conjunctivitis and reddening of the nostrils. Ocular lesions had been present for about three weeks. Three samples of the ocular discharge were collected and cultured. ***Moraxella bovis*** was isolated from all samples. In addition, PCR testing for bovine herpesvirus-1, the cause of **infectious bovine rhinotraceitis** (IBR), was undertaken. Virus was detected in all three samples, confirming a dual infection of *M. bovis* and IBR virus.

An East Coast beef farmer reported trickling deaths of 16 out of 111 rising-one-year-old Friesian bulls grazing above the snowline. Deaths occurred over a period of three months. Post-mortem examination of two dead bulls revealed poor body condition with scant fat stores, but there were no obvious lesions. Multiple tissues were processed for histopathological evaluation. Hepatocyte

cords were thin and atrophic compared to age-matched controls. No significant isolates were identified from culture of intestinal contents, and parasite eggs were not found in faecal egg counts. The history, poor body condition and lack of underlying disease processes supported the clinical suspicion of death due to **starvation and/or exposure.**

One adult Hereford cow from a central Hawke's Bay beef herd developed widespread exudative skin lesions. A skin biopsy was taken and processed for histopathological examination. There was severe plasmacytic and pyogranulomatous dermatitis centred on hair follicles containing numerous fungal spores and hyphae. Fungal organisms were also present in a thick surface crust of hyperkeratosis, degenerate neutrophils and erythrocytes. These findings confirmed a diagnosis of severe **dermatophytosis.** A serum sample tested negative for bovine viral diarrhoea (BVD) virus antigen, excluding persistent infection with BVD virus as a predisposing factor.

Thirteen mixed-age Hereford cows from a Taranaki herd aborted late-term fetuses over a three-week period. The cows had been fed hay with visible mould contamination. Many affected cows retained the fetal membranes and became anorexic and pyrexic. Post-mortem examination of two aborted foetuses showed scaly skin patches on one and no lesions on the other. Histology revealed extensive hyperkeratotic dermatitis with intralesional fungal hyphae in one calf and the other had suppurative bronchopneumonia. Culture of fetal stomach contents in the second case produced a growth of *Bacillus cereus.* Fungal agents (including *Aspergillus fumigatus* and *Mortierella wolfii)* and *Bacillus* spp. are associated with bovine abortion caused by feeding mouldy hay and silage. The diagnosis in this case was **mycotic and bacterial abortion** associated with ingestion of contaminated hay.

Ovine

Fewer sheep abortion samples were submitted from Otago and Southland than in previous seasons. The diagnoses this year were: ***Salmonella* Brandenburg** (52 percent), ***Toxoplasma gondii*** (13 percent), ***Listeria monocytogenes*** (10 percent), ***Helicobacter sp.*** (10 percent), ***Campylobacter fetus fetus*** (9 percent), ***C. jejuni*** (2 percent), ***L. ivanovii*** (2 percent), and ***Yersinia pseudotuberculosis*** (2 percent).

Eight rams died from a mob of 40 over a weekend. The rams were 11 months old and each was being fed 350 grams of concentrates formulated into pellets, in preparation for sale. Evidence of diarrhoea was noted at postmortem. Histopathology identified severe suppurative rumenitis consistent with **ruminal acidosis**. Further questioning of the farm staff revealed that the pellets had been dispensed without the usual close supervision during the weekend, suggesting that some animals had eaten more than intended.

Composite-breed pregnant ewe hoggets in a herd of 1 000 began aborting on a Wairarapa sheep farm. All had been vaccinated to prevent *Campylobacter* and *Toxoplasma* infection. Lambs were collected from the first four ewes that aborted. Post-mortem examination found no gross lesions but histopathology of the placenta revealed liquefactive necrosis associated with dense neutrophil infiltrates. Also, clusters of Gram-positive bacterial rods filling alveoli were found in the lungs. Culture of stomach contents from one lamb isolated ***Listeria ivanovii,*** confirming a diagnosis of **listeria-induced abortion**.

Six out of a flock of 500 two-tooth ewes died suddenly on a Wairarapa sheep farm 3–5 days after being yarded for pregnancy scanning. Gross changes observed at postmortem of one ewe included scant watery intestinal contents, reddened mucosa of the small intestine, and enlarged mesenteric lymph nodes. Histologically there were scattered erosions and mats of necrotic debris along the mucosae of the abomasum, small intestine and colon. There were numerous bacterial colonies among the necrotic debris. Culture of faeces yielded a heavy growth of ***Salmonella* Hindmarsh**, confirming a diagnosis of **enteric salmonellosis.**

Equine

A 19-year-old horse from the Auckland region had a history of chronic diarrhoea, anorexia and weight loss. Histopathology of a rectal biopsy showed diffuse expansion of the epithelium, lamina propria, muscularis mucosa and submucosa, caused by a monomorphic population of proliferating small lymphocytes. These signs were consistent with a diagnosis of **intestinal lymphoma**.

A 14-year-old pony from Northland had bilaterally enlarged submandibular lymph nodes. Fine-needle aspiration of one node showed increased numbers of plasma cells. A subsequent nodal biopsy showed cortical and medullary expansion by well-differentiated but occasionally binucleated or multinucleated plasma cells, consistent with **plasma cell myeloma**.

A mature horse on a Southland property collapsed and was euthanased after being unresponsive to supportive treatment and antibiotics after several days of diarrhoea and pyrexia. Necropsy revealed an extremely haemorrhagic, purple-black colon. Histopathological examination revealed a severe arteritis and thrombosis of blood vessels in the wall of the colon, leading to ischaemic necrosis of the mucosa. ***Salmonella* Typhimurium phage type 56** was cultured from the contents.

A mature donkey pastured with a few others on a small Otago farm was found with severe colic. It was given supportive treatment but the next day it exhibited neurological signs including blindness, stupor, jaw-champing and twitching of the eyes and ears. It was euthanased and a necropsy was performed. This was unremarkable, so a range of fixed tissues including brain were submitted to the laboratory. Histopathological examination revealed malacia in the brain, consistent with an acute **leukoencephalomalacia.** This has been reported overseas in horses, donkeys and mules, and is caused by ingesting fumonisin toxin from a species of the fungus *Fusarium* that grows in warm conditions on cereals such as maize.

A 10-month-old Standardbred colt from Otago had a long history of ill-thrift, ventral oedema and an audible heart murmur. The colt deteriorated further and died. Necropsy revealed marked congestion of the liver and an enlarged right ventricle. On further inspection the heart was found to have incompletely developed leaflets of the right atrioventricular valve, consistent with **congenital valvular dysplasia**.

Cervine

Seven hundred in-calf hinds in three mobs on a large Southland deer farm

were given a copper injection when mustered for tuberculosis testing, as previous investigations had identified copper deficiency on this property. One of them was found dead the next day and on the following day ten more were found dead. Necropsy of several revealed large areas of haemorrhage throughout the viscera and an enlarged, discoloured liver. The copper concentration of the one kidney tested was 138 µmol/kg (reference range 0–157), which was in itself unremarkable, but liver histology from another hind found severe massive hepatic necrosis, which was consistent with **copper toxicity**.

Porcine

Piglets were dying on a small piggery in the Rotorua district. The property was rearing 12 piglets and at nine weeks of age, four had died and one was poorly thriven and had diarrhoea. This piglet was euthanased and necropsied. Histopathological examination of the tissues revealed a necrosuppurative colitis. Silver staining showed spiral-shaped, elongate bacteria in the affected glands and on the surface of the mucosa, where they were associated with erosions and inflammation. These findings were consistent with **swine dysentery** caused by ***Brachyspira hyodysenteriae***.

On another property in Taranaki, five-week-old piglets were rapidly losing body condition, then they developed diarrhoea before dying. Histopathology of an affected piglet found evidence of necrosuppurative colitis with silver-positive bacteria present both in and on the lesions, consistent with swine dysentery induced by ***B. hyodysenteriae***.

Three piglets had died on a small pig farm in the Manawatu rearing 30 piglets, and four others had diarrhoea. Testing of faeces identified ***Escherichia coli* K88** as the aetiology of the diarrhoea.

Llamoid

A two-year-old female Huacaya alpaca from the East Coast was in light condition and was recumbent for long periods. Blood chemistry revealed a marked decrease in serum phosphorus (0.68 mmol/L; reference range 1.35–2.8), suggesting **osteomalacia induced by phosphorus deficiency** that was caused either by a lack of dietary phosphorus or by disease processes that limited phosphorus absorption. Osteomalacia and rickets have been previously seen in New Zealand alpacas with serum phosphorus concentrations of 0.81 ± 0.13 mmol/L (Hill *et al.,* 1994).

Canine

A one-year-old Japanese Akita dog from Auckland had been vomiting for two days was anorexic, lethargic and had developed apparent urinary incontinence. On two occasions the urine was minimally concentrated or isosthenuric (specific gravity 1.010 or 1.018; reference range > 1.030), with mild proteinuria and small quantities of blood found on dipstick examination. Culture of a submitted urine sample did not produce anything significant. Haematology revealed a neutrophilia consistent with inflammation (27.4 x 10^9/L; reference range 3.6–11.5 x 10^9). Serum chemistry revealed severe azotaemia, with elevated urea (27.2 mmol/L; reference range 2.5–9), creatinine (440 µmol/L; reference range 48–109) and phosphorus (3.06 mmol/L; reference range 0.92–1.82), which, given that the urine was minimally concentrated, was consistent with acute renal failure. Ultrasound examination indicated bilateral renal disease, and the dog was euthanased. Histopathology of kidney samples revealed large numbers of refractive, tubular crystals consistent with oxalate, associated with tubular damage and interstitial inflammation. The urine sample tested positive for glycolic acid at a hospital laboratory, confirming a diagnosis of **ethylene glycol toxicity**.

A litter of eight-week-old Catahoula Leopard Dog puppies in Northland presented with one dead and another three drooling, with malaise. They had been vaccinated for canine parvovirus-2 one week previously. Histopathology of post-mortem samples from the dead puppy showed severe diffuse mucosal epithelial loss and atrophy in the small intestine, and multifocal necrosis with centrilobular atrophy in the liver, consistent with **canine parvovirus-2 infection**, and perhaps liver damage caused by secondary sepsis.

A 15-month-old neutered male Bernese Mountain Dog from Otago presented with pyrexia though it was still eating and drinking normally. The pyrexia did not respond to conservative treatment and steroid-responsive meningitis was diagnosed on clinical grounds. Two months later the dog again presented with pyrexia but this time showed frequent episodes of collapse after walking a short distance. This was thought to be the result of spinal cord disease, and **neosporosis** was suspected. A *Neospora* IFAT was >1:2 000 (reference range negative), confirming neosporosis caused by ***Neospora caninum***.

An 18-month-old spayed Weimaraner bitch presented with recurrent haemorrhage including gastrointestinal haemorrhage, periorbital and muzzle swelling with haemorrhage, and nasal haemorrhage. A coagulation profile showed mild thrombocytopenia (150 x 10^9/L; reference range 200–500 x 10^9). Routine haematology showed no anaemia but a marked inflammatory leukogram with increased neutrophils (32.1 x 10^9/L; reference range 3.6–11.5 x 10^9), band cells (1.6 x 10^9/L; reference range 0–0.5 x 10^9), monocytosis (1.6 x 10^9/L; reference range 0.2–1.5 x 10^9) and lymphocytosis (5.3 x 10^9/L; reference range 1–4.8 x 10^9). This resembles the clinical syndrome described as **recurrent and persistent infections in related Weimaraner dogs** and the **hyperinflammatory syndrome of Weimaraners** (Angles 2007; Studdert *et al.* 1984).

Four of a litter of eight Dogue de Bordeaux puppies from Taranaki died over a period of a week. The puppies were two days old when they started dying. Post-mortem examination of one puppy revealed firm consolidation of the left lung and fibrinous adhesions between the lung and parietal pleura. Histopathology confirmed severe regionally extensive fibrinosuppurative and necrotising bronchopneumonia with bacterial colonies. Culture of fresh lung tissue produced a moderate growth of *Escherichia coli* and a light growth of alpha-haemolytic streptococci. A diagnosis of **neonatal bacterial pneumonia** was made. Outbreaks of bacterial infection, including pneumonia, are a major cause of mortality in neonatal puppies. Factors that predispose to infection in puppies include endometritis and vaginal discharges in the bitch, and dystocia, environmental exposure and underlying congenital or viral disease in the pups.

Lagomorph

A nine-month-old Flemish Giant rabbit from Auckland was admitted for routine de-sexing. At surgery, the right uterine horn was found to be replaced by a large cystic structure, and there were multiple

smaller cystic structures grouped around the uterine body. Histopathology showed that the largest cyst was lined with a columnar epithelium, while the smaller cysts contained cestodes with a thick, smooth tegument and a parenchyma containing calcareous corpuscles. These signs were consistent with a **mesonephric duct cyst** concomitant with an infestation of ***Cysticercus pisiformis*** **(cestode)** larvae.

Feline

A one-year-old Burmilla from the Auckland region had a purulent left ocular discharge that was not responding to treatment with a combination of dexamethasone, neomycin sulphate and polymyxin B. A swab was positive for ***Chlamydophila psittaci*** antigen by ELISA, confirming a diagnosis of **chlamydophilosis**.

A five-year-old British Shorthair cat from the Auckland region had a lump on the right forelimb near a digit. Cytological examination of an aspirate from the mass showed pyogranulomatous inflammation with intracellular bacteria consistent with mycobacteria, and PCR testing of a smear identified the presence of ***Mycobacterium lepraemurium***, the agent generally associated with **feline leprosy**.

A four-year-old Domestic Shorthair cat from Auckland had dry, scabby and alopecic areas on its nose and ears. A potassium hydroxide preparation of hair samples yielded fungal hyphae and spores; subsequent culture identified growth of ***Microsporum canis***, the common agent of **ringworm**.

A four-year-old neutered male Burmese cat presented at an Otago veterinary clinic with anorexia and abdominal pain. A radiograph showed abnormal intestinal morphology, so a laparotomy was performed and enlarged mesenteric lymph nodes were seen. Biopsy and histopathology of enlarged mesenteric and colonic lymph nodes identified reactive lymph nodes filled with increased numbers of neutrophils and macrophages in the cortical sinuses. These findings were suggestive of a bacterial **enterocolitis**. Both ***Salmonella*** **Typhimurium phage type 135** and ***Campylobacter jejuni*** were cultured from the faeces of this cat.

