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Editor: Michael Bradstock
Technical Editors: Jonathan Watts, Carolyn Bleach

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

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Editorial

More than an "insect graveyard": the Ministry for Primary Industries' insect reference collections

This editorial sets out to provide an understanding of the importance of the Ministry for Primary Industries (MPI)'s insect collections for biosecurity decision making and aiding bilateral international trade.

Figure 1: Collection room, Christchurch

MPI's biosecurity function is all about protecting New Zealand from harmful pests and diseases. An integral part of this role is plant pest and disease identification and maintenance of invertebrate collections. Plant Health & Environment Laboratory (PHEL) entomologists assist the facilitation of goods across our borders by rapidly identifying submitted specimens. For MPI, the accurate identification of organisms underpins all biosecurity-related activities. Identifying invertebrates is often difficult and complex owing to their diversity, and similarities between species. Expertise is built up over many years, and maintaining a worthwhile collection to support our function takes time and dedication.

MPI has two invertebrate collections, in Auckland and Christchurch, maintained by PHEL within the Diagnostic and Surveillance Directorate. These collections are primarily focused on organisms of biosecurity concern to New Zealand. They include specimens intercepted at the border and post-border, collected by targeted surveillance (e.g. the National Fruit Fly Surveillance Programme), collected from overseas inspections (e.g. from the South Pacific Commission), and also species suspected to be new to New Zealand and collected by the general public.

Figure 2: Queensland fruit fly

How did the MPI invertebrate collections begin? By the 1960s MPI (then the Ministry of Agriculture) had small collections at Auckland, Levin and Lincoln. The Levin collection was closed in the early 1980s and incorporated into the Auckland collection. After this time the Christchurch Quarantine Service started submitting samples to Lincoln for identification, which led to the growth of the overseas invertebrate collection; the Lincoln collection was primarily material collected from surveys of grain stores. When the Department of Scientific Industrial Research (DSIR) was restructured, the insect material was donated to the Lincoln collection, further increasing the variety of New Zealand specimens.

Visitors to public collections enjoy viewing the large, dramatic or colourful pinned specimens, but a proper insect collection is not only made up of pinned insects. Immature or soft-bodied specimens such as caterpillars and spiders are preserved in ethanol to maintain their shape and structure and prevent decay. Specimens too small to properly examine under a dissecting microscope such as aphids, scale insects and mites are preserved on slides and viewed under a compound microscope.

All invertebrate collections require specific environmental and physical conditions: a constant temperature of around 18°C, low humidity and well-fitting lids on drawers housing pinned insect collections. These conditions protect valuable specimens from fungi and pest insects that feed on the dried specimens. Most adult insects have a robust exoskeleton, so they may look the same when dead and dried as when they were alive, but they need to be kept away from direct light, otherwise their colours fade or bleach out and do not provide a true picture of what the species normally looks like.

Tiny labels with collection data are attached to each specimen. Information recorded includes the country of origin, the host (if appropriate), and when and where found. The country of origin for specimens intercepted at the border is especially important. Maintaining the integrity of interception records in our collections requires

Figure 3: Red imported fire ant

accurate labelling, good preparation and curation. If these procedures are not followed correctly, it may take longer to complete an identification. For example, a container from Indonesia was found to contain a nest of the red imported fire ant (*Solenopsis invicta*), a highly invasive species. Specimens were identified by PHEL entomologists, but there was some initial confusion as this species is not known to be established in Indonesia. Further investigation revealed that the container originated from the US and had travelled to New Zealand via Indonesia.

Entomologists identify specimens by viewing their morphological characters and using taxonomic keys in conjunction with the collections. With the advancement of technology in the areas of DNA molecular methods and digital imaging, there is a widespread belief that these two methods alone can be an adequate substitute for actually examining a sample. But, as one British entomologist commented, "You can't extract DNA from an image." All methods have a place in entomology diagnostics; they are complementary. Molecular tests often cannot separate closely related species, and if DNA cannot do that then neither will a digital image. A specimen's internal tissue is required to obtain DNA; depending on its size this can range from removing a leg to crushing the entire specimen. However, recent developments are less destructive and once the extraction process is complete whole specimens remain intact and can be preserved and held in the collection. This method is very useful for old or important specimens where we wish to test the validity of an identification, or when further morphological diagnostic work is to be carried out.

Another complementary tool to aid rapid, accurate identification is the development of an online database – PHELdi (Plant Health and Environment Laboratory diagnostic images) for insects of biosecurity importance to New Zealand and the Pacific. The database aims to deliver high-quality diagnostic images and relevant, peer-reviewed species information for more than a thousand species and is openly accessible to the public.

Carol Muir
Senior Technician & Curator
Christchurch Entomology Collection
Plant Health & Diagnostic Laboratory
Ministry for Primary Industries
Christchurch
carol.muir@mpi.govt.nz

Quarterly report of diagnostic cases

New Zealand Veterinary Pathology

Bovine

A dairy farm in New Plymouth had six abortions over a period of 6 weeks. An aborted female fetus (estimated gestational age 210 days) was submitted for necropsy. The heart was markedly enlarged with increased tortuosity of vessels on the surface of the ventricles and left atrium. Both ventricles were about the same thickness and the aortic valves were hypoplastic with thinning of the aorta. The liver was also markedly enlarged, with a characteristic mottled appearance ("nutmeg liver"). A diagnosis of **congenital heart defect (aortic valve hypoplasia)** with secondary **hepatic chronic passive congestion** was made. Congenital cardiac defects are relatively uncommon in cattle, with ventricular septal defect being the most commonly reported anomaly. It is possible that this case was unrelated to the previous abortions, and further investigation of any additional events would be warranted.

A single 10-year-old Kiwi cross cow from a farm in South Waikato presented with a 1.5-cm-diameter dermal mass on the left shoulder. The lesion had been present for about a year, with intermittent bleeding. Histology revealed a **cutaneous haemangioma** with secondary ulceration and inflammation.

A farm in Thames-Coromandel reported poor body condition, diarrhoea, and anaemia in 11-month-old Friesian calves. Faecal samples from three affected calves yielded 150, 1,250 and 7,800 strongyle eggs/g, indicating **endoparasitism (strongylata)** was likely a significant contributing factor. Other endoparasites were not evident and drenching history was not provided.

A single 8-month-old Friesian heifer calf from a group of 80 on a lifestyle block in Waikato presented with difficulty breathing and inability to eat. A stomach tube could not be passed more than halfway down the neck. The animal was euthanased and post-mortem examination revealed a granulomatous lesion involving the tongue and extending to include three-quarters of the oesophagus. Regional lymph nodes were also enlarged. Histopathology of the tissue confirmed pyogranulomatous inflammation and fibrosis punctuated by accumulations of Splendore-Hoeppli material. The findings led to a presumptive diagnosis of **actinobacillosis**.

Six out of a group of 300 calves in the Far North were affected with mild scouring. Faecal samples from four of the affected calves were all positive for rotavirus on ELISA, and all samples also contained small to moderate numbers of ***Cryptosporidium*** oocysts. Coronavirus ELISA and faecal culture for Salmonella were both negative. A combined diagnosis of **rotoviral infection** and **cryptosporidiosis** was made. Both infections are common in young calves and do not usually cause clinical disease after 1 month of age.

A property in Waikato had an outbreak of severe, sudden onset of illness and death in 2-week-old Friesian calves. From a group of 30 at risk, 15 were affected and five were dead. On post-mortem examination the carcasses were extremely dehydrated, with fluid gut contents. Histopathology of the small intestine revealed large numbers of **cryptosporidia** attached to enterocytes, with concurrent evidence of bacterial infection. A combined diagnosis of **cryptosporidiosis** and **bacterial enteritis** was made.

A 4-year-old cow from Kawerau presented 1 week post-calving with lethargy, poor production, pale mucus membranes and tachycardia. Another cow had presented a week earlier with similar signs and had been euthanased. There was a prior history of theileriosis on the farm. **Severe anaemia** was confirmed, with haemoglobin of 23 g/L (reference range 90–150) and haematocrit of 0.09 (reference range 0.24–0.40). Theileria sp. were evident on the blood smear, consistent with **theileriosis**.

A single Hereford yearling from a group of 12 in Palmerston North presented with ill-thrift and generally stunted growth, diarrhoea, ulcers in the mouth and around the nares, and cutaneous lesions on all four limbs, consistent with dermatophilosis. A serum sample was positive for **bovine viral diarrhoea virus** on PCR, confirming viraemia, which may be transient or persistent. Given the clinical presentation, it is highly likely that this was a persistently infected animal with development of **mucosal disease**.