A three-year-old spayed female domestic cat from Otago was found dead after a short period of anorexia and ataxia. A necropsy revealed consolidated lungs, a dark liver and yellow fluid in the abdomen. Histological examination of the kidney revealed large numbers of oxalate crystals in the renal tubules, confirming **ethylene glycol toxicity**. As this was the third cat in this household to die with similar signs, a malicious poisoning was suspected.

An eight-month-old Bengal cat from the Waikato presented with chronic diarrhoea. ***Tritrichomonas fetus*** was identified in the faeces by PCR testing. In addition, ***Salmonella*** **Typhimurium phage type 56** was cultured, confirming that multifactorial aetiologies contributing to the diarrhoea.

A six-year-old Domestic Shorthair cat from Hawke's Bay presented with lethargy, coughing, vomiting and a thoracic effusion. A fluid sample from the thorax had a nucleated cell count of 85.5 x 10^9/L (reference range < 1.5 x 10^9) and total protein of 54.0 g/L (reference range < 25). A differential revealed 74 percent eosinophils, 4 percent neutrophils and 22 percent lymphocytes. There were very occasional large macrophages. The majority of lymphocytes were small, without obvious nucleoli. The numbers of eosinophils were so high that a paraneoplastic syndrome mediated by interleukin-5 (where neoplastic lymphocytes stimulate the production of, and attract, eosinophils) was suspected. **Lymphoma** was the most likely cause of the **eosinophilic effusion**. No masses were noted in the thorax, but the fluid may have obscured them.

A 12-year-old neutered male Domestic Shorthair cat from the Rotorua district developed a large necrotic area of the skin and subcutis in the axilla. There was under-running of the skin and purulent discharge. Histology revealed extensive multinodular pyogranulomatous inflammation of the dermis and subcutis, with numerous intralesional fungal hyphae and spherical yeast-like structures. A non-*albicans* *Candida* species was cultured from the wound, confirming an unusual case of **subcutaneous candidiasis.**

Avian

An adult kea (*Nestor notabilis*) was found dead in an Otago aviary that it shared with another kea. Necropsy revealed wasting of the pectoral muscles and scant fat reserves. The liver lead concentration was high, at 19 mg/kg (toxic level > 5), confirming **chronic lead toxicity**. The source was found to be lead flashing inside the cage. A blood sample from the other kea tested negative for lead.

A four-year-old male King Parrot (*Alisterus scapularis*) was found dead in its cage in Otago. Necropsy revealed an enlarged liver and numerous pale pin-point foci over the surface. Culture of the liver isolated a heavy growth of ***Yersinia pseudotuberculosis*****.**

New Zealand Veterinary Pathology

Bovine

A group of one-year old cattle in the Taupo district exhibited neurological signs after being drenched with a combination drench that contained levamisole, abamectin and ivermectin. At least one animal died. Histology on the submitted samples revealed a moderate lymphoplasmacytic enteritis and an abomasitis, with numerous coiled **nematodes** visible in abomasal glands. Analysis of the liver revealed a levamisole level of 6.5 mg/kg (normal therapeutic dose level = 8). This was a high level considering it was recorded some time after dosing (though the time scale was not evident from the history), and it suggested that the cattle were likely given significantly more than the normal dose. Toxic doses can be as low as 16 mg/kg by the subcutaneous route, so the therapeutic margin for this drug is very narrow (Parton *et al*., 2006). **Levamisole toxicity** was considered the most likely cause of the neurological signs and deaths in this group of cattle.

Six animals out of a group of 25 yearling steers in Marlborough were pot-bellied, with a low body condition score, diarrhoea and dehydration. One died overnight. Some animals were also febrile. PCR for bovine viral diarrhoea on serum from three of the affected animals was negative. Full faecal analysis was only performed on one of the animals, but it had a strongyle egg count of 2 100 eggs per gram. *Salmonella* and *Yersinia* cultures were negative. **Intestinal parasitism** was diagnosed.

Two dairy calves from New Plymouth exhibited weight loss, diarrhoea and fever. Faecal egg counts were within normal limits, but faecal culture from one of the calves yielded a heavy growth of ***Yersinia pseudotuberculosis*** and **yersiniosis** was diagnosed.

Two mature dairy cows exhibited diarrhoea. Culture of faeces from both animals revealed the presence of ***Salmonella*** **Typhimurium**. **Salmonellosis** was diagnosed.

An R1 heifer in the Waitaki district died acutely on Goliath rape crop. **Nitrate toxicity** was suspected, and was confirmed by post-mortem analysis of the nitrate level in the aqueous humour, which was > 25 mg/L, consistent with death caused by nitrate intoxication.

A seven-year-old cow in south Waikato started losing weight 7–8 days post partum, appeared to be in pain and was euthanased. On necropsy the kidney was found to have multiple small pale foci on the capsular surface, and the abomasal mucosa was reddened, with scattered ulcerated areas. Histopathology on the kidney revealed a multifocal chronic interstitial nephritis with mixed lymphoid, suppurative and granulomatous inflammation. There was also a moderate membranous glomerulopathy. The abomasum was moderately autolysed but showed some evidence of haemorrhage. Histologic lesions were consistent with **multisystemic granulomatous disease**, which has been previously described in the Rotorua region.

Milk from five mature dairy cows with clinical mastitis was submitted for culture. The farm had a history of mastitis caused by ***Prototheca*** **spp.**, which were isolated from one of the samples. The other samples yielded no significant isolates.

A mob of 700 dairy cows in Southland had 12 animals affected by severe weight loss and scouring. Ten animals had died. Faecal swabs and faeces from three of four affected animals revealed the presence of ***Salmonella*** **Typhimurium**. Enteric **salmonellosis** was diagnosed.

Three calves under one week of age from a mob in the Manawatu had diarrhoea. Testing for the various causes of neonatal calf diarrhoea was undertaken. **Rotavirus** and ***Cryptosporidium*** were identified in faecal samples, but there was no evidence of *Salmonella* or *E. coli* K99.

A three-week-old indoor-housed dairy calf in Palmerston North was feeding and growing well, but had numerous scabs present over its entire body. Gram staining of material taken from scabs revealed the presence of Gram-positive organisms with the distinctive "railroad track" morphology of ***Dermatophilus congolensis***.

A group of 1–3-week-old calves in the Waitaki district looked depressed and had marked scour. Seven were tested for *Cryptosporidium* using a modified acid-fast test, and for rotavirus and coronavirus by faecal antigen ELISA. Rotavirus testing was positive in six animals, while all *Salmonella* cultures were negative. Neonatal calf diarrhoea caused by **rotavirus** was diagnosed.

A single milking cow from a herd in the Hauraki district had severe scour and weight loss. Culture of faeces yielded ***Salmonella*** **Typhimurium** and **salmonellosis** was diagnosed.

A group of 12 calves aged 2–4 weeks recently brought on to a property in the Waikato had severe enteritis. Three died. Three samples submitted for faecal analysis and culture all tested positive for ***Salmonella*** **Typhimurium**. Rotavirus was also detected by faecal antigen ELISA. **Enteric salmonellosis complicated by rotavirus infection** was diagnosed.

A calf rearer in the western Bay of Plenty had 25 animals die rapidly within 2 days. The animals were about a week old. Previous testing had been negative for rotavirus, coronavirus, *E. coli* K99 and *Cryptosporidium*. Faecal samples from two animals were submitted for salmonella culture and ***Salmonella*** **Bovismorbificans** was isolated from both.

A four-year-old cow on a Waikato dairy farm had a 15-cm-diameter spherical subcutaneous mass located in the caudal intermandibular space. The mass was primarily composed of densely packed collagen fibres interspersed by numerous small spindle cell nuclei. There was mild anisokaryosis but mitotic figures were rare (< 1 per 10 high-power microscope fields). **Fibroma** was diagnosed, but a well-differentiated fibrosarcoma remains a differential. Soft-tissue sarcomas are only rarely identified in the bovine.

Two four-month-old calves in the Manawatu exhibited diarrhoea and weight loss. They had been drenched 10 days previously and had been treated with Baycox. Faecal samples were submitted for culture and ***Yersinia pseudotuberculosis*** was isolated from both animals.

Ten out of 100 eight-week-old calves in the Rotorua district exhibited a watery, green scour. There was a previous history of *Giardia* on the farm, but the calves were bright. Faecal antigen ELISA for *Giardia* was positive. ***Campylobacter jejuni*** was also isolated from two cultured faecal samples but it is not unusual for this pathogen to be cultured from clinically normal calves. ***Giardia*** is the pathogen that was most likely to be significant in this case.

A group of 4–5-day-old calves in the Waikato exhibited severe scouring and four died. Testing for rotavirus and *Cryptosporidium* revealed very high numbers of **cryptosporidia** in faecal samples. Rotavirus was not detected.

A group of calves in the Kaipara area exhibited severe scouring. Calf scour panels performed on two animals were negative for *Cryptosporidium*, rotavirus and coronavirus but ***Salmonella*** **Typhimurium** was identified from both. Phage typing indicated that the serovar did not conform to any previously recognized phage type, and it was assigned the type name **RDNC-Sept 15**. The same phage type was also isolated from two scouring calves in the Rodney district.

Two 4-year-old cows from a herd in the Western Bay of Plenty were losing condition. Haematology showed a mild anaemia and *Theileria* spp. organisms were visible on red blood cells. Both cows also had moderate hypoproteinaemia and tested positive with a Johne's antibody ELISA. Protein-losing enteropathy caused by **Johne's disease** complicated by **theileriosis** was diagnosed.

A two-year-old steer from a research facility in the Auckland area exhibited nervous signs, with circling to one side and collapse. Histology of the brain revealed a marked encephalitis affecting the brain stem, consistent with **listeriosis**.

Six dairy cows in the Western Bay of Plenty appeared anaemic. Haematology revealed a moderate to marked anaemia, with the most severely affected cow having a haematocrit of 0.07 (normal range 0.24–0.40) and haemoglobin of 22 g/L (reference range 85–130). *Theileria* spp. organisms were visible on the haematology smears in five of the six cows. **Theileriosis** was diagnosed.

A group of 3–4-day-old dairy calves in Whangarei exhibited diarrhoea. Faecal

antigen ELISA testing revealed the presence of rotavirus and *E. coli* K99. Cryptosporidia were not present and cultures were negative for *Salmonella*. Enteritis caused by **rotavirus** and **enterotoxigenic *E. coli*** was diagnosed.

Ovine

Three sheep aged about eight months in Palmerston North died suddenly out of a group of 220. All were moderately autolysed by the time of necropsy, but the livers appeared dark and swollen. The animals had been grazed in a commercial orchard that was regularly sprayed with copper sulphate. Liver copper concentration in one animal was 6 200 µmol/kg (reference range 95–2 000), consistent with **acute copper toxicity**.

In a similar case, a hemorrhagic disorder was described in a group of ewes grazing in an orchard in Marlborough. The orchard was known to use copper sulphate. Necropsy of one animal that died suddenly revealed an enlarged, friable liver with marked jaundice. The kidneys appeared black. Kidney copper concentration was 910 µmol/kg (reference range 0–157), consistent with **acute copper toxicity**.

Fetal loss took place in 660 out of 1 100 hoggets on a property in Palmerston North. Culture of stomach contents from one of the aborted fetuses revealed no evidence of *Campylobacter*, *Listeria* or *Salmonella*. Histology on tissue from two ewes revealed multifocal aggregates of neutrophils and macrophages within the liver, accompanied by foci of necrosis in one animal. Examination of the placentas of both ewes revealed a necrosuppurative placentitis with leukocytoclastic vasculitis. An infectious aetiology, likely **salmonellosis**, was the primary rule-out.

A hogget from the Waipa district aborted. The flock had been vaccinated for toxoplasmosis and campylobacteriosis. Serology on heart blood from the aborted fetus was negative for *Leptospira* Hardjo and *L.* Pomona. Culture of stomach contents from the fetus yielded a pure growth of ***Listeria innocua***. While *L. innocua* is generally considered non-pathogenic, it has sometimes been associated with encephalitis. Its presence in a pure culture in this case supports the likelihood that it had a role in the abortion.

Forty hoggets out of a group of 500 animals in Marlborough died. Three that were necropsied by the referring veterinarian were markedly dehydrated and had watery caecal contents. Histology on one animal revealed multifocal suppurative hepatitis with cholangitis, suggestive of sepsis that may have been of gastrointestinal origin. The other animal examined histologically had evidence of a suppurative bronchopneumonia with a milder multifocal non-suppurative hepatitis. Culture of intestinal lymph nodes was negative for *Salmonella*, *Campylobacter* and *Yersinia* but ***Listeria ivanovii*** was isolated from the pooled caecal contents of two affected animals. *L. ivanovii* is associated with abortion in ruminants but, unlike *L. monocytogenes*, it has not previously been associated with gastroenteritis (Staples, 1997). Its role in the deaths of these animals remains unknown.

A 2 000-ewe flock in the Waitaki district experienced some abortions. The ewes were of mixed age, but only younger animals (hoggets and two-tooths) had been vaccinated for toxoplasmosis and campylobacteriosis. Histology on one of the submitted fetuses revealed the presence of scattered foci of gliosis in the brain, which may have been consistent with *Toxoplasma* abortion. Serology on fetal heart blood revealed a toxoplasma titre of 1:64, indicating in-utero exposure to *Toxoplasma*. Cultures for *Campylobacter* were negative. **Toxoplasmosis** was considered the most likely cause of abortion.

Two ewes in the Waitaki district died after aborting. One fetus had evidence of septicaemia, with intravascular proliferation of bacilli visible in several sections. This lamb's dam had a necrosuppurative endometritis and a multifocal ulcerative colitis. Histologic findings on the other fetus and ewe were less clear-cut but suggestive of **fetal septicaemia**. Culture of stomach contents and tissue from both fetuses yielded ***Salmonella* Brandenburg**, which was the likely cause of the abortions in this case.

An eight-week-old lamb from the Nelson area was recumbent, on a property where the farm manager reported a few "floppy" lambs each year. A post-mortem examination performed by the referring veterinarian revealed an absence of body fat. Histology revealed a marked severe chronic enteritis and coccidiosis with an acute hepatopathy that was characterised by the presence of cytosegrosomes. There was also evidence of cerebral oedema, likely secondary to hypoxia. **Coccidiosis** with evidence of terminal hypoxia was diagnosed.

Six out of 1 500 Merino hoggets brought on to a property in Marlborough showed hindlimb ataxia. They had come off vineyards and were being run with 1 800 hoggets from two other properties. None of the other hoggets had any clinical signs. Histology of the brain and spinal cord of these sheep showed low numbers of spheroids in the brainstem, accompanied by axonal swelling in the lateral and dorsal funiculi of the spinal cord and Wallarian degeneration in the ventral funiculi. These changes are most consistent with a **segmental axonopathy**, which has been previously described in New Zealand Merino sheep and is presumed to be a genetic disorder (Jolly, 2006).

Equine

A one-year-old Thoroughbred colt from the Waikato had marked hypoproteinaemia, with total serum protein of 31 g/L (reference range 52–72), albumin 16 g/L (reference range 28–38) and globulins 15 g/L (reference range 21–39). Serum amyloid A was markedly elevated at 3 409 mg/L (reference range 0–8), suggesting acute inflammation. There were also moderate electrolyte abnormalities including hyponatraemia and hypokalaemia, which were likely secondary to an enteritis. A sample tested positive by PCR for ***Lawsonia intracellularis***. In one study of *L. intracellularis* diagnosis in pigs, the sensitivity of PCR on faecal material was only 0.15, but the specificity was very high, at 0.97 (Swart *et al.*, 2009). Such high test specificity means *L. intracellularis* was the most likely cause of the enteropathy in this horse, but negative PCR tests would not have ruled out the disease.

A yearling Thoroughbred filly in south Wairarapa had a history of grade 4/5 lameness, with stifle joint effusion in the hindlimb. The serum amyloid A level was markedly elevated, at 382 mg/L (reference range 0–8). Examination of the joint fluid revealed a nucleated cell count of 52.7 x 10^9/L (reference range 0–0.5 x 10^9), which was primarily made up of

neutrophils. Cytology demonstrated rare small cocci visible within the neutrophils. Culture confirmed **septic arthritis** caused by ***Streptococcus equi* ssp. *zooepidemicus***.

A 15-year-old pony gelding in Palmerston North presented with colic, consistent pyrexia and occasional diarrhoea despite treatment with penicillin, gentamicin and metranidazole. Faecal culture revealed the presence of ***Salmonella* Typhimurium**. **Salmonellosis** was diagnosed.

A 16-year-old Clydesdale cross mare had an enlarging, ulcerated wound on the lateral aspect of the left rear cannon bone. Histology on a wedge biopsy taken from this wound revealed the presence of a **squamous cell carcinoma**, an uncommon diagnosis at this site in the horse.

Cervine

A deer farm in the Buller district experienced deaths across several age groups including yearlings and stags. There was no evidence of scours. One animal found dead in a paddock one morning was necropsied and samples were submitted by the referring veterinarian for histologic examination, which revealed the presence of a **lungworm** (***Dictyocaulus*** spp.) infection. ***Ostertagia*** spp. strongyles were visible histologically in the abomasum. A second submission from the same property also had evidence of a severe lungworm infestation. Endoparasitism, including gastrointestinal parasitism and lungworm, appeared to be a problem for this property.

Caprine

A dairy goat operation in the Waikato had clinical evidence of fatty liver disease in does coming up to kidding. Blood samples from five animals all had marked increases in beta-hydroxybutarate ketone bodies (up to 14.7 mmol/L; reference range 0.1–1.5), indicating a negative energy balance. Calcium levels were also decreased (down to 1.84 mmol/L; reference range 2.00–2.54), which likely reflected a decreased feed intake. Marked **ketosis** was diagnosed.

A dairy goat operation in the Waikato had a problem with scours and decreased production in yearling goats. Faecal cultures for enteric pathogens on three animals revealed the presence of ***Yersinia pseudotuberculosis*** in one animal and ***Campylobacter coli*** in the other two. *C. coli* is only rarely identified as a cause of enteritis in ruminants, and is a common contaminant of goat carcasses at slaughter (Lazou *et al.*, 2014). *Y. pseudotuberculosis* may be the more important isolate in this case.

A castrated male Boer goat used for research in Auckland was in good body condition but was found moribund, lying on its side and unable to rise. Necropsy by the submitting veterinarian revealed the presence of abundant fluid in the chest and a hard, "grapefruit-sized" mass in the mediastinum. Histology revealed that the mass was composed of lymphoid cells and **mediastinal lymphoma** was diagnosed.

Several two-year-old goats on a Waikato property exhibited central nervous system signs. They were treated for polioencephalomalacia with thiamine and there was some evidence of a response. Histology on one of the affected goats revealed the presence of a chronic lymphoplasmacytic and suppurative encephalitis, consistent with **listeriosis**.

Canine

A litter of two-month-old puppies in Whangarei developed diarrhoea and anorexia. They had been fed raw chicken mince. Faecal egg counts revealed large numbers of ***Toxocara canis*** eggs (1 400 eggs/g) and occasional coccidial oocysts, and ***Campylobacter jejuni*** was also isolated from the faeces. **Campylobacteriosis** and **roundworm infection** was diagnosed.

Lagomorph

A litter of rabbits exhibited progressive neurological disease, with head-tilt, rolling and splayed legs. Sections of brain tissue submitted for histology revealed several small foci of gliosis in the grey matter of the cerebrum. These lesions were consistent with ***Encephalitozoon cuniculi*** infection.

Amphibian

An Archey's frog (*Leiopelma archeyi*) from a zoological collection in Auckland had a swollen right leg with bone lysis seen on X-ray. Histologic examination of tissues from the leg revealed marked granulomatous inflammation with intracellular acid-fast bacilli, consistent with ***Mycobacteria* spp. Mycobacteriosis**, likely arising from a penetrating skin wound, was diagnosed.

Zoo animal

A red-rumped agouti (*Dasyprocta leporina*) held in a zoological collection was lethargic, with diarrhoea and muffled heart sounds. Faecal culture isolated ***Campylobacter jejuni*** and ***Yersinia pseudotuberculosis***. Given the systemic illness affecting this animal, **yersiniosis** was considered the most likely cause of the clinical signs.

A spider monkey (*Ateles geoffroyi)* in a collection in the Auckland area died suddenly. Histology revealed a necrosuppurative hepatitis with intralesional short bacilli. Similar lesions were also seen in the mesenteric lymph node. The lung also had evidence of a suppurative interstitial pneumonia with intralesional bacilli. No lesions were found in the intestine. ***Yersinia pseudotuberculosis*** was isolated from the intestinal contents, lymph node, peritoneal fluid and liver of this animal. Septicaemic **yersiniosis** was diagnosed.

An 11-year-old cheetah (*Acinonyx jubatus*) held in a collection was euthanased after a history of polydipsia and inappetence for about three days. Blood chemistry performed before euthanasia indicated marked azotaemia. Histology revealed a chronic active pyelonephritis with medullary interstitial amyloid. A moderate to marked lymphoplasmacytic gastritis was also present. Special stains confirmed the presence of amyloid in the kidney, and spiral-shaped bacteria in the gastric glands. A **chronic lymphoplasmacytic gastritis with *Helicobacter* and *Helicobacter*-like organisms** is described as an important cause of mortality in North American and South African captive cheetahs. **Renal amyloidosis** appears to occur secondarily to the chronic inflammation/immune stimulation caused by the gastritis (Lane *et al.*, 2012).

Feline

A four-year-old Domestic Shorthair cat in the Whakatane district exhibited recurrent diarrhoea at approximately monthly intervals. Testing of a faecal sample submitted to the laboratory gave a positive result on a giardia antigen ELISA test. **Giardiasis** was diagnosed.

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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 2015.

Exotic vesicular diseases ruled out

A veterinarian contacted MPI's exotic pest and disease hotline after examining a recently-calved three-year-old dairy cow with pyrexia and oral lesions. An Initial Investigating Veterinarian (IIV) visited the property, inspected the herd and clinically examined the affected cow and five others selected because they were either lame or in sub-optimal condition. The affected cow had calved 24 hours earlier and had since been down and unable to stand. The mouth lesions were limited to the tongue. On the dorsum of the tongue, lesions consisted of small (~ 5 mm) round to oval ulcerated areas with slightly raised edges. Lesions under the tongue consisted of small (< 5 mm) grey-white proliferative areas. The remainder of the mouth, including the gums, dental pad and hard palate, were normal, as were the feet, udder and vulva. No lesions were identified in any of the other cows examined.

Exotic vesicular disease was excluded on clinical and epidemiological grounds. Serum biochemistry indicated significant acute muscle damage but was otherwise unremarkable. Antigen and antibody ELISA tests for bovine viral diarrhoea virus (BVDV) gave negative results, and a molecular assay for ovine herpesvirus-2 (OvH-2, the cause of malignant catarrhal fever) was also negative. The cow received supportive care and routine antibiotic and NSAID therapy, but remained unable to stand and was euthanased four days later. Exotic disease was excluded and the investigation was stood down.

An experienced bovine practitioner called the MPI exotic pest and disease hotline to report suspected exotic vesicular disease in a milk cow in the Rangitikei. The three-year-old Ayrshire cow was part of a herd of 700 on the property. The practitioner had been contacted by the contract milker, who had noticed coalescing ulcerations affecting up to 50 percent of the lower muzzle and gingival mucosa. Photos sent by the veterinarian were reviewed by the Incursion Investigation team. Owing to the high risk that exotic vesicular-type diseases pose to New Zealand's primary industries, an IIV was dispatched to the property to examine the cow, herdmates and other farm stock, and to obtain travel and disease history. Meanwhile, the investigating team contacted Fonterra and put a stop order on milk pickup from this farm. The IIV reported to the Incursion Investigator, investigation details were discussed and the farm was determined to be negative for foot and mouth disease (FMD), based on clinical and epidemiological data. Timing included: notification by hotline at 13:19; IIV arrival at farm at 15:30; and negative status declared at 17:15, after which the stop order was immediately reversed. As part of follow-up, blood samples for the cow and two herdmates were sent to the Animal Health Laboratory (AHL) IDC (Wallaceville), where serology and PCR for endemic FMD "look-alike" diseases (including BVDV) were performed, along with tests for OvH-2 and infectious bovine rhinotracheitis (IBR) virus. Serology for IBR was positive for all three cows but all other tests were negative. However, the implications of this are unclear since IBR is endemic and exposure to it is not unlikely. IBR can cause nasal and gingival erosions and haemorrhage, but ulceration of the type seen here would be an uncommon manifestation.

A private veterinarian notified MPI of calves that were ill-thrifty, some of them presenting with what appeared to be oral erosions. The affected property reared about 2 500 calves received from six dairy farms, and about a hundred calves from all six farms were affected. On clinical examination calves were in poor condition and afebrile, with evidence of diarrhoea. A small proportion had erosive and proliferative lesions on the hard palate and tongue. Post-mortem examination revealed erosive lesions on the oesophagus. Gross examination of the abomasum indicated evidence of parasitism. Spleen and lesion tissue samples collected from two postmortemed calves were negative for BVD virus by PCR but were positive for papular stomatitis virus. Blood was also negative by PCR for BVD virus and OvH-2.

Blood biochemistry showed that the calves had low blood albumin, supporting the post-mortem finding of parasitism. Thus the calves were affected concomitantly with both a high parasite burden and papular stomatitis. Exotic disease was ruled out and the investigation was stood down.

BSE ruled out

A pathologist notified MPI of an inconclusive finding for transmissible spongiform encephalopathy (TSE) on histology of a cow brain as part of MPI's TSE Surveillance Programme. The two-year-old cow had been ataxic in the hind limbs, hyperactive, and became aggressive before dying three hours after the veterinarian's examination. Vacuolation of small numbers of neurons, consistent with TSE but most likely due to autolysis, was noted by the pathologist. The brain samples were sent to the Animal Health and Veterinary Laboratories Agency in the UK for histopathology and immunohistochemistry. No TSE-specific labelling that would indicate the presence of abnormal prion protein was detected

on immunohistochemistry, and second-opinion histopathology confirmed that the brain changes were most likely due to autolysis, so the cause of the vacuolation was determined to be autolytic.

Anthrax ruled out

A veterinarian contacted MPI to request exclusion of anthrax from a dairy cow with a bloody discharge from the nostrils and anus. The animal had been acutely affected with the syndrome and was unable to stand, but was alive. A whole-blood EDTA sample was collected from the peripheral blood vessels, and blood smears were made. No organisms consistent with *Bacillus anthracis* were seen on microscopic examination after staining with polychrome methylene blue at the AHL (Wallaceville), and the blood sample tested negative by culture for *Bacillus anthracis*. No historic cases of anthrax (> 50 years ago) had occurred in the same district as the affected cow, though there had been cases in the same region. No further acute deaths have occurred on the property. Anthrax was excluded as a cause of the clinical signs observed.

Enzootic bovine leukosis excluded

An MPI Verification Services veterinarian at a meatworks called the MPI exotic pest and disease hotline to report a suspect enzootic bovine leukosis (EBL) case. A one-year-old dairy heifer had multiple severely enlarged lymph nodes and enlarged kidneys at postmortem. The carcass had been condemned and a lymph node was collected. Histopathology at Massey University revealed malignant lymphoma (lymphosarcoma). EBL is caused by bovine leukaemia virus (BLV), an agent thought to be absent from New Zealand's dairy herd. However, there is also a sporadic, non-contagious form of lymphoma, and it is not possible to differentiate between them on the basis of histology. EBL is highly unlikely to occur in cows less than 2–3 years of age, but becomes much more likely in older BLV-infected animals, typically 5–6 years or older. ELISA testing through the Livestock Improvement Corporation had been carried out in conjunction with the histopathology, and was negative for BLV.

Brucella melitensis investigated

An AHL scientist called the MPI exotic pest and disease hotline to report a *Brucella* culture sample submitted by the Auckland District Health Board (ADHB) for *Brucella* species identification. The sample was from a 24-year-old man who had been diagnosed with brucellosis. Zoonotic *Brucella* species are not present in New Zealand production or companion animals. *B. melitensis* was confirmed following culture, PCR and gene sequencing. This agent occurs in sheep and goats and is most commonly found in Mediterranean countries and in the Middle East, central Asia and South America. The principal route of human infection is infected raw milk and dairy products made from raw milk. With input from an MPI Incursion Investigator, an ADHB Medical Officer of Health interviewed the patient, an Auckland-based foreign student who had been back to Kuwait during summer and was diagnosed with brucellosis on return. He did not have any connection with New Zealand farms, farm animals or animal-product processing, nor had he consumed raw milk or raw milk products in New Zealand. The ADHB and MPI were satisfied that the infection was acquired overseas and the investigation was closed.