An outbreak of scouring and death affecting 15 percent of dairy calves in a mob of 130 in a Manawatu calf-rearing operation was diagnosed by faecal cultures yielding ***Salmonella* Bovismorbificans**. The calves showed poor response to electrolyte treatment and antimicrobials owing to high bacterial challenge, and some calves were initially positive for ***Cryptosporidium*** infection.

Another outbreak of ***Salmonella* Bovismorbificans** was confirmed in a mob of 40 one-month-old Angus cross calves on a Taranaki property. Twelve showed signs of severe scouring and two had died. The *Salmonella* species was cultured from faecal samples from three calves. A less common *Salmonella* serotype, ***Salmonella* Emek**, was isolated from 3-week-old scouring dairy calves in the Waikato. The bacterium was isolated from one of three faecal samples submitted from three of the scouring calves and was likely related to potential intermittent shedding.

A Waipa property had ill-thrift affecting first-lactation cows, and samples from 10 affected animals were submitted as part of the investigation. Pooled serology for ***Fasciola hepatica*** yielded an S/P ratio of 92.18 percent (80–150 is correlated with a "medium" prevalence of liver fluke infection, i.e. 20–50 percent). In addition, serum vitamin B12 was < 12 pmol/L in all animals (reference range 93–1,000). A combined diagnosis of **fascioliasis** and **hypovitaminosis B12/cobalt deficiency** was made.

Over a period of 1 week, four 4-week-old calves from a property in Whangarei developed seizures and died. Five separate pens of 40 calves were on the

property, but only one pen was affected. Lead paint was on the walls of this pen, and was covered as soon as the first calf died. Post-mortem examination of a calf revealed no gross lesions. Histopathology of the brain revealed subtle changes of laminar cortical neuronal hypereosinophilia and gliosis. The blood lead level was measured at >0.6 mg/L (> 0.35 consistent with **lead toxicosis**).

Equine

A single mature gelding from Central Otago presented with a history of recurrent laminitis and hirsutism. Endogenous ACTH was increased (177 pg/mL; normal results in autumn < 47 and at other times of the year < 29), leading to a diagnosis of **pituitary pars intermedia dysfunction** (PPID). Despite the history of laminitis, insulin was normal (62 pmol/L; reference range < 143). Insulin resistance can develop in horses with PPID, and increased insulin levels may be predictive for the development of laminitis.

An abortion occurred on a property in Matamata-Piako. On examination of the aborted fetus the thymus was enlarged with areas of liquefaction, which on histopathology were confirmed to be necrosis. In these areas some of the thymic reticuloendothelial cells contained basophilic intranuclear inclusion bodies. Similar inclusions were seen in hepatocytes associated with small foci of hepatocellular degeneration and necrosis. The histological findings were consistent with abortion caused by **equine herpesvirus 1 (EHV-1)** infection.

A yearling Thoroughbred filly was confirmed as the second case of **strangles** on a Taranaki property. Cultures of abscess material from the filly's swollen submandibular lymph nodes yielded ***Streptococcus equi* ssp. *equi*** in an outbreak of this highly contagious disease. All equine yards should have a strangles prevention and outbreak control plan for this globally common disease (Slater, 2015). The control plan should include zoning of areas of risk with quarantine measures of clinical cases, identification of in-contact non-clinical horses, biosecurity measures between zones and identification of carriers at the end of the outbreak. Although the strangles vaccination does not provide 100 percent immunity, it greatly reduces the disease incidence and severity in an outbreak, and a robust, regular vaccination programme is key to prevention.

Ovine

Multiple ovine abortions occurred on a property in Waipa. Examination of an aborted fetus and placenta revealed extensive changes in the fetal membranes, with extensive pale plaques in the intercotyledonary placenta and flattened, pale cotyledons (**Figure 1**). Histopathology of the affected tissue revealed extensive necrosis and mineralisation with vasculitis and intralesional branching, septate fungal hyphae. A diagnosis of mycotic abortion was made. A potential source of infection is spoiled or mouldy silage, and there has been a report of an abortion outbreak attributed to fungal infection (Gardner, 1967). Abortion caused by fungal infection is considered extremely rare in sheep.

Figure 1: Necrotising placentitis. Image courtesy of Alan Julian.

Another abortion occurred on a different farm in Waipa. Examination of the placenta revealed areas with thickened intercotyledonary tissue. Histology revealed intercotyledonary necrosis with foci of mineralisation, as well as foci of necrosis and mineralisation within cotyledons. Occasional cells within cotyledons contained cysts laden with tachyzoites, warranting a diagnosis of **necrotising placentitis** caused by **toxoplasmosis**.

A property in Hastings also had abortions affecting eight out of 120 two-year-old East Friesian ewes. There was a history of vaccination for *Campylobacter* but not *Toxoplasma*. Serology on all eight ewes showed positive ***Toxoplasma*** titres of > 1:128 in seven animals and 1:32 in one animal. Fetal stomach content culture from two of the abortions did not reveal *Campylobacter* or *Listeria*. **Toxoplasmosis** was considered to be an important contributor to abortions on this property.

A farm in the Far North had an outbreak of sudden death in ewes prior to lambing, with four dead and 4,000 at risk. Ocular fluid from three of the dead ewes revealed moderate to marked hypocalcaemia (calcium 0.73–1.80 mmol/L; reference range 2.00–2.70). **Hypocalcaemia/milk fever** in ewes can occur from about 2 months prior to lambing until 1.5 months after, but cases are most common in the 2–4-week period immediately prior to lambing.

A single 10-year-old pet wether from a group of six in Kapiti was found seizuring, with regurgitation. On routine blood work, the most significant abnormality was hypomagnesaemia (0.45 mmol/L; reference range 0.80–1.15), accompanied by slight hypocalcaemia (1.97 mmol/L; reference range 2.00–2.70). **Hypomagnesaemic tetany** was considered to be the most likely cause of neurological signs in this case. This could be due to decreased intake (e.g. spring grass low in magnesium) or decreased rumen absorption; the latter may be due to high dietary intake of potassium, elevated rumen pH, and/or high levels of easily degradable protein. The diet of this animal was not known.

Caprine

An 11-month-old female *Toggenburg* goat from Invercargill had a prior history of neurological signs, which responded to treatment, but about 2 months later re-presented with sudden-onset right hindlimb paresis progressing to bilateral hindlimb paralysis. No gross lesions were found on post-mortem examination, but histology of the brain revealed chronic nonsuppurative meningoencephalitis. A presumptive diagnosis of **listeriosis** was made.

A single 4-month-old male goat from Palmerston North presented with a flank abscess. Culture revealed a moderate growth of a gram-negative coccobacillus identified by the Bruker Maldi Biotype method as ***Bibersteinia trehalosi***, as well as a light growth of an **alpha-haemolytic *Streptococcus* sp.** *B. trehalosi* is a potential cause of pneumonia in sheep and cattle as well as septicaemia in lambs, but has occasionally been isolated from cutaneous and subcutaneous lesions.

Canine

A 9-year-old female toy poodle from Auckland presented with a history of persistent glucosuria with normoglycaemia. Urine was maximally concentrated and slightly acidic, with marked glucosuria. Mild proteinuria was considered unlikely to be significant at this level of urine concentration. A voided urine specimen yielded a scant growth of coagulase-negative *Staphylococcus*, considered most likely to be contaminants. The presentation was strongly suggestive of Fanconi-like syndrome, and on further investigation the dog was found to regularly consume chicken jerky treats. **Acquired Fanconi syndrome** has previously been reported in association with long-term consumption of jerky treats, with resolution of glucosuria following withdrawal of the treats (Yabuki et al. 2017).

A single 2-year-old female Labrador retriever from Palmerston North presented with acute-onset neurological disease, with recumbency, stupor and neck pain. Cytological examination of cerebrospinal fluid revealed marked blood contamination and haemorrhage, but also a predominantly eosinophilic pleocytosis (leukocytes 780 x 106/L; reference range 1.5–5 x 106), containing 69 percent eosinophils, 14 percent lymphocytes, 9 percent neutrophils (non-degenerate) and 8 percent large mononuclear cells. Scattered yeast organisms were present within the sample, characterised by a thick, non-staining capsule and occasional narrow-based budding. A diagnosis of **cryptococcosis** was made. This can be a multi-systemic disease in dogs, but there is a predilection for the CNS. Altered mental status, as seen in this case, is a negative prognostic indicator.

Reptile

A 14-year-old captive male red-eared terrapin (*Trachemys scripta elegans*) from the Auckland area presented with a lesion of shell rot with abscessation. ***Morganella morganii*** was isolated from enrichment culture of a swab from the lesion. This organism has been reported to be associated with cell lesions in chelonians, and abscesses in other reptiles. Such lesions may contain a variety of aerobic and anaerobic bacteria. Anaerobes were not isolated from this case.