Scrapie ruled out

A member of the public called the MPI exotic pest and disease hotline after three of seven goat kids aged eight months showed signs of neurological disease. Two had died, while the third had improved transiently when treated with penicillin and B vitamins. Testing by ELISA for caprine arthritis and encephalitis (CAE) was negative. Given the history and non-response to treatment, under the direction of an Incursion Investigator the property veterinarian euthanased the third kid and collected tissues for further investigation. Histopathology at the AHL (Wallaceville) revealed a severe bilateral fibrinosuppurative and necrotising ventriculitis and encephalitis, considered most likely of bacterial origin. Potential endemic pathogens included *Histophilus somni*, *Listeria* sp., *Pasteurella* sp. and other environmental agents that most likely accessed the choroid plexus and ventricles via the bloodstream. The lesion distribution was not typical of *Listeria,* and immunohistochemistry for *Histophilus somni* was negative. Transmissible spongiform encephalopathies were excluded on histopathological, clinical and epidemiological grounds. The affected kids, two of which were not born on the property, had all been hand-reared and weaned at four months of age, while the four unaffected kids had been reared on their dams. As the initial two kids were not examined post-mortem, there was no way of determining whether their lesions were similar to those identified in the third case. As a result there was no aetiological diagnosis and the investigation was closed after excluding exotic disease.

Exotic blood parasites excluded

A veterinary pathologist notified MPI of a possible blood parasite in a goat with severe anaemia. Blood smears showed small structures adherent to erythrocytes, which were thought to resemble mycoplasma-like structures. The blood parasite *Mycoplasma ovis* (formerly *Eperythrozoon ovis*) can cause anaemia in sheep and goats. It is known to be in New Zealand sheep, but has never been reported here in goats. Blood work from this goat showed severe anaemia (PCV 14 percent; reference range 27–42) and hyperglobulinaemia (74 g/L; reference range 19–63), possibly indicating chronic immune stimulation after blood loss or RBC destruction. PCR testing at the AHL (Wallaceville) was negative for generic haemotropic mycoplasmas, *Anaplasma phagocytophilum*, *Babesia* spp. piroplasm-type organisms (semi-nested) and *Theileria* spp. Common parasite agents of ruminant erythrocytes were also ruled out. Neither the identity of the structures nor the cause of the anaemia in this goat were determined. However, there are numerous non-parasitic causes of erythrocyte "dots" or stippling, including stain precipitates, residual ribosomal material (left over from the RBC maturation process), intoxication (e.g., with lead, caused by increased reticulocyte count and hence ribosomal material) and unexplained

artefacts. The identity of the dots was not further investigated in this case and the investigation was stood down.

EIA/EVA ruled out

A veterinarian called the MPI exotic pest and disease hotline to report a horse for surveillance of equine viral arteritis (EVA). The horse was a New Zealand-born eight-year-old Thoroughbred with oedema of the distal limbs. It was in contact with a horse that had recently been imported from Australia, where EVA is known to occur. Blood collected from the affected horse 10 days after clinical signs were first observed tested negative for EVA (EVA VNT < 1:2). The cause of the signs observed was not determined but the horse made an uneventful recovery.

A veterinary pathologist contacted MPI to report severe anaemia of unknown cause in a six-year-old horse. The horse was severely anaemic, with a haematocrit of 12 percent (reference range 29–46) and a mature neutrophilia (17.6×10^9/L; normal range $3–7 \times 10^9$) but a high-normal fibrinogen. The horse also had a unilateral anterior uveitis. The neutrophilia was suspected to be caused by inflammation, but this was questionable owing to a lack of hyperfibrinogenaemia. Exotic causes of marked anaemia in the face of inflammation include EIA and EVA, and there are many unusual endemic causes including autoimmune haemolytic anaemia or haemorrhage in the face of an inflammatory lesion. Samples were sent to the AHL (Wallaceville) for rule-out of EIA and EVA; tests for both agents were negative. On follow-up the vet indicated that the horse had been in somewhat poor body condition when first examined, had improved substantially on antibiotics and dewormers, and was now considered to be recovered. No cause of the clinical illness was determined but it is likely to have been multifactorial. The investigation was stood down.

Equine influenza ruled out

In September a report was posted on ProMED-mail, a website run by the International Society for Infectious Diseases, regarding the equine influenza outbreak (caused by EIV) in Malaysia. In the report, Malaysian Veterinary Services authorities suggested that the source of the infection could have been four horses imported from New Zealand via Singapore. An investigation was conducted into these horses and the properties from which they were exported, to confirm New Zealand's EIV-free status.

The horses were traced back to four properties in the Waikato, which were visited by MPI Incursion Investigation veterinarians, assisted by the properties' regular veterinarians. Horses at each property were examined for signs of respiratory illness, and any health events in the preceding few months were assessed. Horses that had previously been in contact with the exported horses were identified and blood samples were collected. There were no signs of respiratory illness on any of the properties, and there had been no recent illnesses of concern. In addition, Singaporean authorities were able to confirm that the horses that had been on the same flight as the Malaysia-bound horses showed no signs of respiratory illness after arriving in Singapore.

Blood samples from the in-contact horses were tested at the AHL (Wallaceville). All horses were negative by haemagglutinin inhibition testing for antibodies to EIV. The exported horses had tested negative for EIV by PCR of nasopharyngeal swabs as part of export requirements. As part of the follow-up investigation, blood samples collected during pre-export quarantine were tested for antibodies to EIV, all with negative results.

The timeframes of the horses' travel and quarantine periods were reviewed in relation to the incubation and infectious periods for equine influenza and the timing of the onset of clinical disease. This assessment supported the clinical and laboratory findings detailed above, indicating that it was not plausible that the horses could have carried infection from New Zealand to Malaysia. The assessment indicates that exposure would have occurred either during the horses' quarantine period in Malaysia, or soon after.

These findings confirmed New Zealand's EIV-free status. (See also the article "MPI reaffirms New Zealand is free from equine influenza", *Surveillance*, this issue, pages 4–5.)

EHV-1 myeloencephalitis excluded

In July an equine veterinarian called the MPI exotic pest and disease hotline to report sudden onset and rapidly progressive neurological signs in a three-year-old filly five days after the horse arrived on a stud farm. When standing, the horse tucked her hindlimbs under her abdomen and would only walk short distances, knuckling over on her fetlocks before falling into lateral recumbency. Apart from a heart rate of 60 there were no other clinical abnormalities, with normal mentation, normal temperature, good tail and anal tone and no cranial nerve deficits. The horse was euthanased. She had not been imported and the time of year further precluded exotic arboviral diseases. None of the other 60 equines on the property exhibited signs of disease. While EHV-1 is not an exotic disease, there has only been one recorded outbreak of its neurological manifestation (equine herpesvirus myeloencephalopathy) in New Zealand and it is a highly transmissible disease requiring prompt biosecurity measures to control spread, so further investigation was carried out. PCR tests on nasal swabs, cerebrospinal fluid and EDTA blood, along with a serum ELISA, were negative for EHV-1 at the AHL (Wallaceville). A subsequent necropsy, histopathological examination and EHV-1 PCR on a range of tissues at the Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University, Palmerston North, again ruled out viral neurological disease. The only abnormality seen was mild to moderate Wallerian-type demyelination of the dorsal and ventromedial fasciculi of all regions of the spinal cord, resembling that seen in wobbler's syndrome. A neurotoxin could also be responsible for the demyelination. Since EHV-1 infection had been ruled out, the investigation was stood down.

Exotic flaviviruses excluded

A veterinary pathologist called the MPI exotic pest and disease hotline to report neurological signs consistent with CNS lesions in a seven-year-old Miniature mare. The mare had presented with depression, pyrexia, anorexia and muscle fasciculations. She subsequently became

ataxic and had difficulty standing, so she was euthanased. The horse was New Zealand-born and had been on the one property since she was six months old. There was no history of risk movements onto the property and three in-contact horses remained clinically normal. Histopathology revealed severe multifocal non-suppurative meningitis with multifocal necrotising vasculitis in the brain, and spinal cord lesions, which collectively suggested an immune-mediated or viral aetiology.

The rare neurological manifestation of EHV-1 infection was ruled out by PCR testing at IVABS and by immunohistochemistry (IHC) testing at an overseas laboratory. Exotic flaviviruses including West Nile virus and alphaviruses were ruled out by PCR and IHC testing conducted at, or co-ordinated by, the AHL (Wallaceville). Given these findings and the lack of further cases, the lesions were considered likely to be the result of an immune-mediated encephalitis. Exotic disease was ruled out and the investigation stood down.

Ehrlichia canis confirmed

An AHL scientist informed the duty Incursion Investigator that a high positive IFAT titre (1:10 240) to *Ehrlichia canis* had been identified in a six-year-old Whippet bitch during routine pre-export testing. Follow-up with the owner determined that the dog had lived in Australia, Singapore and Korea before being bought to New Zealand two and a half years ago. Under the direction of an Incursion Investigator, routine haematology and biochemistry profiles were carried out. The blood work identified changes in the haemogram (mild non-regenerative anaemia (HCT 0.32), thrombocytopenia, lymphocytosis and neutrophilia) and serum biochemistry (mild azotaemia, mild hyperglobulinaemia and hypoalbuminaemia). These changes were potentially indicative of chronic-stage ehrlichiosis, but no parasites were identified in blood smears. A molecular assay was positive for *Ehrlichia* spp., and sequencing confirmed *E. canis*. The dog was given doxycycline therapy (10 mg/kg) for eight weeks and blood parameters returned to normal, apart from a persistent mild azotaemia and hypoalbuminaemia consistent with a nephropathy resulting from chronic *Ehrlichia* infection. Although *E. canis* is exotic to New Zealand and an Unwanted Organism under the Biosecurity Act 1993, no response was initiated because the risk was considered negligible owing to the lack of a suitable vector (the brown dog tick, *Rhipicephalus sanguineus*) in New Zealand and the effective treatment with antimicrobial therapy. In consultation with MPI's Response Team, the investigation was stood down.

Canine distemper excluded

A veterinarian called the MPI exotic pest and disease hotline to report an ill five-year-old Huntaway dog with no history of vaccination. Clinical signs were consistent with a potential aetiology of distemper, including pyrexia, bilateral nasal discharge, coughing and neurological signs including collapse and trembling. An immunochromographic assay carried out at a regional veterinary laboratory was positive for antibodies to distemper virus. Blood, conjunctival and nasal swab samples were collected and submitted to the AHL (Wallaceville). The virus neutralisation test confirmed the initial results and returned a weak positive titre (1:56) for canine distemper antibodies. However, PCR tests on whole blood and the swabs were negative for distemper virus. The dog was given routine antibiotic therapy and recovered uneventfully over the following few days. Distemper virus was excluded and the investigation was stood down.

Brucella canis excluded

An NZVP pathologist contacted MPI after receiving a submission of semen for bacterial culture from a recently imported dog that exhibited poor semen motility. The dog was otherwise clinically healthy, with no abnormalities detected in the reproductive organs. Serum and semen were submitted to the AHL (Wallaceville), where *Brucella canis* was excluded after both the *B. canis* rapid slide agglutination test (RSAT) and a molecular assay for *B. canis* returned negative results. The dog's poor semen quality was considered likely to be the result of travel-induced stress associated with importation from the US. Exotic disease was excluded and the investigation was stood down.

In another case, a veterinarian called the MPI exotic pest and disease hotline after neutering a dog and identifying unilateral swelling of the epididymis suggestive of epididymitis. The dog was New Zealand-bred. Serum was submitted to the AHL (Wallaceville), where *B. canis* was excluded by a negative *B. canis* RSAT. Exotic disease was excluded and no further action was required.

Haemorrhagic pneumonia investigated

Possible haemorrhagic pneumonia in a Greyhound dog was reported to MPI by a pathologist via the exotic pest and disease hotline. The dog was in a kennel with seven other Greyhounds, some of which had also become sick and were treated with antibiotics. An X-ray revealed severe consolidation of the lungs and other signs included bleeding from the mouth, haematuria and haemolysed red blood cells. The dog rapidly went into a coma and was euthanased. No postmortem was done, but culture of oropharyngeal swabs taken earlier yielded *Streptococcus equi* ssp. *zooepidemicus*. This bacterium has been linked to acute fatal pneumonia in dogs in several countries, and can cause outbreaks in kennelled dogs. It is not often isolated in New Zealand and even more rarely associated with clinical signs that are consistent with haemorrhagic pneumonia, which means it could be considered an emerging disease. The other seven dogs recovered fully with antibiotic treatment. The case was referred to Massey University researchers studying the causes of respiratory infections in New Zealand dogs.

Feline sporotrichosis excluded

A veterinary pathologist reported a six-year-old cat with suspected sporotrichosis, a dermatomycosis caused by the fungus *Sporothrix schenckii*. This disease occurs worldwide but a definitive diagnosis in animals has never been made in New Zealand. The cat presented with nodular skin lesions that were histologically consistent with sporotrichosis. Multiple fungi and yeasts were isolated on culture (including *Cystofilobasidium* sp., *Cladosporium* sp. and *Mucor* sp.) but *S. schenckii* was not detected by PCR.

Ornithobacterium rhinotracheale confirmed

A poultry veterinarian phoned the duty Incursion Investigator to discuss cases of upper respiratory disease he had seen in backyard fancy-breed chickens in Canterbury. The notifier had information from a poultry conference in Australia (Blackall 2015; Groves 2015), where the poor sensitivity of standard laboratory approaches (culture and conventional PCR) for detecting *Ornithobacterium rhinotracheale* had been noted, and details of a real-time PCR assay had been made available. Tracheal swabs from a historical case had been stored frozen, and fresh upper respiratory tract swabs were collected from a second, more recent case. Both sets of swabs were submitted to the AHL (Wallaceville) for testing, which identified a mixture of endemic bacterial respiratory pathogens, as well as *O. rhinotracheale* in two samples. Also, *Avibacterium paragallinarum* was detected in all five samples. *O. rhinotracheale* was detected using a newly implemented real-time PCR protocol (Adelwhab *et al*., 2013) after conventional PCR failed to detect the pathogen.

The outbreak was characterised by upper respiratory disease including sinusitis and localised exudative lesions ("canker") affecting areas of the mouth, tongue or pharynx, without death. Flocks responded well to routine antibiotic therapy. *O. rhinotracheale* has not previously been detected in New Zealand, and is an Unwanted Organism under the Biosecurity Act 1993.

As part of delimiting surveillance, respiratory swab extracts held at the AHL from previous poultry disease investigations were tested. Fifty-nine swabs from three commercial poultry farms (broiler, free-range layer, barn layer) that had upper respiratory tract disease associated with *A. paragallinarum* were tested for *O. rhinotracheale* by PCR, and 12 samples from each farm tested positive in the real-time PCR, while only one conventional PCR test was positive. The investigation concluded that *O. rhinotracheale* is widely distributed in both the commercial and backyard poultry sectors, and that during the current outbreak it was only detected thanks to more sensitive laboratory techniques than the tests used in historic investigations. Disease in these cases is thought to have been caused by a mixture of pathogens, including *O. rhinotracheale*. These findings were consistent with the literature (Chin *et al*., 2008), where *O. rhinotracheale* is most commonly identified as a co-infectant associated with other respiratory agents. In consultation with MPI's Response Team, the investigation was stood own.

Avian influenza excluded

A District Health Board called the MPI exotic pest and disease hotline to report that an influenza patient had more than half of her backyard chicken flock die in a short period. An Incursion Investigator interviewed the flock owner, who confirmed that 30 of 62 chickens had died over a six-month period. Initial deaths were among the 8–16-week-old age group, but later older birds died. Typical findings included weight loss and weakness, progressing to recumbency and death. In general, affected birds remained appetent. There were no clinical signs consistent with respiratory, gastrointestinal or neurological disease. Two moribund chickens were submitted for necropsy. Gross and histopathological findings were consistent with ill-thrift and emaciation caused by parasitism. Based on epidemiology, clinical signs and post-mortem findings, avian influenza and Newcastle disease were ruled out. The owner's influenza was subsequently typed and confirmed as regular seasonal influenza A subtype H3N2. Further investigation attributed the mortalities in the flock to the tapeworm *Davainea proglottina*.

AI/ND excluded

An AsureQuality veterinarian called the MPI exotic pest and disease hotline regarding an increased mortality event in day-old chicks at a poultry-breeding Transitional Facility. Only one line of chicks was affected, with lethargic birds and an initial 2 percent mortality rate that increased to almost 4 percent by day 5, whereas the other lines had almost nil mortalities. Postmortems conducted by the facility's veterinarian showed no sign of infectious disease pathology other than some lung congestion, but gross clinical signs might not be apparent with such a rapid onset of death. Dead chicks were submitted to the AHL (Wallaceville) for rule-out of exotic disease agents. Tests for avian influenza and avian paramyxovirus-1 (the cause of Newcastle disease) were negative by PCR on mixed tissues and intestine, and *Salmonella* culture on liver, yolk and intestine was negative. A moderate mixed growth of Gram-negative bacteria, including *E. coli*, was seen on pooled lung culture. Histopathology subcontracted to Massey University revealed severe acute Gram-negative bacterial meningitis and septicaemia in all the submitted chicks. It was considered likely that the deaths resulted from a humidity problem with the incubator used for this line of chicks, leading to excessive moisture or dryness of the eggs. This could favour bacterial overgrowth during incubation and external contamination of the eggs, leading to infection during the hatching period.

Lotmaria passim confirmed

An AsureQuality apiary officer notified MPI in late October 2014 of a bee mortality event occurring in the Coromandel district. Bee samples were collected from some of the most severely affected colonies and tested for exotic agents. Samples were negative by PCR for European foulbrood, Israeli-associated paralysis viruses and tracheal mite. However, *Lotmaria passim,* an agent not previously detected in NZ, was identified. This agent is a trypanosomatid that infects the honeybee and has previously been misclassified as *Crithida mellificae*. First detected in Australia in the 1960s, it has only recently been associated with winter losses in bees. However, the present investigation did not show an association with bee mortality, and no interaction with high levels of *Nosema ceranae* was found, even though interactions reported in the literature for winter losses relate to summer levels of these agents. *L. passim* has subsequently been identified in beekeeping operations outside of the Coromandel district, suggesting it has been here for some time but only recently detected thanks to new developments in molecular diagnosis. The reported mortality in this operation is likely to have been associated with the high levels of *N. ceranae* present in affected colonies.

Honey bee mortalities investigated

An Apiculture Officer was contacted by a beekeeper following bee mortality in a single colony in an apiary. The affected apiary was located in a remote area of the west coast of the South Island where there was no obvious pathway of introduction for an exotic agent. Adult bee samples were negative by PCR to the endemic agents *Nosema ceranae*, *N. apis*, *Lotmaria passim*, black queen cell virus and chronic bee paralysis virus, and the exotic agent Israeli acute paralysis virus (IAPV). However, they tested positive for deformed wing virus and Kashmir bee virus. Samples were examined for mites and a few (< 10) *Varroa destructor*, mould mite (*Tyrophagus* sp.) and soil mites (unidentified species of the order Oribatida) were found associated with dead bees. No exotic parasites were found on a sample of bees examined for tracheal mite and small hive beetle. It appears likely that varroa mites and bee viruses were involved in the mortality observed.

A North Island beekeeper called the MPI exotic pest and disease hotline in late winter to report failing hives. Of 400 hives at multiple sites, 240 were affected, with only just enough bees to cover brood. The beekeeper saw evidence of diarrhoea in some hives and noted there were more dead bees outside the hives than would be expected for the numbers in the hives.

In consultation with an AsureQuality Apiculture Technical Adviser (ATA), bees were submitted for testing. Samples were taken from three failing hives and one strong hive. The exotic agents Israeli acute paralysis virus and tracheal mite (*Acarapis woodi*) were excluded after testing at the AHL (Wallaceville) and Plant Health and Environment Laboratory (PHEL) (Tamaki), respectively. Concurrent quantitative PCR assays for endemic agents revealed high to very high levels of *Nosema apis* in the failing hives, with only moderate levels in the strong hive. High to very high levels of *Lotmaria passim*, deformed wing virus and Kashmir bee virus were identified in all four hives but *N. ceranae* was not detected. The diarrhoea was attributed to *N. apis* infection of virus-stressed bees.

The ATA discussed the results and hive management with the notifier. High endemic virus loads reflect poor autumn management of varroa mites: treatment must be done early in the autumn so that healthy winter bees can be reared that will survive into spring. In this case, varroa treatment had been applied late in autumn. ATAs are finding that varroa mite is a growing problem that stems from a combination of beekeeper inexperience and poor timing of varroa management. There are also anecdotal reports of emerging miticide resistance. Exotic disease was excluded and the investigation was stood down.

A hobbyist beekeeper called the MPI exotic pest and disease hotline to report seeing dead bees around his hives daily over the previous two to three months, predominantly near the entrance to one of his two hives. The number of dead bees seen daily had recently dwindled from about 200 to about 60. An AsureQuality Apiary Advisory Officer (AAO) visited the apiary and assessed both hives as being in normal to strong condition. The beekeeper said he had seen evidence of parasitic mite syndrome (PMS, caused by *Varroa destructor*) in the hives in autumn (three months earlier), with the infestation resolving after routine treatment.

Bees were collected from both hives and submitted to the PHEL (Tamaki) and AHL (Wallaceville). A search for tracheal mite (*Acarapis woodi*) and external mites (particularly the exotic species *Tropilaelaps clareae*) was negative. Molecular assays were positive for deformed wing virus in one hive and black queen cell virus in the other, while both hives were positive for the trypanosome *Lotmaria passim*. These findings were consistent with an infection of endemic virus and other agents as a sequel to a varroa infestation, which can serve as a vector for pathogens. Exotic disease was excluded and the investigation was stood down.

A commercial beekeeper called the MPI exotic pest and disease hotline to report a large proportion of dead and ill-faring hives when carrying out the spring check of his approximately160 hives. An AAO visited the apiary, collected samples from four affected and two unaffected hives, and submitted them to the PHEL (Tamaki) and AHL (Wallaceville). Examination for the exotic mites *Acarapis woodi* (tracheal mite) and *Tropilaelaps clareae* (Asian bee mite) was negative in all samples, but external bee washings (from about 150 bees) yielded about a hundred *Varroa destructor* mites on bees from each unhealthy hive. Molecular assays were negative for the exotic Israeli acute paralysis virus. Testing for endemic agents identified deformed wing virus and black queen cell virus in bees from all hives tested. The trypanosome *Lotmaria passim* was present in five of the six hives, while chronic bee paralysis virus was present in two unhealthy hives. On further questioning it was determined that the beekeeper's staff had mistakenly used only one Apistan varroa treatment strip per hive in the autumn, instead of the required two. These findings were consistent with an infection of endemic virus and other agents as a sequel to poor varroa control. Exotic disease was excluded and the investigation was stood down.

A Wairarapa beekeeper called the MPI exotic pest and disease hotline to report that two of his three hives were failing. He reported a patchy brood pattern with chewed cappings, evidence of sac brood virus and mature but dead, unhatched bees. A brood sample was submitted to the AHL (Wallaceville), where molecular assays for the endemic American foulbrood and exotic European foulbrood returned negative results. An ATA discussed colony management with the beekeeper and concluded there had been poor varroa mite management in the autumn, resulting in high loads of endemic viruses that shortened the lifespan of the bees. In this case the colony had tried to rear some brood but this had largely failed owing to limited numbers of adult bees. The beekeeper changed his varroa mite control programme and subsequently reported that the affected colonies were showing signs of recovery.

A beekeeper on the west coast of the South Island called the MPI exotic pest and disease hotline to report the failure of his one hive, with an increased number of dead bees observed. An investigation was instigated with input from an AsureQuality ATA. A sample of dead bees was submitted to the PHEL

(Tamaki) to rule out the exotic tracheal mite (*Acarapis woodi*). Although that test was negative, significant numbers of varroa mites were seen. Once again, it was concluded that inappropriate varroa control during autumn had resulted in a proliferation of endemic viruses and played a role in the loss of the colony. Dead bees around the hive could also be consistent with a poisoning event contributing to the colony loss, but the beekeeper did not pursue this. Exotic disease was ruled out and the investigation closed.

Exotic insects excluded

A Christchurch beekeeper called the MPI exotic pest and disease hotline to report a hive that had lost its bees and contained unusual insects and larvae. These were identified at the PHEL (Tamaki) as wax moth larvae and bumble bees. Wax moths are opportunist scavengers that will take over a weak hive. An AsureQuality Apiary Officer attributed the weak state of this hive to varroa mite mismanagement. Also, in winter, bumble bees can attempt to hibernate in warm beehives but are attacked and killed by bees. There was no indication of exotic disease and the investigation was closed.

Exotic ticks excluded

A veterinarian contacted MPI to report finding ticks on a dog soon after it had been walking in an Auckland park. The veterinarian was concerned that the ticks might not be the common endemic New Zealand cattle tick. Five ticks were collected and submitted to the PHEL (Tamaki), where they were identified morphologically and by molecular assay as *Haemaphysalis longicornis,* the New Zealand cattle tick. Exotic ticks were excluded and the investigation was stood down.

Exotic ticks intercepted

A missionary who had recently returned from six years living in Thailand called MPI's exotic pest and disease hotline to report having found two ticks. The un-engorged ticks were found on her arms about 10 days after her return, and soon after she had put on clothing from a suitcase. She had already squashed and discarded both ticks but one was retrieved and submitted to the PHEL (Tamaki). This tick was identified morphologically and by molecular assay as *Rhipicephalus sanguineus* (the brown dog tick), which is an Unwanted Organism under the Biosecurity Act 1993. In Thailand, the missionary had had a dog and five cats, on which she occasionally found ticks. The preferred host for this tick species is dogs. In consultation with an expert acarologist, fumigation of the premises and treatment of clothing and a suitcase was carried out. Establishment was prevented and the investigation was stood down.

A GP in Christchurch called the MPI exotic pest and disease hotline to report removing two ticks from a patient who had returned from a holiday in Australia. The ticks were identified by the PHEL (Christchurch) as nymph stages of the exotic *Amblyomma triguttatum* (ornate kangaroo tick). Macropods are the preferred final host of this species. In Australia, the tick has been identified as a carrier of *Coxiella burnetii* (the agent of Q fever), so the ticks were tested by PCR for this bacterium at the AHL (Wallaceville), with a negative result. A reference acarologist observed that, given what is known about the behaviour of this species and its climatic requirements, removing and destroying the ticks would have terminated the incursion. The GP was notified of the results and reported that the patient remained in good health. The investigation was closed.

Risk goods investigated

A member of the public called the MPI exotic pest and disease hotline to report some beeswax that might have originated from overseas. The caller alleged that a beekeeper had illegally imported a foundation wax press from Russia and that this equipment contained wax. An Incursion Investigator interviewed the beekeeper to assess the credibility of the allegation. The beekeeper advised that he had imported a foundation wax press from Latvia, and it had contained a small amount (60 grams) of beeswax that was used to align the silicone matrices during transit.

While it is not illegal to import new products for use in the bee industry, bee products and material that has been in contact with bees overseas are prohibited. In this case, even the small amount of beeswax that accompanied the press could harbour exotic pathogens that would pose a biosecurity risk. The beekeeper was aware of this risk, had alerted the exporter accordingly, had destroyed the wax by burning and had buried the ash. The Incursion Investigator also contacted the exporter to reiterate New Zealand regulations regarding bee-related products. The exporter responded that the wax had been sterilised, but agreed in future to use an alternative (i.e., paraffin wax), or not export the product to New Zealand. As any biosecurity risk had been mitigated and the pathway had been closed, the investigation was stood down.

A beekeeper alerted an AsureQuality Apiculture Officer to a New Zealand product that could potentially act as a vector for bee diseases. The product is a feed for wild birds that includes honey powder and pollen. If these ingredients originated from overseas the product could potentially harbour exotic pathogens. If the ingredients were sourced from within New Zealand, and were not heat-treated, endemic diseases such as AFB could be spread. An Incursion Investigator contacted the manufacturer to determine the origin of the bee-related ingredients and the composition of the product. The wild bird feed contained both honey powder and pollen. The honey powder was sourced from New Zealand honey and the pollen was bee-collected from hives in Oamaru and frozen and dried to the standard required for human consumption. The Apiculture Officer reviewed this information and agreed that no further action was required by MPI, but the Management Board of the National American Foulbrood Pest Management Plan may wish to do a risk assessment. Having established that there was no risk of exotic pathogens, the investigation was closed.