SVS Laboratories

Bovine

An adult dairy cow from Waipa was found dead. Differential diagnoses for sudden death in this animal were nitrate toxicosis and **salmonellosis**. Aqueous humour was submitted and had a normal nitrate level of < 10mg/L (> 25mg/L is consistent with toxicity). Intestinal samples were submitted for culture and were positive for ***Salmonella* Enterica**.

Twelve of 200 heifers aborted over a 2-week period in Buller. Two fetuses were submitted for post-mortem examination and tissues were collected for culture. Neither fetus had fluid in the stomach. Culture of both fetal lungs yielded moderate growths of ***Salmonella* Brandenburg**. **Salmonellosis** is not uncommon cause of abortion in areas of the South Island where cattle have contact with infected sheep.

Five cows that aborted in Whakatane had concurrent mastitis. Culture of fetal stomach contents from one aborted calf yielded a heavy growth of ***Streptococcus bovis, Escherichia coli*** and ***Enterobacter* sp.** In the absence of histology lesions in the submitted fetal tissues the cause of abortion is suspected to be haematogenous spread of **mastitis** pathogens.

A 2-year-old heifer from Canterbury was diagnosed with a fractured humerus in late September. In a liver sample sent to the laboratory the liver copper level was 56 umol/kg (reference range 45–85 umol/kg). While this was a marginal level, **copper deficiency** was suspected to be a likely contributor to the humeral fracture.

Caprine

A 7-year old female Saanen goat from Matamata-Piako was pregnant with four kids and was recumbent for 5 days prior to euthanasia. An on-farm post-mortem examination showed a fatty liver. Fixed liver was submitted for histology. Hepatic histopathologic changes included variably sized clear cytoplasmic vacuoles up to 20 microns in diameter that often displaced hepatocyte nuclei to the periphery. **Hepatic lipidosis** caused by **pregnancy toxaemia** was most likely the cause of clinical signs in this goat. As the fetal need for glucose increases, the doe's insulin level decreases. This spares glucose for fetal needs and stimulates lipolysis and gluconeogenesis. Ultimately, recumbency in the doe is due to hypoglycaemia and hyperketonaemia. Mobilisation of lipids for energy in the doe is suspected to contribute to hepatic steatosis.

A de-horning injury was suspected in a 1-month-old female goat kid from Whakatane and the head was submitted for post-mortem examination. The horn buds were dark grey, up to 3 cm in diameter and lacked hair. The adjacent tissue had yellow to tan serocellular material. When the skin was reflected, there was a 5-mm-diameter hole in the calvarium subjacent to the right dehorning site. The hole extended through the dura and leptomeninges and into the brain, and purulent material oozed from the lesion. Based on the gross findings a diagnosis of **suppurative encephalitis**, **meningitis** and **osteomyelitis** with coagulative necrosis of the overlying epidermis was made. These lesions were consistent with the clinically suspected de-horning injury, with secondary bacterial infection resulting in brain abscessation.

In a similar case, two 1-month-old female goat kids from Matamata-Piako also presented with suspected dehorning injuries. The kids were ill-thrifty and were euthanased owing to a poor prognosis. An on-farm post-mortem examination revealed discolouration of the cerebral cortex subjacent to the horn

buds in one kid, and cerebral malacia in the other. Fixed brains were submitted for histology, resulting in a diagnosis of **encephalomyelitis**. The lesions were characterised by coagulative necrosis and/or loss of the glial limitans with malacia, fibrosis and increased numbers of gemistocytic astrocytes and gliosis. In this case, cerebral necrosis caused by heat trauma was consistent with the clinical concerns for de-horning injuries.

Two goat kids aged 2 and 6 weeks from Waikato died suddenly in early August. A postmortem was conducted, and fresh and fixed lungs were submitted to the laboratory. The histology was consistent with **fibrinosuppurative bronchopneumonia** and **pleuritis**. ***Mannheimia haemolytica*** was isolated from both lungs.

Cervine

Tissues from a dead weaner red deer from Bay of Plenty were received for histological examination. The deer had apparently died suddenly. The only significant abnormality seen on histology was marked haemosiderosis in the spleen. Analysis of the liver revealed < 50 umol/kg of copper (reference range 100–2,000), consistent with **copper deficiency**. Copper deficiency has previously been associated with formation of Heinz bodies, which could lead to increased turnover of red blood cells or haemolysis and could potentially explain haemosiderosis of the spleen. Unfortunately, no antemortem blood samples were available in this case.

Equine

A 4-year-old Warmblood mare from Waikato presented with signs of fibrosis and visceral pleuritis following suspected aspiration pneumonia. A bronchoalveolar lavage (BAL) was performed and the washings were submitted to the laboratory for cytology. A layer of low-density material was present on top of the saline, consistent with a lipid or other oily substance such as paraffin. Cytologically, mixed inflammation and lipid material was present. A wedge biopsy of the affected lung lobe was submitted 3 weeks after the BAL. Findings were consistent with a diffuse chronic fibrinous and necrotising pneumonia with intrahistiocytic lipid globules, type II pneumocyte hyperplasia, fibrin, haemorrhage and oedema. Both cytology and histology were consistent with **exogenous lipid pneumonia** (synonyms: lipoid pneumonia; paraffinoma) in this case.

Poultry

Two female adult commercial chickens from Auckland were reported to be unco-ordinated. Histopathology revealed **meningoencephalomalacia**, characterised by replacement of neuropil and neurons by hypereosinophilic amorphous material admixed with heterophils, macrophages and myriad bacilli. The adjacent neuropil also had malacia and perivascular lymphocytic cuffing. Similar inflammation expanded the meninges. In chickens, bacterial meningoencephalomalacia is commonly associated with *Salmonella* (including *S.* Arizonae and paratyphoid species) and *Escherichia coli*. Fresh tissues were not submitted to identify the causative bacterium in this case. Fixed tissue was submitted from adult chickens. The tracheal mucosa was regularly eroded or ulcerated. The surface was covered by a thin layer of necrotic material admixed with fibrin, heterophils and viral syncytial cells or individual epithelial cells with intranuclear inclusion bodies and small amounts of haemorrhage. Similar changes were seen in the tertiary bronchioles, which were regularly filled and distended by syncytial cells with intranuclear viral inclusions and inflammatory cells. A diagnosis of **bronchopneumonia** and **tracheitis** with epithelial syncytia and intranuclear inclusion bodies consistent with **infectious laryngotracheitis virus** (ILT) was made. ILT is an acute, highly contagious disease of chickens characterised by respiratory distress, coughing, expectoration of bloody, mucoid exudate and high mortality. It is caused by gallid herpesvirus type 1, an alpha-herpesvirus. Outbreaks range from highly pathogenic epizootic forms to mild enzootic disease. It can affect birds of all ages, but it is most common in 4–18-month-old birds. Morbidity is high and mortality is variable, depending on strain pathogenicity. The virus is spread via aerosolisation of respiratory secretions, and recovered birds often have latent virus within the trigeminal nerve ganglion. Virus reactivation may occur with immunosuppressive conditions and stressors.

Canine

In July, a 2-month-old Sharpei cross puppy from Bay of Plenty presented with marked jaundice and petechial haemorrhages. The dog had been treated with an anthelminthic a week before and had been vomiting the day prior to presentation. Serum biochemistry revealed a moderate azotaemia (urea 37.7 mmol/L, reference range 2.6–10.2; creatinine 446 umol/L, reference range 45–145). Liver enzymes were elevated (ALP 654 U/L, reference range 0–85; ALT 153 U/L, reference range 0–75) and bilirubin was markedly elevated at 203 umol/L (reference range 0–6). The dog had a moderate regenerative anaemia, with an RBC count of 4.0 x 10^{12}/L (reference range 5.2–7.9 x 10^{12}), haemoglobin 85 g/L (reference range (124–192) and a reticulocyte count of 116 x 10^{9}/L (reference range 0–60 x 10^{9}), and it had a platelet count of < 10 x 10^{9}/L (reference range 200–500 x 10^{9}). A rapid assay for leptospiral IgM antibodies was positive. The dog had received its 6-week vaccinations, but its *Leptospira* vaccination history was not known. **Leptospirosis** was suspected, given the chemistry and serology results, with possible secondary immune-mediated haemolytic anaemia and immune-mediated thrombocytopenia.

In September, a 3-year-old Heading dog from Bay of Plenty presented with decreased appetite and watery diarrhoea containing blood. It was straining to defecate. The dog had recently been on a sheep-and-beef farm, but other dogs on the property were not affected. A faecal test identified *Giardia* antigen and faecal culture identified ***Salmonella* Bovismorbificans** and ***Campylobacter jejuni***. All of these organisms are potentially zoonotic.