A beekeeper contacted MPI via the exotic pest and disease hotline with concerns about an imported honey product used in the food industry. The beekeeper believed that the product was not regulated by MPI and therefore had not been treated to kill potential bee pathogens. An Incursion Investigator requested advice from the MPI Animal Imports Team on the treatment and regulatory requirements for imported

bulk dehydrated honey. Honey powder has been imported into New Zealand for a long time and is used in baked goods, sauces and mixes. MPI Import Standard assessment ensures that the powder has been spray-dried, which means that it has been processed at extremely high pressure and heat, making it a negligible-risk commodity. This product does not require a permit for importation, but the importer is required to provide a manufacturer's declaration that records the time and temperature of treatment for each consignment before it is released. As the product met these standards, there was no biosecurity risk and the investigation was closed.

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Quarterly report of biosecurity responses

The Biosecurity Response Group was managing 42 high-priority active responses and six low-priority active responses (i.e., full responses were not initiated) at the end of the July to September 2015 reporting period. During that period five new responses were initiated and three were closed or stood down. **Figure 1** shows the number of active biosecurity responses from September 2014 to September 2015.

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

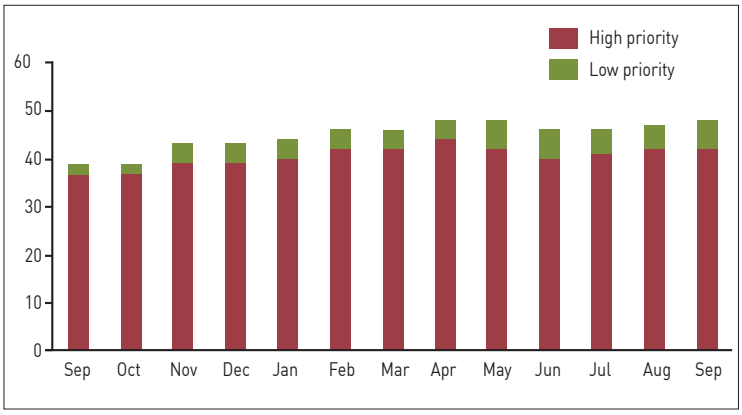

Figure 1: Active biosecurity responses from September 2014 to September 2015.

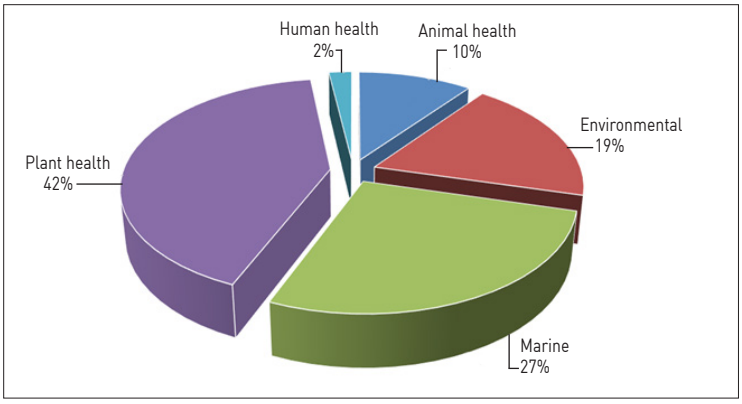

Figure 2: Sector breakdown for open responses at the end of the July – September 2015 reporting period.

Table 1: National interest pests responses, 2015

Response name	Scientific name	Sector affected	Organism type
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* ssp. *dioica*	Environment	Plant

The Group manages biosecurity responses affecting plant, environment, animal and marine sectors (**Figure 2**). The one human-health-related response was to imported grapes infested with spiders.

The Biosecurity Response Group also manages the National Interest Pests Responses, which are eradication programmes for nine pests of national interest (**Table 1**). These programmes run for several years. The species remain unchanged from 2014.

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Snapper mortalities: finding a cause

Background

The common inshore fish known as snapper or tamure, *Chrysophrys auratus* (Roberts *et al*., 2015) is found predominantly in the warmer waters of the North Island of New Zealand, but can also be found, though more sparsely, in the South Island from approximately Christchurch north (Crossland, 1981). A popular food and sports fish, it belongs to the sea bream family (Sparidae), which includes species from Australia, Asia and the Mediterranean.

In late December 2014 snapper were reported washing up on the shores of Doubtless Bay in Northland (**Figure 1**), a known breeding ground for this species (Crossland, 1981). The fish were reported to be in poor condition, with reddening along the belly and a distended abdomen filled with fluid. Snapper was the only species reported to be affected, so a pathogenic rather than an environmental cause was suspected. If poor environmental conditions were the sole cause of the mortalities, it would more likely impact on many different species in the area at the same time, whereas a pathogenic agent is more likely to affect a specific host.

Figure 1: Location of snapper mortalities

The Ministry for Primary Industries (MPI) was contacted through the pest and disease hotline and samples were sent to the Animal Health Laboratory (AHL), Wallaceville. Photos of the fish were received (**Figure 2**) and these showed the fish had reddening around the belly and fins, suggestive of a bacterial septicaemia infection.

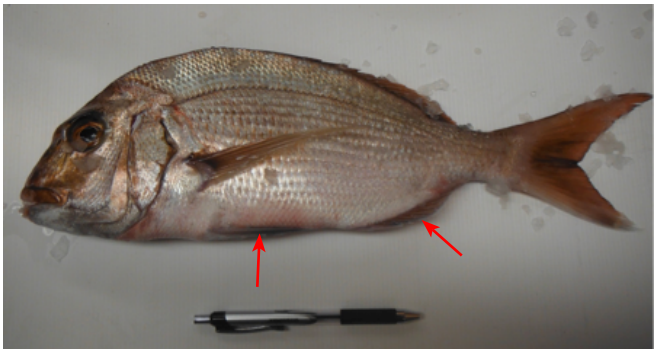

Figure 2: Affected fish showing reddening along the belly and fins (arrowed)

Diagnostic testing methods

The AHL received three fish: one freshly dead and chilled specimen, one fixed in 10 percent neutral buffered formalin, and one frozen specimen. Two swab samples were also submitted: one from the kidney and one from the vent of the freshly dead fish, taken before transport to AHL.

The frozen fish was unsuitable for histopathology and bacteriological testing but kidney tissue was sampled for molecular testing. Histopathology was performed on two fish: the fish fixed in formalin and the chilled specimen.

DNA was extracted from kidney tissue and PCR assays for the presence of *Megalocytivirus* (Gias *et al*., 2011) and *Streptococcus iniae* (designed in-house) were carried out.

Organs from the fixed fish were excised and processed for histopathology at the Institute for Veterinary and Biomedical Sciences (Massey University), as per standard protocols. Slides stained with haemotoxylin and eosin (H&E) were examined at the AHL.

The two swabs were inoculated onto multiple different nutrient media plates for bacterial culture and incubated at 22°C for five days. Isolates of interest (dominant or pure growth) were sub-cultured and the biochemical and molecular tests were carried out to identify the bacteria of interest.

Biochemical tests included Gram stain, cytochrome oxidase test, catalase test, indole spot test, oxidative-fermentative tube test to determine metabolites of carbohydrates; motility test, sensitivity to the vibriostatic agent 0/129, assimilation of certain sugars (bile esculin, arabinose and sucrose); utilisation of citrate as a sole carbon source and ability to produce the enzyme urease.

An API®20NE (API, Biomerieux) strip test with 20 different miniature biochemical tests was also used for identification.

Results

Kidney tissue tested negative for both *Megalocytivirus* and *Streptococcus iniae* by PCR. A Gram-negative bacterium was isolated in light pure growth from the kidney swabs and among a heavy mixed growth from the vent swabs.

Biochemical tests conducted on the Gram-negative isolate revealed a motile organism that was sensitive to the vibriostatic agent 0/129 and tested positive for oxidase, catalase and indole. The isolate assimilated the sugars esculin, arabinose and sucrose; it was positive for the utilisation of citrate; it produced urease and was able to metabolise carbohydrates by fermentation.

The API® 20NE strip test showed a 99.9 percent identity to *Vibrio parahaemolyticus*. However, API® test

strips have a database primarily for mammalian bacterial identification and must be used with caution when identifying aquatic bacteria.

These biochemical results led to an identification of *Vibrio harveyi* but DNA sequencing of the atpA gene (Thompson *et al.*, 2007) was also carried out for confirmation. This sequencing revealed a 99 percent homology to the *V. harveyi* ATCC®33843 strain (GenBank® accession CP009467.2).

In addition to *V. harveyi*, the vent swabs also yielded a mixed growth of environmental *Vibrio* spp., *Photobacterium* spp. and species of the family Enterobacteriaceae.

Histopathology revealed a mass of bacteria in the gut lumen, proliferating into the bloodstream. It appeared that toxins produced by the bacteria had destroyed the architecture of the cells in the liver, kidney and spleen, and had caused clotting in the heart that led to the death of the fish (**Figures 3 and 4**).

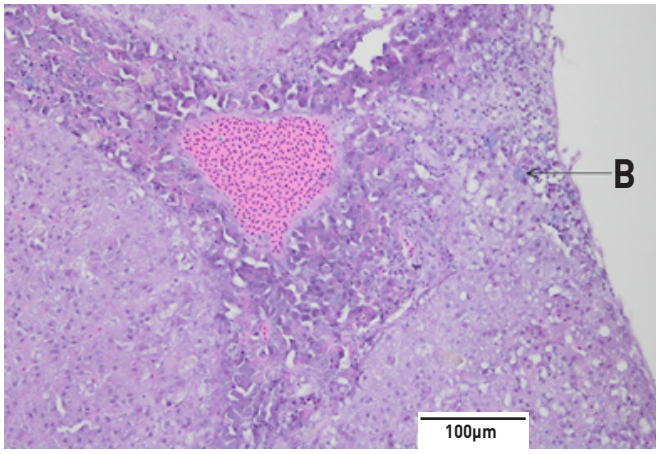

Figure 3: Histological section of the liver stained with H&E with clumps of rod-shaped bacteria (B) and necrosis of the tissue

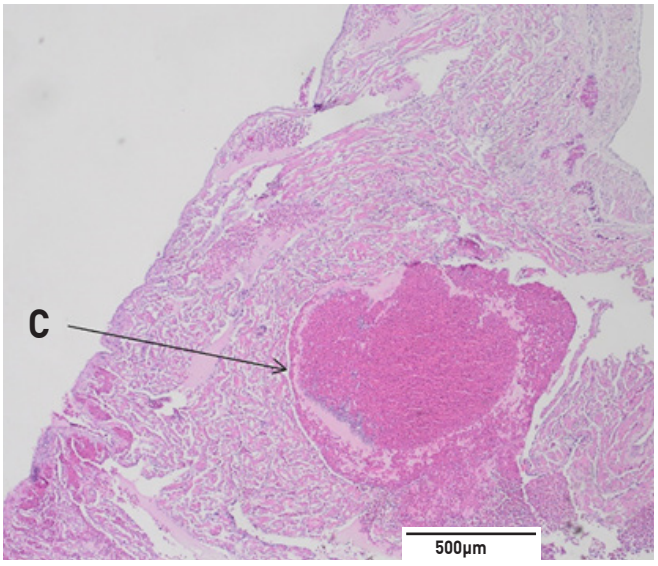

Figure 4: Histological section of the heart stained with H&E with large blood clots (C)

Discussion

V. harveyi is a serious pathogen of marine fish and invertebrates and has been associated with chronic low mortality linked to bloat in snapper at an aquaculture facility (Stephens *et al.,* 2007). It has also been implicated in gastroenteritis in grouper (*Epinephelus coioides*), black sea bream (*Acanthopagrus schlegeli*), yellowfin sea bream (*Acanthopagrus latus*), Japanese sea bass (*Lateolabrax japonicus*) and red drum (*Sciaenops ocellatus*) (Lee *et al.*, 2002), and as the causative agent of tail-rot disease in sea bream (*Sparus aurata*) (Haldar *et al.,* 2010).

In summer flounder (*Paralichthys dentatus*), gross pathology has been described that was similar to the signs reported here (reddening around the anal area, distended abdomen filled with opaque fluid), with *V. harveyi* being identified as the causative agent (Soffientino *et al.*, 1999; Lee *et al.,* 2002). In this particular case the taxonomic name of *V. carachariae* was used, which is a junior synonym for *V. harveyi* (Austin *et al.,* 2006).

Despite the small sample number of fish tested at the AHL, all indications were that the cause of death was a systemic bacterial infection caused by *V. harveyi*. What remains unknown, however, is how and why these fish became infected. No further investigation into these aspects of the outbreak could be conducted because the mortalities ceased, no further samples or detailed information could be collected and the investigation had to be closed (Bingham, 2015).

Disease in wild fish is a result of the interaction between the environment, host and pathogen (Hedrick, 1998).

Environmental factors such as water quality and temperature as well as spawning can contributed to fish being immunocompromised and therefore more susceptible to a bacterial infection such as *V. harveyi,* which is a known pathogen of snapper (Stephens *et al.*, 2007).

As spawning of snapper occurs in New Zealand from October to January, with the peak spawning time occurring in November (Crossland, 1981), this is also likely to have played a role in the infection of these wild fish.

With the limited sample size and information available, no definitive conclusion can be drawn as to what factors contributed to this fish kill event.

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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 2015.

Flavobacterium branchiophilum ruled out

A veterinary pathologist notified MPI after identifying lesions in salmon fry that were consistent with a potential aetiology of bacterial gill disease caused by *Flavobacterium branchiophilum*. The salmon fry were submitted from a hatchery that had been experiencing low levels of mortality (< 1 percent/day). The fish were described as having ulcerated or missing peduncles, but were otherwise in normal body condition. Initial histopathology revealed acute deep-tissue necrosis in the gills, with little inflammatory response and the presence of green filamentous Gram-negative organisms. *F. branchiophilum* is not known to be in New Zealand, so an Incursion Investigator arranged for more clinically affected salmon fry to be sent to the AHL for molecular diagnostics and bacterial culture.