Feline

In early September, a 4-month-old Ragdoll kitten from Cambridge presented with chronic intermittent diarrhoea. The kitten was otherwise well and eating normally. A faecal ELISA assay was positive for ***Cryptosporidium*** antigen and a faecal culture identified ***Salmonella enterica* ssp. *enterica* (I) serovar 4,12 :-: 1,2**. This is a monophasic variant of *Salmonella* Typhimurium. Like most *Salmonella* species, this is a zoonotic organism. It has been identified in human and bovine samples in New Zealand, and overseas has been associated with food-borne salmonellosis outbreaks.

Lagomorph

An adult female rabbit from Auckland presented with alopecia, hyperpigmentation and flaky skin on its ears 3 days after parturition. A hair sample was submitted for PCR analysis for ringworm. The sample was positive for ***Microsporum canis*** and a diagnosis of **dermatophytosis** was made.

Reptilian

A 4-year-old bearded dragon (*Pogona* sp.) from Matamata-Piako presented with a hard lump in the right axilla that was slowly growing over a 1-month period. When the lesion was aspirated, a small amount of viscous fluid was obtained. Wedge biopsies were submitted for histopathology. The mass was composed of interlacing bundles, streams and whorls of spindle cells that were supported by a moderate to abundant myxomatous stroma. The lesion was diagnosed as a **myxosarcoma**, a type of soft tissue sarcoma that arises from mesenchymal precursor cells. The lesions often exude a clear mucoid fluid, as was reported clinically. While they are often unencapsulated, poorly circumscribed, and locally infiltrative lesions, they are rarely reported to metastasise.

Zoo animal

A 28-year-old female tapir (*Tapirus* sp.) was euthanased owing to declining health, including suspected seizure activity. Limited fixed tissues were submitted for histology. Renal changes were characterised by mesangial thickening in addition to periglomerular fibrosis and tubular proteinosis. A diagnosis of **membranoglomerulonephropathy** was made. Chronic renal disease is not uncommon in aged captive animals. Atherosclerosis was also seen in the cross section of a renal artery. Unfortunately, ante-mortem blood and urine would have been needed to assess the extent of renal function. The liver had numerous random foci of hepatic **haemochromatosis** characterised by hepatocellular degeneration and abundant intracytoplasmic Perls-positive granules (iron). Haemochromatosis is a common lesion caused by iron accumulation in the liver that is seen in many captive perissodactyls. Unfortunately, the pathogenesis of iron-overload in tapirs has been studied less extensively than in other species.

An adult female meerkat (*Suricata suricatta*) from Hamilton was tested for antibodies to *Toxoplasma*. ELISA testing on serum had a titre of greater than 1:2,048. Meerkats are highly susceptible to *T. gondii* infection and multiple outbreaks with high mortality have been described in captive groups. Clinical signs include respiratory distress and neurologic signs. Given the high titre, this meerkat must have been recently exposed to ***Toxoplasma gondii.***

Gribbles Veterinary Pathology

Bovine

Four of 50 Friesian heifer calves between 1 and 7 days old presented with fever, mental dullness, recumbency and swollen, painful carpi on a farm in Northland. Culture of the joint fluid from one calf yielded a heavy growth of non-haemolytic *Bibersteinia trehalosi*. To our knowledge, **septic polyarthritis due to *Bibersteinia trehalosi*** has not been previously reported in cattle. This organism is a commensal of the tonsils and upper respiratory tract of sheep and is most frequently associated with septicaemia and pneumonia in lambs. Disease in cattle is uncommon but pneumonia in young stock has been reported, along with single case reports of fatal necrotising hepatitis in an adult cow (Watson, 2010) and subcutaneous botryomycosis in a steer (Spagnoli, 2012). Experimental studies suggest the organism is a secondary opportunistic rather than primary pathogen in bovine pneumonia (Hanthorn, 2014). Cattle strains tend to be non-haemolytic, as in the present case, whereas isolates from sheep are usually haemolytic. Given the age of the calves, the carpal infection was considered likely secondary to septicaemia, although a site of origin for this could not be identified. There was no evidence of umbilical infection, lung disease or diarrhoea. Penicillin and anti-inflammatory treatment resulted in rapid resolution of the clinical signs.

A farmer checking on her cattle on a Banks Peninsula property found one dead. She saw another two die while she was calling a veterinarian. Necropsy of two cows revealed several branches of tutu (*Coriaria arborea*) in the rumen, supporting a diagnosis of **tutu toxicity**. Almost all parts of this plant are toxic and the primary toxin, tutin, has a picrotoxin-like character, acting as an antagonist at amino-acid receptors within the central nervous system. Poisoning has been reported in many species, including humans, but cattle are most commonly affected.

An unwell febrile Friesian dairy cow from South Canterbury presented with "black", i.e. **gangrenous mastitis**, in her left back quarter. Culture of a milk sample from this quarter yielded a heavy, pure growth of ***Staphylococcus aureus***. This is the organism most commonly associated with gangrenous mastitis, but other bacteria can be involved.

Histological examination of tissues from a fetal calf from South Canterbury revealed a small segmental cerebellar lesion consisting of necrosis of outer granular layer cells and a mild lymphoid infiltrate. **Bovine viral diarrhoea virus** was detected in the fetal stomach contents by PCR and it was considered likely that this was the cause of the cerebellar lesions and the abortion.

An abnormality was noticed during butchering of a home-killed animal from Golden Bay, and a sample of fixed bovine muscle was submitted to the laboratory for examination. The muscle had areas of yellow/green discolouration grossly and histological examination revealed a severe **eosinophilic myositis**. Within the areas of eosinophilic inflammation were smaller areas of necrotic muscle fibres surrounded by abundant eosinophils. Sarcocysts were more frequent in these foci of necrotic fibres than they were in the normal muscle fibres. Sarcocysts without any associated inflammation reaction are very common as a background finding in bovine muscle, but it has long been speculated that they may be responsible for these uncommon cases of eosinophilic myositis.

Five of 400 dry dairy cows on a Nelson farm died overnight. They were on a kale crop and samples of aqueous humour from two of the dead cows had high levels of nitrate (25 and 50 mg/L, reference < 5.7), consistent with **nitrate poisoning**.

The cows in the aforementioned nitrate-toxicity case were also being fed some hay and, prior to these acute deaths, five cows had aborted. Histopathological examination of placentae from two of these cows revealed lesions typical of **fungal abortion**, with necrotising and

suppurative placentitis, vasculitis and intralesional fungal hyphae. ***Aspergillus fumigatus*** was recovered from the stomach contents of the two aborted fetuses. There have been many cases of *Aspergillus* abortion in cattle in Canterbury this year, more than seen in most typical years.

Another case of ***Aspergillus*** **abortion** occurred on a farm in North Canterbury. Twenty of a group of 1,000 mixed-age Friesian cows had aborted when two calves were received at the laboratory for examination. One was late-term (fully formed) and one was early-term (hairless). ***Aspergillus fumigatus*** was isolated from fetal stomach contents.

Three mixed-age cows aborted over a period of 4 days on a Horowhenua dairy farm. Gross examination of a fetus and placenta revealed areas of intercotyledonary thickening and opacity, with cotyledonary exudate and haemorrhage. Histopathological examination showed fibrinosuppurative inflammation, vasculitis and intralesional fungal hyphae, confirming **mycotic abortion**.

Histological examination of samples from an aborted fetus from a North Canterbury farm revealed small lymphoid infiltrates around some airways in the lung. This is often seen in the few cases of *Ureaplasma* infection seen in the Christchurch Gribbles laboratory each year. ***Ureaplasma diversum*** was detected in the fetal stomach contents by PCR.

A single abortion occurred at 7 months' gestation in a cow on a Mid-Canterbury property. Culture of the fetal stomach contents yielded a heavy pure growth of ***Streptococcus uberis***, supporting a **streptococcal abortion**. Although *Streptococcus* spp. are recognised as causing sporadic abortion in cattle, this is a rare finding at the Christchurch Gribbles laboratory.

Four of 300 cows in late gestation on a Canterbury dairy farm were noticed with reduced mentation, twitching and blindness. A veterinarian was called to investigate. The cows were being fed kale and fodder beet. Sulphur-induced polioencephalomalacia was suspected. Lead poisoning was also considered, although this is rare in adult cows in New Zealand as, unlike calves, they are less likely to be exposed to lead, e.g. in areas around farm sheds. Histological examination of the brains of two cows revealed only one non-diagnostic small focal area of paler and slightly vacuolated parenchyma in the cerebral cortex. A kidney sample tested for lead had a staggeringly high concentration of 205 mg/kg (toxic level > 5 mg/kg), diagnostic of ***lead toxicity***. The farmer had not thought lead toxicity likely and an initial search of the kale grazing area revealed no source, but a more thorough search eventually located a broken-up commercial battery among several piles of rocks. Two additional kidney samples also had very high lead concentrations. As some of the cows had recently calved and many were due to calve, this discovery raised concern about the milk, and the milk company and MPI were notified. In total, 19 cows were affected and all either died or had to be euthanased. Fortunately the farm had other groups of cows that had not grazed the contaminated area and were not affected.

A 2-year-old dairy cow on a Canterbury property presented with a fractured humerus. The liver copper concentration was 32 umol/kg (adequate range 95–3,000), confirming **copper deficiency**, which may have contributed to bone fragility.

On a South Canterbury property 20 of 150 seven-day-old bull calves died and another 30 had diarrhoea, sometimes containing blood. Culture of faeces from two affected calves yielded a moderate growth of ***Salmonella*** **Bovismorbificans**, confirming **salmonellosis**.

Blood and faecal samples from two young cattle from Marlborough were received for testing. Both were markedly azotaemic, with elevated creatinine (1,100 and 746 umol/L; reference range 39–181) and urea (109.1 and 63 mmol/L; reference range 2.7–11.9) as well as high phosphate (4.36 and 5.39 mmol/L; reference range 1.3–3.3), low calcium (1.02 and 1.18 mmol/L; reference range 2.00–2.7), high magnesium (1.19 and 1.18 mmol/L; reference range 0.59–1.08), low sodium (117 and 126 mmol/L; reference range 136-146) and low chloride (63 and 76 mmol/L; reference range 90-104). Both had evidence of recent muscle damage, with serum creatine kinase concentrations of 5,060 and 1,089 IU/L (reference range 127–537) but only marginally increased aspartamine aminotransferase. One calf had moderately increased GDH (242 IU/L; reference range 5–35) while the other had a marginal increase to 41 IU/L. On further investigation it was discovered that superphosphate had been applied to the pasture 4 days previously, supporting a diagnosis of **renal failure caused by fluorosis**.

A pregnant dry cow from Manawatu had ascites and an elevated heart rate. A sample of the abdominal fluid was consistent with a transudate on gross appearance. Concurrently collected serum and ascitic fluid were tested for creatinine to exclude uroperitoneum. The concentration of creatinine in the abdominal fluid was 1,963 umol/L, which was 6.5 times greater than the concentration in the serum (300 umol/L; reference range 3–181). A ratio > 2 is considered diagnostic so this confirmed **uroperitoneum**. Causes of bovine uroperitoneum include dystocia, necrotising cystitis, bladder obstruction, urachal rupture (at any age) and traumatic bladder perforation (breeding accidents, accidental introduction of insemination pipettes, etc). Often, urination and urine collection remain possible as the bladder does not completely empty. Prognosis is variable and depends on the cause.

Eight of 20 one-week-old Friesian calves from a South Canterbury farm became lethargic and pyrexic and five died over 24 hours. Only one had diarrhoea. Necropsy of one calf revealed red (possibly bloody) urine. Faecal culture yielded ***Salmonella*** **Typhimurium**, supporting a diagnosis of **salmonellosis**. We have seen several other outbreaks of salmonellosis in calves this spring.

A 2-year-old beef animal from Northland was observed to be scouring and very ill-thrifty. It was about half the liveweight of its herdmates. A BVD antigen ELISA test on a serum sample showed a strongly positive result consistent with **persistent infection by bovine viral diarrhoea virus**.

On a Northland farm, three out of 200 calves aged 1–3 weeks that had been brought on to the property at age 4 days, showed varying neurological signs including tremor and stupor progressing to recumbency and paddling. One was euthanased. Necropsy revealed a small area of pneumonia and meningeal opacity. Histopathological

examination revealed a **neutrophilic bronchopneumonia** consistent with aspiration, and a **neutrophilic meningitis** with associated gram-negative bacilli. Such findings are commonly seen in septicaemia caused by bacteria such as *Escherichia coli.*

Ten adult dairy cows from Tokoroa were moved to a farm in the Kaipara district. Three lost weight and had low milk production after calving. Clinical examination revealed marked icterus and pale mucous membranes. Haematocrits were low (0.11–0.17; reference range 0.24–0.4), as were haemoglobin concentrations (37–58 g/L; reference range 85–130). There were up to 69 ***Theileria*** per 1,000 red blood cells, indicating clinical **theileriosis**.

Twenty of 120 dairy calves under 1 month of age from Manawatu/Horowhenua died within a 48-hour period. Prior to death they were frothing at the mouth, tachycardic, hyperthermic and seizuring. Necropsy on three calves found extensive pulmonary oedema with abundant foam in the trachea, interlobular emphysema and pallor and discolouration of the heart muscle. Histological examination revealed significant monophasic multifocal degenerative and mineralising lesions in the myocardium, along with pulmonary oedema. This was consistent with ionophore toxicity. Further investigation found that the deaths started within 24 hours after the farmer added lasalocid (an ionophore) to the milk fed to the calves. Ionophores assist with ion transportation across cell membranes. Monensin is the most commonly used ionophore but others include lasalocid, salinomycin and maduramycin. Derived from the fermentation products of *Streptomyces cinnamonenis*, they are used in livestock industries as growth promoters (by altering the ruminal flora), coccidiostats and antibiotics. Heart and skeletal muscle are the main target organs for toxic effect, and the range of outcomes, which include sudden death and congestive heart failure, depends on the duration and magnitude of the overdosing.

In another case, on a Southland dairy farm, 23 of 100 calves aged 2–3 weeks died 7–9 days after an unknown quantity of monensin powder was added to their milk. Necropsy of one calf revealed marked lung oedema, hydrothorax, and pale areas over the surface of the heart. Histopathological examination revealed severe lung oedema and scattered irregular foci of acute myodegeneration, consistent with **ionophore toxicity**.

Four of 500 calves from North Otago died within 24 hours of being administered injectable copper, selenium, vitamin B12 and a pour-on ivermectin/abamectin anthelmintic. Necropsy and subsequent histopathological examination revealed massive diffuse acute hepatocellular necrosis and haemorrhage. The kidney copper concentration was not abnormal, at 111 umol/kg (reference range 0–157), but copper toxicity was still considered the most likely diagnosis with this history and these lesions. Calves are particularly sensitive to **copper toxicity**. Individual variation, rate of absorption and potentiating effects of co-administered products may explain why the kidney copper concentration was not elevated.

Two of 60 dairy heifer calves aged 4–6 weeks from Manawatu were found dead and 10 others in the group were listless and pyrexic. Histopathological examination of tissues from the two dead calves revealed a severe neutrophilic meningoencephalomyelitis with vasculitis and thrombosis, suggestive of infection with ***Chlamydia pecorum***, the causative agent of **sporadic bovine encephalomyelitis** (SBE). Outbreaks of SBE are occasionally seen in New Zealand and pre-weaned calves are most at risk. Fibrinous polyserositis and a meningoencephalomyelitis are characteristic. PCR testing for the organism on fresh brain tissue, which could not be done in this case, can be helpful, as the lesions may resemble those of other bacterial septicaemias such as those caused by *Pasteurella multocida* and *Histophilus somni.*

Two 9-month old Angus calves on a North Canterbury farm collapsed and died 3 days after receiving a clostridial vaccination and copper injection. Necropsy and histopathological examination revealed centrilobular to massive hepatocellular necrosis. The kidney copper concentration was 181 µmol/kg (reference range 0–157). Together, the findings confirmed **acute copper toxicity**.

Six of 65 Wagyu cross yearling steers on a Rangitikei farm died after developing diarrhoea and foaming at the mouth. Clinical examination of an affected steer that remained alive revealed poor body condition, oral ulceration, diarrhoea and ocular discharge. The steer was euthanased and necropsied. Histopathological examination revealed oral, oesophageal, ruminal and enteric ulcerative lesions and aspiration pneumonia. Subsequent blood testing of the whole group detected 15 animals with high positive results in the bovine viral diarrhoea virus antigen ELISA test, confirming an outbreak of **bovine viral diarrhoea mucosal disease**.

A 9-month-old Scottish Highland steer on a Waikato property died 2 days after receiving a multi-mineral injection containing copper, selenium, cobalt and manganese, as well as an oral ivermectin drench and a copper capsule. Histopathological examination revealed acute myocardial degeneration. The liver selenium concentration was 41,156 nmol/kg (reference range 850–15,000), supporting a diagnosis of **selenium toxicity**.

A rising 1-year-old dairy heifer grazing on a Central Hawke's Bay farm was euthanased after a period of chronic ill-thrift. Necropsy revealed a large, cream-coloured, lobulated mass in the cranial mediastinum. Histopathological examination of the mass revealed densely packed round cells, confirming **thymic lymphoma**. This is a sporadic form of bovine lymphoma, usually affecting cattle between 6 and 24 months old, with mediastinal mass, weight loss, anorexia, dyspnoea and oedema. Progression to leukaemia may occur.