General aquatic bacterial culture yielded no significant growth and the species that were isolated were considered most likely to be environmental organisms. Molecular diagnostics revealed that eight of the 13 fish submitted were positive by real-time PCR for *Flavobacterium psycrophilium*, as the causal agent of cold water disease (CWD), a bacterial disease that affects a wide range of fishes in cold fresh waters (< 16°C). CWD typically affects salmonid fry (e.g., rainbow trout, brown trout and chinook salmon) and has also been reported from eels, lamprey and carp. It typically appears where large numbers of fish are confined, for example in fish farms and hatcheries. Environmental factors such as stress and poor water quality are often contributing factors. In New Zealand, *F. psychrophilum* has only been reported from farmed chinook salmon fry and there have been no reports of disease outbreaks in wild fish in waterways associated with salmon farms. Simple husbandry and hygiene measures can be incorporated into hatchery management routines to minimise the risk of disease outbreaks involving endemic pathogens such as *F. psychrophilum*. Exotic disease was excluded and the investigation was closed.

Perkinsus excluded

A Licensed Fish Receiver (LFR) in Marlborough noticed an unhealthy-looking paua (*Haliotis iris*) with a slightly retracted mantle. The paua had been landed from the Challenger (Nelson/Marlbrorough) fishery area (PAU7). The LFR informed the Paua Industry Council, who informed MPI. As this was unusual for the area, the whole previously frozen paua was submitted to IDC&R for diagnosis. Samples were examined for gross pathology and histopathology and tested by PCR. Several of the slides displayed signet-ring-like structures suggestive of *Perkinsus,* but gross pathology did not demonstrate the lesions normally associated with a *Perkinsus*-like infection, and PCR and *Perkinsus* culture methodologies were negative. *Perkinsus* spp. are protozoan parasites that are notifiable under the Biosecurity Act. As the results were inconclusive, the investigation was stood down.

New to New Zealand organisms identified

A vessel was reported to have very high levels of surface biofouling, so it was closely inspected. Before reaching its intended port of first arrival in NZ, the vessel had broken down and been towed to Port Nelson. Samples were then taken to determine whether there were any high-risk organisms present. Some large clumps of biofouling were removed by hand, then captured and removed with suction. Further cleaning was deemed too difficult, with a high risk of dropping material on to the seabed or encouraging the release of propagules. Among the organisms sent for identification, 11 non-indigenous species were identified, of which three were new records from New Zealand. These included two species of the bryozoan *Schizoporella*, which have the potential to be an invasive species (especially *S. japonica*), although one cannot predict how any species will behave in a new environment. Although the level of risk was determined to be low the finds were communicated to the MPI response team.

An unknown species of ascidian was found while the Marine High Risk Site Surveillance programme was being undertaken in Tauranga during April. This species was sent to the Marine Invasive Taxonomic Service for identification and identified as an undescribed ascidian of the genus *Polyandrocarpa*. Since this is a native species, there was no biosecurity risk and the investigation was closed. At the time of writing the ascidian has still not been identified to species level.

Fish mortality investigated

An ornamental fish breeder contacted MPI after observing mortalities in juvenile African cichlids 30–50 mm long. Four of 90 fish had died overnight and another four were showing jerky movements followed by loss of equilibrium in the water; then they died within 12 hours. Only one 1 000-litre tank was affected out of several on the property. Two *Labidochromis caeruleus* and one *Aulonocara* sp. "Stuartgranti Maleri" from the affected tank were euthanased and submitted for histology. Only one fish exhibited notable pathology, with muscle wasting and calcified bacterial granulomas of

the gut. This suggested the fish had a chronic or historical bacterial infection of the gut, which was likely to have affected its appetite and/or ability to absorb nutrients, leading to the muscle wasting. There was no evidence of serious exotic or emerging disease in the fish, and meanwhile the breeder reported that mortalities had resolved, so the investigation was closed.

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The possibility of a dengue outbreak occurring in New Zealand

Background

Dengue fever (DF), one of the mosquito-borne viral diseases, has become a leading global health problem, as more than a third of the world's population live in regions at risk for infection (Centers for Disease Control and Prevention, 2015). The World Health Organisation (WHO) suggests that there are about 390 million DF infections each year (95 percent confidence interval = 284–528 million) and about 2.5 percent of those affected die (WHO, 2015a).

Geographically, DF is found in tropical and subtropical regions such as Central and South America, Africa, the eastern part of the Middle East, the Indian Subcontinent, Southeast Asia, south China, northern Queensland and the Pacific Islands (**Figure 1**). The disease is now endemic in more than a hundred countries, putting more than 40 percent of the world's population at risk (WHO, 2015a).

DF is caused by any of four serotypes of the DF virus, specified as DEN-1, DEN-2, DEN-3 and DEN-4 (WHO, 2009a). The disease typically presents with flu-like symptoms, but may develop into the potentially lethal dengue haemorrhagic fever (DHF), which was first diagnosed during DF epidemics in Thailand and the Philippines in the 1950s (WHO, 2009b).

Both DF and DHF are transmitted by DF-virus-infected mosquitoes of the species *Aedes aegypti* (the primary vector) and *Ae. albopictus* (the minor vector) (**Figure 2**). Both species breed in containers.

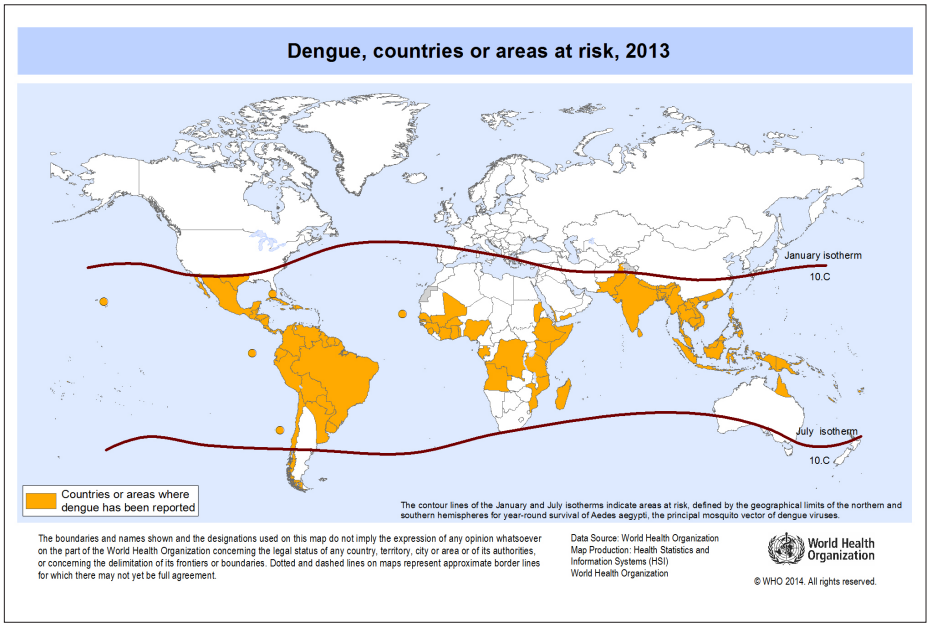

Figure 1: Global DF distribution, 2013 (WHO, 2014)

Figure 2: *Aedes aegypti* and *Ae. albopictus*: two vectors of DF virus (Lounibos & O'Meara, 1999)

An alarming picture

In New Zealand, most DF cases are reported in Auckland, the main gateway for international passengers arriving in the country. Statistics compiled by the Auckland Regional Public Health Service (ARPHS) show that the number of DF notifications is steadily increasing, with all notifications being acquired overseas so far (ARPHS, 2015).

In the Pacific, the DF outbreak situation is regularly updated by ARPHS (ARPHS, 2015). The latest update highlights the alarming situation in Fiji and Samoa: 11 confirmed deaths and 10 000 cases of infection since a DF outbreak started in Fiji in December 2013 (Australian Broadcasting Corporation, 2014; Inter Health Worldwide [IHW], 2014) and 543 confirmed cases had been reported as of 29 April 2015 (ARPHS, 2015). In Samoa, 773 new cases were confirmed between 11 June and 27 September 2015 (ARPHS, 2015).

In Australia, the latest DF outbreak was confirmed as DEN-1 in Townsville, Queensland, on 2 July 2015 (Queensland Health, 2015). It may have shown a new trend, as cases were reported during the winter season, which was previously thought unusual, though all cases are still being reported from northern Queensland. Over the past decade, large outbreaks have occurred in the cities/towns of Cairns, Mossman, Port Douglas, Innisfail, Tully, Townsville and Charters Towers, and in Torres Strait. It is also noted that 1 000 laboratory-confirmed DF cases were reported in Australia in the year to 1 July 2015 (WHO, 2015b).

Table 1: Summary of DF vector interceptions, 2013–2015*

Year	Date	Mosquito species		Specimen type	Risk item/location
		Ae. aegypti	*Ae. albopictus*		
2013	26 May		✓	Larva	Secondhand boat, ex USA, Ports of Auckland
2014	7 January		✓	Larva	Used tyres in container, ex South Korea, Transitional Facility
	20 December	✓		Adult	Baggage tracing unit, Auckland International Airport
2015	31 March	✓		Adult	Passenger's baggage, MPI X-ray unit, Auckland International Airport
	27 June	✓		Adult	Passenger's baggage, Customs inspection area, Auckland International Airport
	7 October	✓		Adult	Fresh pineapple in container, ex Philippines, Transitional Facility

* Source: Updated ARPHS mosquito interception database

To deal with the outbreak, Queensland Health focused on treatment of containers to get rid of the breeding habitat of *Ae. aegypti*. In 2009 alone, 106 000 breeding sites were treated on more than 48 000 properties; more than 6 000 interior surfaces were sprayed and more than 23 600 letters were delivered to Cairns residents to advise them of the risk and the precautions they should take (Montgomery, 2009).

ARPHS roles and responsibilities

DF vector mosquitoes have been targeted for surveillance by ARPHS. Both species are yet to become established in New Zealand but have been intercepted in arriving passengers' baggage at Auckland International Airport, in international cargo from containers at Ports of Auckland, and/or at Ministry for Primary Industries (MPI)-approved Transitional Facilities in the Auckland region. **Table 1** summarises the recent DF vector mosquito interceptions responded to by ARPHS over the past three years.

Routine mosquito surveillance at AIA and POA

ARPHS has been operating a surveillance programme targeting both *Ae. aegypti* and *Ae. albopictus* at Auckland International Airport and Ports of Auckland twice a week for the last decade. Surveillance at Ports of Auckland trapped one *Ae. albopictus* in March 2007.

ARPHS also undertakes monthly mosquito surveys at both these locations and carries out a mosquito survey jointly with MPI and Auckland International Airport at the airport annually.

Efficient responses to suspected exotic mosquito interceptions

ARPHS has responded to a number of suspected exotic mosquito interceptions over the years, largely notified by MPI. MPI quarantine inspectors notify ARPHS of any mosquito-related incidents at the border by following a memorandum of understanding with the Ministry of Health before handing over the responsibility to ARPHS. ARPHS conducts a delimiting survey, removes all potential and apparent mosquito-breeding habitats and arranges for enhanced surveillance around the interception site.

Identification of possible risk factors

It appears that the following five major factors might contribute to future establishment of DF virus in New Zealand:

- climate change may modify the vector mosquito's range of habitat or life pattern;
- there may be unpredictable interceptions of either *Ae. aegypti* or *Ae. albopictus* at the New Zealand border;
- increasing number of passengers are coming from overseas;
- there is ever-growing international trade with Australia, the Pacific countries, Asia and the Americas, which is only likely to increase after New Zealand joins the TPP Deal; and
- there are numerous local breeding habitats that could be used by exotic mosquitoes.

Climate comparison between New Zealand and Australia

Global warming is recognised as one of the major risks, so it may be insightful to compare the temperature regimes of New Zealand and Australia (**Table 2**).

It appears that the daily maximum average temperature for mid-summer in New Zealand cities is much lower than in all the northern Queensland cities where DF outbreaks are frequently declared by Queensland Health.

New Zealand's far cooler climate is unsuitable for the DF mosquito, although this situation could alter with significant climate change (Yu, 2009). However, the climate appears unlikely to change significantly in the near future. There might be some factors associated with ongoing DF outbreaks in northern Queensland (compared with the rest of that state) and even the rest of Australia because historical data indicates that transmission is still limited within Queensland (Russell *et al.*, 2009; Safetravel, 2015).

Discussion

Conditions in New Zealand are unfavourable for the establishment of the DF vectors, *Ae. aegypti* and *Ae. albopictus,* which means the risk of human infection from a locally transmitted source remains very low.

New Zealand has learnt a lot from the incursion of southern saltmarsh mosquito, *Ae. camptorhynchus*, found in Hawke's Bay in December 1998. The 10-year+ high-profile government

Table 2: Comparison of mean maximum temperature in New Zealand and Australian cities and towns

New Zealand cities*	Mean maximum temperature*(°C)	Australian cities/towns†	Mean maximum temperature§ (°C)
Auckland	22.8	Cairns	31.4
Wellington	20.3		
Christchurch	22.6	Port Douglas	30.3
Hamilton	23.0		
Dunedin	18.9		
Palmerston North	20.1–22.9	Townsville	31.3
Tauranga	23.7		
Hastings	24.0	Innisfail	30.8
Rotorua	23.0		
Napier	24.5		
Invercargill	18.5	Tully	31.3
New Plymouth	21.7		
Nelson	22.2	Charters Towers	30.3
Whangarei	23.9		
Whanganui	20.1–22.9	Bamanga	30.8
Gisborne	24.8		
Timaru	20.1–22.9		

* Source: Kay et al., 2013

† All located in northern Queensland

§ Source: Wikipedia, https://en.wikipedia.org/wiki/Cairns

eradication programme has cost more than $70 million.

ARPHS works closely with MPI and major operators at the largest ports of entry, such as Auckland International Airport and Ports of Auckland, and high-risk Transitional Facilities where regular monitoring and treatment of local mosquito-breeding habitats is recommended to reduce the number of mosquito interceptions.

MPI quarantine officers have maintained a high level of competence in keeping exotic mosquitoes out of New Zealand. International shipping containers are fumigated or properly treated whenever a live insect is found, in accordance with MPI standard operating procedures or protocols for pest control.

ARPHS has been running an effective and efficient mosquito surveillance programme for more than two decades while enjoying a sound work relationship with MPI.