During July and August there were numerous cases of diarrhoea in young cattle in Waikato that prompted laboratory investigations. ***Yersinia pseudotuberculosis*** was commonly isolated from fecal samples in these cases and there was often concurrent infection with strongyle parasites and/or coccidia.

A 6-day-old calf on a Waikato farm was found to have meningitis and a thickened navel when examined by the local veterinarian. ***Salmonella* Bovismorbificans** was isolated from the faeces of this calf and one other. Tests for *E. coli* K99, rotavirus and cryptosporidia were negative. Serum immunoglobulin G concentrations were low in both calves at 3 mg/dL (adequate level > 1,450),

indicating **failure of passive transfer of immunity**, which was likely due to inadequate colostrum intake in the first few hours of life.

Two Friesian cows on a Taranaki farm were noticed to have copious bloody urine, jaundice and pale mucous membranes, while another cow in the same group had pale membranes but her urine was not discoloured. Theileriosis was ruled out when PCR tests for *Theileria orientalis* (Ikeda) were negative on blood samples from all three cows. **Postparturient haemoglobinuria** was considered to be the most likely cause. This condition, associated with intravascular haemolysis, is seen sporadically in early lactation in dairy cattle and is often associated with hypophosphataemia. A low serum phosphate concentration of 0.6 mmol/L (reference range 1.3–3.3) was demonstrated in one of the affected cows, supporting this diagnosis.

Twenty three of 180 heifers on a Southland farm developed a hindlimb ataxia of variable severity. One was found recumbent with a fractured humerus and was euthanased. The farm was on peat and sand soils and had a history of mineral deficiencies. Samples from nine heifers had very low serum copper concentrations (1–4 umol/L with a group mean of 2; adequate range 7–20). The whole group was treated with copper. Some of the mildly affected heifers appeared to recover but the more severely affected animals did not. Older cows on the property were not affected. In previous years, injectable copper supplementation had been used on all the cows but this year none was given as it was thought that the feeding of palm kernel expeller, which usually has a high copper content, would be sufficient. The clinical and laboratory findings were considered suspicious of **enzootic ataxia**, in which severe copper deficiency results in damage to the spinal cord. This condition has been reported in sheep and deer, but we are not aware of previous reports in cattle.

On a Southland dairy farm, almost all of a group of 100 eight-week-old calves developed severe diarrhoea over a 48-hour period. They were living outside on pasture and being fed cow's milk. Necropsy of one calf showed severe mucosal changes in the colon, and histopathological examination revealed large numbers of coccidial organisms in the epithelium, consistent with **coccidiosis**.

Fifteen of 200 heifers on a Southland dairy farm aborted over a 2-week period. The fetuses were markedly decomposed. There were also some calves born alive but weak at full term. The heifers were being fed whole-crop silage. Culture of stomach contents from one calf yielded a heavy growth of ***Bacillus licheniformis***. Unfortunately histopathological examination was not possible. This organism is ubiquitous and, in the absence of evidence of lesions in the fetus, its presence could be the result of sample contamination, but the history in this case suggests the find was significant. The source of infection was most likely the whole-crop silage, a common source in clinical cases.

Salmonellosis was a common diagnosis in Otago and Southland dairy cattle during this quarter. Two cases involved ***Salmonella* Bovismorbificans** (adult cows with severe diarrhoea), eight cases involved ***Salmonella* Brandenburg** (aborting heifers and, in one case, sick calves), and six cases involved ***Salmonella* Typhimurium** (sick calves).

Ovine

A 7-year-old Suffolk ram from Auckland was lethargic and subsequently developed signs of haematuria, epistaxis, melena, tachycardia, tachypnoea and "muddy" mucous membranes. It had a history of exposure to bracken fern, and had not received any recent copper supplementation. It was euthanased and necropsy revealed haemorrhagic intestines, a brown, lobulated, small liver and grey/blue/black kidneys. Histopathological examination showed a chronic hepatopathy with bridging fibrosis, portal inflammatory cells including pigmented macrophages, hepatocyte karyomegaly and anisokaryosis, centrilobular necrosis and cholestasis. In the kidney there was intratubular haemoglobin with haemosiderosis and interstitial nephritis. Kidney copper concentration was excessive at 351 µmol/kg (reference range 0–157). A diagnosis of **chronic copper toxicity** with a superimposed haemolytic crisis was made. Bracken fern can cause haemolysis, but not a hepatopathy. The liver likely suffered a pyrrolizidine alkaloid insult with possible additional **sporidesmin damage**. The copper may have accumulated from a pelletised diet or agricultural spray.

A group of 700 pregnant mixed-age ewes from Central Otago were yarded for pre-lambing anthelmintic treatment. After several hours six were found recumbent, frothing at the mouth, seizuring and diarrhoeic, and three others were dead. It was thought that the drinking water may have been contaminated with zinc sulphate. A blood sample from one of the dead ewes had a serum zinc concentration of > 1,000 umol/L (adequate range 9–19), supporting a diagnosis of acute **zinc toxicity**.

A Rangitikei sheep farmer reported finding 14 aborted lambs over a 10-day period, from a group of 92 two-tooth (rising 2-year-old) primigravid ewes. No abortions had occurred among older ewes on the same property. The affected ewes had been vaccinated against toxoplasmosis last year but not during this year. Histopathological examination of tissues from an aborted lamb revealed neutrophilic and lymphohistiocytic meningitis, neutrophilic bronchopneumonia, and neutrophilic and lymphohistiocytic hepatitis, compatible with an infectious (likely bacterial) aetiology. ***Campylobacter fetus fetus*** was detected by PCR on fetal abomasal contents, confirming ***Campylobacter* abortion**.

A **listerial abortion** outbreak occurred in a flock of 221 dairy ewe hoggets on an Otorohanga farm. At least 13 hoggets aborted about one month before the planned start of lambing. They were on a diet of chicory, red clover and silage, and vaccinated against toxoplasmosis and campylobacteriosis. Histopathological examination of five aborted lambs and placentae showed fibrinosuppurative inflammation and vasculitis of all placentae, and one lamb had suppurative hepatitis. ***Toxoplasma gondii*** was detected by PCR in the stomach contents of one lamb but not the other four. Bacterial culture of stomach contents from five lambs yielded moderate to heavy growths of ***Listeria monocytogenes***.

In Otago and Southland, sheep abortions were a common reason for laboratory investigations during this quarter. There were 33 outbreaks of ***Salmonella* Brandenburg abortion** recorded, almost twice as many as in the 5-month autumn-to-spring gestation period for sheep

in 2018. Also seen were six outbreaks of *Toxoplasma* sp. abortion (cf. 20 last year), 11 caused by *Campylobacter fetus* ssp. *fetus* (14 in 2018) and four small outbreaks associated with *Helicobacter* spp. Most of the ewes affected in the *Toxoplasma* and *Campylobacter* abortion outbreaks had not been vaccinated against these infections.

Campylobacter abortion was diagnosed on several farms in Canterbury during this quarter. In one case, on a North Canterbury property, 30 older ewes in a group of unspecified size aborted near full term. Culture of the fetal stomach contents of two lambs yielded **Campylobacter *fetus* ssp. *fetus***. In another case, two of a group of 550 twin-bearing mixed-age ewes on a South Canterbury property aborted 6 days after a feed change from kale to Italian ryegrass. The farmer was concerned about salmonellosis but the ewes remained well. Culture of fetal stomach contents again yielded *Campylobacter fetus* ssp. *fetus*, confirming campylobacter abortion.

Porcine

A veterinarian was asked to investigate deaths in weaner piglets that were 3–5 weeks old on a free-range piggery in Nelson. Two litters were involved. Two of 10 piglets died from one litter and three of 11 from another. Histopathological examination revealed lesions in the spleen and brain that were consistent with infection related to **porcine circovirus 2**, previously known as post-weaning multisystemic wasting syndrome. No lesions suggestive of swine fever or African swine fever were detected (epidemics of African swine fever have recently been reported in several countries, especially in Asia).