Conclusion

Based on the above analysis, there is little likelihood of a DF outbreak in New Zealand in the near future. Hopefully New Zealand can continue enjoying its advantageous position of having no locally transmitted arboviral diseases.

Acknowledgement

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Four in one pot! Management of an ant incursion hotspot at Ports of Auckland

The National Invasive Ant Surveillance (NIAS) programme is an ongoing targeted survey of international points of arrival for cargo into New Zealand (Peacock *et al.*, 2015). 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, airports and Transitional Facilities (TFs) that are considered the most likely places where exotic ant species will first be found – typically several times per year at each port.

The programme surveys sites using small GPS-tracked baited lures placed in systematic grids. This system provides temporal and spatial data on exotic ant incursions, including the patterns of invasive detections and how they relate to human activities.

Over the years it has been noted several times that hot spots of invasive ant activity frequently appear at port facilities (Gunawardana *et al.*, 2013). This article describes a recent invasive ant hotspot found at the Ports of Auckland (POA) and discusses future management.

Initial detection of brown crazy ant

Brown crazy ant (*Paratrechina longicornis*) is considered a high risk owing to its pest status in other countries, adaptability, reproductive capacity and likely ability to establish in New Zealand. It is frequently intercepted by border authorities and is the ant species most commonly intercepted in the NIAS programme (Craddock, 2014).

It was therefore no great surprise when brown crazy ants were found at the POA on 21 January 2015 in a NIAS surveillance lure deployed in the multi-cargo area to the south of Freyberg Wharf. MPI initiated an "Urgent Measures" response and on 28 January FBA Consulting visited the location.

Ants were first found during NIAS alongside a line of concrete barriers and a lightstand next to a tarsealed container storage area. The containers stored nearby were undergoing fumigation treatment, which had not been carried out before at this particular location. Visual inspection revealed suitable ant habitat around the base of the barriers, with small weeds and accumulated dirt and detritus, but the area was generally fairly clean as the barriers had obviously not been in position for long (i.e., less than 6 months).

Three more species found

The area was very dry, and when liquid ant bait was deployed, almost immediately foraging brown crazy ant workers could be seen moving about. Soon three more ant species were noticed. A single ghost ant (*Tapinoma melanocephalum*) worker was collected, along with several white-footed ants (*Technomyrmex albipes*). A few minutes later, dozens of individuals of a fourth species, yellow crazy ant (*Anoplolepis gracilipes)* were found. Thus four different exotic ant species not established in New Zealand were collected at the same location in just 20 minutes (**Figure 1**).

The probable location of the brown crazy ant nest was found by following trailing ants under one of the barriers. About eight metres away the probable location of the yellow crazy ant nest was also found, under another concrete barrier. However, the ghost ant and white-footed-ant nests could not be found.

Because of the number of exotic ant species found, the area was immediately and thoroughly treated with toxic ant bait and a residual spray application. All ant species were confirmed as destroyed during follow-up inspections.

Future management

There had been no previous ant detections in the area where these ants were found, and the majority of the ant habitat (i.e., concrete structures) had only recently been placed there. When shipping containers require fumigation it is because contaminants have been found in them (often including insects), so it seems likely that these containers were the source of the ants found. Fumigation areas have previously been noted to be exotic ant hotspots (FBA Consulting, 2007).

In response, a number of management measures were implemented. The MPI-funded preventative treatment programme undertaken at the POA since 2006 was extended to include this new hotspot. Regular preventative treatments have been shown to significantly reduce exotic ant detections in treated areas (Thomas *et al.*, 2009). NIAS mapping systems were updated

Figure 1: Barrier line where the exotic ants *P. longicornis, A. gracilipes, T. melanocephalum* and *T. albipes* were found. Nest locations are shown for *P. longicornis* (upper arrow) and *A. gracilipes* (lower arrow).

to include the structures in this area so it could be earmarked for further NIAS surveillance, and the MPI Quarantine Service was informed of the hotspot as they frequently visit this area and check fumigation activities.

This case underlines the importance of ongoing border surveillance for high-risk invasive ants. It also highlights the need for biosecurity systems to be adaptable to the sometimes rapid changes in surveillance environments and the human activities occurring there that increase the risk of exotic species establishment.

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Plants and environment investigation report

The Ministry for Primary Industries' (MPI) Incursion Investigation (Plants & Environment) and Plant Health Environment Laboratory teams investigate and diagnose suspected exotic pests and diseases in the plant and environment sectors. Investigators and scientists are based in Auckland and Christchurch. These teams provide field investigation, diagnostic testing and technical expertise with regard to new pests and diseases affecting plants and the environment. They also have surveillance and response functions and carry out research and development to support surveillance and incursion response activities.

Spider establishment prevented

Spiders found on bagged bulk mail at NZ Post's International Mail Centre near Auckland airport were identified as the false widow spider *Steatoda nobilis*, a species not present in New Zealand. Although this species is not venomous, it can inflict a painful bite and is frequently confused with the venomous black widow spider. A registered pest control operator visited the facility and found no more of the spiders but applied bendiocarb as a precautionary treatment. The mailbags were wrapped in plastic and incinerated to mitigate any remaining biosecurity risk.

New fungi found

Foliage, stem and soil were sampled from a Waikato stand of redwood trees (*Sequoia sempervirens*) after a Scion scientist noted unusual dieback symptoms. The potential causal agent *Phytophthora cinnamomi* was detected in the soil, but no other potential pathogens were found. However, two new to New Zealand fungal species were isolated from the leaf and branch samples: *Pestalotiopsis chamaeropis* (Xylariales: Amphisphaeriaceae) and *Phaeosphaeria podocarpi* (Pleosporales: Phaeosphaeriaceae). Both have been isolated overseas from plant foliage, but the nature of the association is unclear. There is no evidence to suggest they are pathogenic, and many *Pestalotiopsis* spp. are epiphytic or endophytic so *P. chamaeropis* may not be pathogenic. This species was only recently described and New Zealand is its second locality (Maharachchikumbura *et al.*, 2014). *Phaeosphaeria podocarpi* was also only recently described, from *Podocarpus latifolius* in South Africa (Crous & Wood, 2014). At present no urgent measures are considered necessary to contain or attempt eradication of these species and it is considered most likely that the primary cause of the observed die-back symptoms was *Phytophthora cinnamomi*.

An Auckland entomologist caught a single specimen of a fly belonging to the Milichiidae family ((Diptera: Schizophora), at the University of Auckland Tamaki campus. This information became available after the find was posted on the Naturewatch website. Little published information is available on milichiid flies (sometimes known as freeloader flies). A definitive text on New Zealand Diptera (Harrison, 1959) lists only one genus of Milichiidae, yet there are specimens of at least three genera deposited in Landcare Research's National Arthropod Collection (NAC) awaiting description. This case is thought to be another species, possibly exotic. The larvae of milichiid flies feed on rotting vegetation and the adults are described as kleptoparasites, feeding on the bodily fluids of their prey. Neither habit is likely to have any biodiversity consequence in New Zealand. As yet there is no evidence of a population present in New Zealand no further action by MPI is planned. Owing to the lack of available information the specimen will not be identified beyond family.

Status of peach viroid clarified

Peach foliar samples (*Prunus persica* cv. Spring Lady) were sent to MPI by a Hastings nursery because they displayed symptoms consistent with peach calico disease, which is caused by peach latent mosaic viroid (PLMVd) (Avsunviroidae). Samples tested positive for PLMVd using molecular diagnostic methods that were confirmed by independent sequencing (sample isolates had 99 percent similarity to PLMVd isolates published in GenBank). This result validated a recent published record of PLMVd present in a 1956 herbarium sample from Central Otago labelled "virus in peach foliage" (Guy, 2013). PLMVd was not recorded here before 2013 but a 2006 publication recorded peach calico disease as a "virus-like" disease, based on evidence from symptoms and indexing results. Coupled with the new find, the evidence suggests that a strain of the viroid has been present in New Zealand peach trees since at least the 1950s, having probably arrived originally in budwood material, so it is likely to be widespread. PLMVd is an economically important disease in some countries, reducing fruit quality and tree vigour. The present economic impact of PLMVd on the local stonefruit industry is not known, but is likely to be low as it has not been an issue before. The viroid is probably already widespread, so no urgent action is considered appropriate and MPI plans to revoke its regulated status.

New mycorrhizal fungus reported

An amateur mycologist reported a suspect new to New Zealand fungus, *Ramaria subaurantiaca* (Gomphales: Ramariaceae). This is a mycorrhizal coral fungus associated with eucalyptus tree roots, native to Australia and not a plant pathogen. It was found near eucalyptus trees alongside a busy suburban road in the Bay of Plenty. Before MPI was notified of this detection a record had been posted on the Naturewatch website, including photos of the fungus and GPS co-ordinates of where it was found. Identification of a sample sent to MPI could not be confirmed by molecular diagnostic methods, owing to a lack of publicly available sequence data, but

was confirmed by morphology. This organism is not considered a biosecurity risk. Isolates were provided to Landcare Research for the Fungal and Plant Disease Collection.

New tomato disease ruled out

A commercial tomato grower observed unusual disease symptoms on tomato plants grown from seed imported from Denmark. Symptoms were first seen in a single cultivar, then in two more cultivars. Following advice from a horticultural consultant, the grower submitted symptomatic and non-symptomatic samples to MPI. There were two fungi present, *Alternaria* sp. and *Stemphylium* sp., known saprobic species that were considered unlikely to be the cause of the symptoms. Tests undertaken by MPI's virology team were also negative, using electron microscopy, herbaceous indexing and PCR. However, growing plants in different media and environmental conditions indicated the symptoms were induced by abiotic factors. The grower was advised of the findings and advised to consider plant nutrition testing.

Insect found in fresh curry leaves

A Blenheim retailer reported a large live insect found in fresh curry leaves (*Murraya koenigii*) imported from Fiji. MPI arranged urgent identification of the insect and contacted the importer to review the importation documentation and procedures. Fresh curry leaves are imported from Fiji weekly in plastic bags. The fixed customer base enabled the risk consignment to be rapidly identified; investigation revealed it had already been distributed for use and that border clearance had been in accordance with the relevant Import Health Standard. This clearance had involved visual inspection of a 600-piece sample of the leaves, taking two hours, indicating a high likelihood that other insects would have been found if present. The insect was identified as the leaf-footed bug *Leptoglossus gonagra* (Hemiptera: Coreidae) (synonym *L. australis*), a regulated pest not present in New Zealand. MPI's Border Clearance team was advised of the outcome to ensure more vigilant future inspection of fresh curry leaves. No further live insects have since been found in curry leaves, suggesting this interception was a one-off case. The notifier and importer were advised and the case was closed.

Increasing risk from on-line seed sales

When a consignment of plant seeds purchased online arrived by post, the labels indicated it had been sent directly from Greece, by-passing New Zealand's seed-importation procedures. The seeds included Mexican sour gherkin (*Melothria scabra*), a species not listed on MPI's Plant Biosecurity Index and therefore a prohibited import. The seeds were sent to MPI for destruction. Although the website from which the seeds were ordered has a New Zealand domain name, it actually operates out of Greece, beyond the direct regulatory influence of MPI, so that the onus is upon the importer, in this case a member of the public, to comply with New Zealand's requirements. Fortunately the Greek business responded to MPI's emails and subsequently removed all prohibited seed from sale. The MPI border intelligence team has been advised.

Scorpion found

Guests at a Queenstown hotel found a scorpion alongside their luggage and coats after having arrived from Melbourne, Australia, the previous day. MPI staff searched the apartment but found no more scorpions. After asking the tourists where their luggage had been, it was concluded unlikely that the scorpion had originated from their residence in Australia, but was more likely to been picked up en route as an opportunistic "hitchhiker". A further possibility was that it had arrived with the previous occupiers of the hotel room, who were also from Australia. The scorpion was identified as the forest or wood scorpion *Cercophonius squama* (Scorpiones: Bothriuridae), a native of South Australia, Victoria, New South Wales, ACT and Tasmania. No further action was considered necessary.

Exotic ants found

Small black ants arrived in Southland in a parcel of canvas originating from the UK. The approximately 30 live ants were associated with the packaging material, which at the time of notification had been placed outside in a domestic waste wheelie bin. The packaging was destroyed by incineration and the ants were identified as *Camponotus sexguttatus*, a species absent from New Zealand. Although this species is not considered to be established in the UK, it is regularly intercepted there. As it is mainly a tropical species, it was considered unlikely to survive outside in Southland during winter, but the notifier was advised to contact MPI again if they found any more ants. No further action was considered necessary.

Border treatment addresses possible risk from mandarins

Flying insects were found during a routine compliance audit of an MPI-accredited Transitional Facility holding imported fresh produce from Australia in a coolstore. Two insects found in a consignment of mandarins were submitted to MPI's diagnostic entomology team with an urgent identification request because of uncertainty over whether they were of local or Australian origin. The fruit was held in containment pending the outcome. The insects were identified as a sciarid fly (*Epidapus* sp.) and a parasitic wasp of the family Encyrtidae that was not identified to genus level. The lack of conclusive identification meant either or both insects could have originated from Australia, so methyl bromide fumigation was immediately arranged for both the mandarins and another produce consignment stored alongside. The produce was then granted border clearance.

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PEST WATCH: 11 August – 23 October 2015

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 & 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 11 August 2015 – 23 October 2015. 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
Chromista	*Phytopythium litorale* no common name	*Rhododendron* sp.	Bay of Plenty	Scion	Saprophyte; of no significance on this host
Insect	*Phacophallus parumpunctatus* rove beetle	Found on grass clippings	Auckland	S. Thorpe (General Surveillance)	Has a wide distribution, has been recorded from Africa, Europe, Asia, North America, West Indies and Australia.
Insect	*Telsimia subviridis* ladybird beetle	*Acacia* sp. Wattle	Auckland	S. Thorpe (General Surveillance)	An Australian ladybird species.
Insect	*Cassida compuncta* tortoise beetle	*Calystegia sepium* ssp. *roseata* Pink bindweed	Bay of Plenty	IDC & R (General Surveillance)	First collected in late 2014. An Australian species that is also present in some parts of the Pacific.

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

Veterinary Diagnostic Laboratories

gribbles veterinary pathology

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- CHRISTCHURCH
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NEW ZEALAND VETERINARY PATHOLOGY

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  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:

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

Investigation and Diagnostic Centre – Tamaki
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