Camelid

A 2-year-old alpaca from Rodney died after a period of intermittent weakness. Immediately before death it was hypothermic, with congested mucous membranes. Possible access to toxic plants including karaka (*Corynocarpus laevigatus*), woolly nightshade (*Solanum mauritianum*) and oleander (*Nerium oleander*) was reported. Antemortem testing had shown azotaemia, with urea 18.1 mmol/L (reference range 4.1–9.8) and creatinine 282 µmol/L (reference range 60–145), increased muscle enzymes (AAT 584 IU/L; reference range 129–235) and creatine kinase 1,965 IU/L (reference range 42–139). A leukogram was consistent with acute inflammatory response, with neutrophils 21.9 x 109/L (reference range 3.1–14.4 x 109) and band neutrophils 1.3 x 109/L (reference level 0). Necropsy showed pulmonary congestion, pericardial effusion, gastrointestinal tract reddening and 1–3-cm-diameter nodules in the uterus. Histopathological examination revealed multifocal myocardial necrosis, pulmonary congestion and oedema and congestion of other organs, all consistent with **oleander toxicity**. Oleander contains oleandrin, a cardiac glycoside with a chemical structure similar to digoxin. The uterine masses were spindle-cell tumours consistent with **leiomyomas or leiomyosarcomas**. The latter has been previously reported in an alpaca.

Caprine

Several 3–4-week-old kids from South Auckland died suddenly. Necropsy revealed severe fibrinous pleuritis and pericarditis in one while in another the meninges were opaque. Histopathological examination showed a fibrinosuppurative pleuritis and pericarditis and a neutrophilic meningitis, with ***Mannheimia haemolytica*** cultured from both sites, supporting ***Mannheimia* bacteraemia**. This organism is reported to cause pneumonia and pleuritis in kids, usually peracute, in late autumn or winter.

Bacterial meningitis was also diagnosed in a group of goat kids on a farm in King Country. The kids were 2 days to 2 weeks old and had neurological signs that included circling, lack of ocular menace response, unilateral drooping of ears or eyelids, head-shaking and recumbency. Ten of the group of 500 had died when a veterinarian was asked to investigate. Histopathological examination revealed severe diffuse subacute fibrinosuppurative meningitis and meningoencephalitis in samples from two animals. Intralesional bacteria (gram-positive cocci) were seen but unfortunately culture was not possible so the organisms could not be definitively identified.

A 14-year-old Cashmere doe in King Country had a rapidly-growing mass on the upper lip, close to the nasal philtrum. The mass was dermal, well-circumscribed and 1 cm in diameter with a scabbed surface. Cytological examination of a fine-needle aspirate revealed many pleomorphic spindle-shaped cells containing melanin pigment, consistent with a **melanoma**. Melanomas are occasionally reported in goats and are usually aggressive, with local infiltration and frequent metastasis to regional lymph nodes and internal organs.

Two Saanen cross goats from South Auckland had enlarged abscessed retropharyngeal lymph nodes, as well as coughing and exercise intolerance. Bacteriological culture of samples from the lesions in both animals yielded ***Corynebacterium pseudotuberculosis***, consistent with **caseous lymphadenitis**. This is a contagious, potentially zoonotic disease of sheep and goats, usually associated with infection through penetrating wounds.

Cervine

Neurological signs suspicious of ryegrass staggers developed in deer on a Canterbury property. They were being fed a mixture of grass and silage to which 4–5 kg of ryegrass straw per animal per day had recently been added. Within two days of adding the ryegrass straw signs of head tremor and a markedly ataxic gait developed. The affected animals were lethargic and inappetant and many became recumbent. Over a period of 2 weeks, 50 mature stags died out of a group of 600. In a similar-sized group of younger deer the clinical signs were similar but less severe. Four died. These younger animals had been offered about 2 kg of ryegrass straw per animal per day. Histological examination of the brains of two animals in the early stages of disease revealed no lesions (as is often the case with ryegrass staggers) but the brain of one deer examined later had lesions consistent with ryegrass staggers (swollen Purkinje cell axons and vacuolation in the molecular layer). Analysis of the ryegrass hay found no trace of lolitrem B, the classic toxin causing ryegrass staggers, but epoxy janthritrems, which have also been associated with ryegrass staggers, were detected. Surviving affected deer slowly recovered over about 4 weeks. The clinical, pathological, and toxicological findings were considered consistent with a diagnosis of **ryegrass staggers**.

Nine hinds from a farm with 3,000 deer were found dead 48 hours after being injected with a copper supplement. Two more were alive but lethargic and ataxic

and they died soon afterwards. The concentrations of copper in the kidneys of these two animals were 235 and 556 umol/kg (reference range 0–157) and histopathological examination showed massive hepatic necrosis, confirming **copper toxicity**.

Equine

A 10-year-old Friesian horse from Auckland developed a hot, swollen and painful udder and was pyrexic. Culture of a milk sample yielded small numbers of ***Streptococcus dysgalactiae***, consistent with **streptococcal mastitis**. Most cases of equine mastitis are reported to be caused by haemolytic gram-positive cocci.

A 10-year-old Warmblood gelding from Northland developed a firm swelling of the entire right hindlimb after an injection. Serum biochemistry showed markedly increased muscle enzymes including CK 10,601 IU/L (reference range 0–310) and AST 2,778 IU/L (reference range 233–567). Areas of gas within the limb were suspected on sonography. Culture of a swab collected through a sterile incision yielded ***Clostridium* sp**. bacteria, supporting a diagnosis of **clostridial myositis**.

Two Thoroughbred mares on a South Taranaki property produced stillborn foals. Histopathological examination of tissues from one of the foals revealed a necrotising bronchopneumonia and hepatitis with numerous intranuclear viral inclusions, confirming herpesvirus abortion. Most cases of **herpesvirus abortion** in New Zealand are attributed to equine herpesvirus-1 (EHV-1) but EHV-4 may rarely be involved. EHV-1 may be associated with sporadic abortions or, especially if carriers are introduced to naïve herds, more widespread abortion outbreaks.

A 13-year-old Appaloosa from the Bay of Plenty had a well-demarcated 5-cm ulcerated fleshy protruding mass on his sheath. When the horse was purchased a year earlier the mass had been much smaller, only 1 cm in diameter. Histopathological examination of a biopsy revealed an unencapsulated, infiltrative neoplastic proliferation of squamous epithelial cells, forming anastomosing islands and lobules and supported by a dense fibrovascular stroma, confirming **squamous cell carcinoma**.

A 1-year-old Thoroughbred filly from Waikato developed pyrexia and a purulent nasal discharge. ***Streptococcus equi* ssp. *equi*** was isolated from a nasopharyngeal swab, confirming a diagnosis of **equine strangles**.

Canine

A 15-month-old spayed female Spoodle with a history of urinary tract infection presented with diarrhoea and was treated with an anti-diarrhoeal preparation containing potentiated sulphonamides for 3 days. Four days after the treatment finished she re-presented with pollakiuria. Urine sediment examination revealed numerous sulfadiazine crystals with the characteristic "sheaf of wheat" morphology. Urinalysis was otherwise unremarkable, with urine specific gravity of 1.047, pH 6.5, minimal protein and no evidence of haematuria, pyuria or bacteriuria. The antidiarrhoeal preparation used (Scourban™ Bayer Animal Health) contains sulfadiazine, among other ingredients. The crystalluria resolved uneventfully. In humans **sulfadiazine crystalluria** has been reported as an asymptomatic finding, as well as with acute kidney injury (precipitation of crystals within the kidney) and obstructive uropathy. Risk factors for nephropathy include hypovolaemia/dehydration (which may have been a factor in this diarrhoeic patient), excessive dosing, hypoalbuminaemia and pre-existing renal insufficiency. Treatment involves cessation of sulfadiazine treatment, volume replacement and urine alkalisation.

Feline

A sixteen-year-old lioness from a zoological park was noted to be polydipsic and exhibiting abnormal behaviour including licking the ground. Despite treatment she became anorexic and isolated herself from the pride. Examination under anaesthesia revealed a palpable abdominal mass and she was euthanased. Necropsy revealed a markedly enlarged spleen, enlarged liver and discoloured left lung. Histopathological examination showed sheets of neoplastic lymphocytes in the spleen and liver, consistent with lymphoma. Blood vessels in the kidneys and lungs were also noted to contain intraluminal neoplastic lymphocytes, suggesting an associated lymphoid leukaemia. It was unclear whether lymphoma or leukaemia was the primary disease process.

A chronically unwell 1-year-old Domestic Longhaired cat presented with recent-onset neurologic signs including staring into space, head-tilt, wide stance, difficulty eating and falling into the food bowl. Blood glucose was normal. Serum biochemistry and a complete blood count revealed markedly elevated sodium (207 mmol/L; reference range 147–160) and chloride (170 mmol/L; reference range 113–127). There was a mild increase in urea (19.3 mmol/L; reference range 6.6–12.6) but creatinine was normal at 107 umol/L (reference range 67–150). Differentials for severely increased sodium include neurologic disease (limited access to water or central diabetes insipidus), water loss from severe gastrointestinal disease, and (although considered less likely in this case) chronic kidney disease or non-oliguric acute kidney injury. The lack of creatinine elevation in this case may have been due to reduced muscle mass in this chronically unwell young cat. The neurologic signs could have been primary, or secondary to the hypernatraemia (intracranial disease can cause hypernatraemia, which in turn can cause neurologic disease.) Acute hypernatraemia carries a risk of CNS haemorrhage or thrombosis. Chronic cases require slow fluid resuscitation to avoid cerebral oedema. On further questioning it emerged that the cat had been observed licking a Himalayan salt lamp which had recently been moved to a lower level. The cat was re-hydrated and the neurologic signs abated, supporting a diagnosis of **acute salt toxicity**. Salt toxicity in companion animals is uncommon and reported more commonly in dogs than in cats, possibly because cats have more fastidious tastes. Reported causes of salt toxicity in dogs include ingestion of seawater, paint balls, playdough and home-made emetic solutions.

An 8-year-old female Domestic Shorthair cat from Rangitikei developed chronic draining nodular masses in the inguinal region. Histopathological examination of biopsies revealed pyogranulomatous cellulitis with rare gram-positive bacteria and there was a scant growth of ***Nocardia* sp**. on bacterial culture, confirming the clinical suspicion of **nocardiosis**. This is a rare disease in cats, often developing

in body locations subject to bite and scratch injuries. Immunosuppressed cats appear more susceptible to infection (Malik et al., 2006).

A 10-year-old neutered male Domestic Shorthair cat with a long history of midline dorsal pyoderma had been treated for an unspecified period of time with cefovecin. Cytology by the attending veterinarian revealed abundant neutrophils, moderate numbers of bacterial cocci and lower numbers of bacterial rods. Culture of a skin swab yielded an isolate of ***Stenotrophomonas maltophilia*** that was resistant to multiple antibiotics. This is an opportunistic pathogen often found in water or aqueous environments (including antiseptic solutions and liquid soaps). It has been associated with nosocomial infections in human hospitals, most commonly in immunocompromised patients.

Avian

A rainbow lorikeet (*Trichoglossus moluccanus*) thought to be between 2 and 5 years old was found dead in the aviary. It had appeared normal that morning. Necropsy was unremarkable. Histopathological examination of multiple tissues revealed widespread **haemosiderin deposition** in multiple organs. This takes place when there is an increase in serum iron, particularly in association with haemolysis. It can be an incidental finding in pet birds but appeared excessive in this case. One possible cause is avian malaria. No malarial organisms were noted in the examined tissues but they are not always identified. Other causes of haemolysis include zinc and copper toxicity. The cause was unfortunately not able to be definitively identified in this case.

A 2-year-old galah (*Eolophus roseicapilla*) became lethargic and anorexic. Despite treatment it became recumbent and died later. Necropsy revealed a large, pale liver with areas of haemorrhage beneath the capsule. These findings were consistent with a fatty liver (**hepatic lipidosis**) caused by excessive accumulation of lipid metabolites within hepatocytes and eventual liver failure. The most common cause is a high-fat diet. In this case the bird was being fed entirely on sunflower seeds, which are high in oil.

Lagomorph

A 3-week-old Lop rabbit bought from an Auckland pet store was lethargic. It was immediately taken to a veterinarian for treatment but died 2 days later. At necropsy the rabbit was noted to be emaciated and dehydrated. Histopathological examination revealed a moderate multifocal subacute tubulointerstitial nephritis with tubular intracellular gram-positive protozoa consistent with ***Encephalitozoon cuniculi***. Similar organisms with accompanying inflammation were seen in the brain, heart, liver, adrenal glands and mesenteric lymph node. *E. cuniculi* is an intracellular microsporidian protozoan recognised as a significant opportunistic pathogen of immunocompromised humans. Spontaneous infections have been documented in rabbits, mice, rats, muskrats, guinea pigs, hamsters, ground shrews, goats, sheep, pigs, horses, domestic dogs, wild and captive foxes, domestic cats, exotic carnivores and nonhuman primates. The life cycle in mammals is postulated to be simple and direct, with most infections acquired through ingestion or inhalation, transplacentally, or, rarely, through trauma to the epithelium. In naturally infected rabbits, it is primarily acquired through ingestion of infected urine, and is most commonly spread from infected dams to neonates. Spores are excreted in the urine for about 63 days and early infection is largely confined to the lung, kidney, and liver, with later infection most commonly localised to heart, brain and kidney. The disease is often subclinical but clinical signs may include those of vestibular disease (ataxia, opisthotonos and torticollis), paralysis, phacoclastic uveitis, renal failure or weight loss.

The owners of a Mini Lop rabbit-breeding establishment in Taupo became concerned by reports of young rabbits from the property dying after being shipped to the South Island. Coccidiosis was suspected. Faecal samples from a number of animals on the breeding establishment were collected over several days to investigate. Small numbers to very high numbers of coccidia were seen in these samples, confirming **coccidiosis**.

Piscine

An aged inanga (*Galaxias maculatus*) from a zoological collection was euthanased as its internal organs appeared to be prolapsing through the vent. At necropsy the coelom contained a gelatinous substance. Histopathological examination revealed a **large-intestinal adenocarcinoma** that had metastasised to the peri-renal tissues, coelom and skeletal muscle. Neoplasia is common in older fish.

Marsupial

A four-year-old Tasmanian devil (*Sarcophilus harrisii*) from a zoological collection was euthanased because of deteriorating mobility. Histopathological examination of the brain and spinal cord showed Wallerian-type degeneration in white-matter tracts, consistent with **degenerative leukoencephalopathy and myelopathy**, which has previously been described in Tasmanian devils and quolls.

Amphibian

Three Australian whistling or brown tree frogs (*Litoria ewingii*) were moribund and underweight. The frogs were euthanased. Necropsy showed no gross lesions but histopathological examination revealed epidermal intracorneal fungi in the skin of all three, consistent with **chytridiomycosis** caused by *Batrachochytrium dendrobatis*. This pathogen is associated worldwide with declining amphibian populations and was first identified in New Zealand in Canterbury in 1999. It has since spread into other frog populations.

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Quarterly report of investigations of suspected exotic diseases: July to September 2019

Exotic disease investigations are managed and reported by the Ministry for Primary Industries (MPI) Diagnostic and Surveillance Services Directorate, Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from July to September 2019.

Enzootic bovine leucosis excluded

A veterinary pathologist called the MPI exotic pest and disease hotline to report a case of possible enzootic bovine leucosis (EBL) after identifying lymphoma in multiple biopsy samples submitted from a dairy heifer. The heifer had presented with pyrexia, an unusual posture and a multicentric lymphadenopathy, noticed at calving. Histopathologically a large proportion of the normal lymph-node architecture was replaced by a diffuse, uniform proliferation of round cells resembling large lymphocytes. EBL is caused by bovine leukaemia virus but there is also a sporadic, non-contagious form of lymphoma, and it is not possible to differentiate between them on the basis of histology. Samples of the lymphoma from multiple sites including the abdomen, hind limb, shoulder and mammary tissue were submitted to MPI's Animal Health Laboratory (AHL) (Wallaceville) and subsequently to the Elizabeth MacArthur Agriculture Institute in New South Wales for PCR testing. As a precaution, testing for bovine ephemeral fever was also completed. All samples were negative in the molecular assays. EBL has been the subject of an industry-led eradication programme and is now thought to be absent from New Zealand's dairy herd. Bovine ephemeral fever (three-day stiff sickness) is an exotic arthropod-borne disease of cattle in northern Australia, Africa and Asia. Affected cattle may exhibit periorbital swelling and oedema of superficial lymph nodes, along with a temporary fever, stiffness and lameness. Bovine leukaemia and ephemeral fever were excluded and the investigation was stood down.

Exotic causes of bovine abortion excluded

A veterinary practitioner contacted MPI via the exotic pest and disease hotline to discuss an abortion storm in a group of heifers. About a hundred heifers out of a mob of 300 had aborted over a 10-day period, 2–3 weeks before their due date. Calves were found either dead or non-viable, with no gross deformities. Heifers aborted without assistance and most retained their placentas. The farm veterinarian was satisfied that the heifers had not been exposed to any toxic agents, including isocupressic acid from ingestion of macrocarpa, Leyland cypress or pine needles.

A routine bovine abortion investigation work-up at a commercial veterinary laboratory, which included histopathology and culture of stomach contents for known endemic abortion agents, was unrewarding. Fetal tissues were submitted to the AHL, where they tested negative by PCR for the exotic agents *Brucella abortus*, the abortifacient strain of bovine herpesvirus-1 and *Coxiella burnetti*. In addition, *Mycoplasma bovis* was ruled out by negative PCR results from fetal tissues and negative serology (ELISA) from sera collected from 143 animals. Subsequently *Aspergillus fumigatus* was isolated at the veterinary laboratory, from stomach contents of two later abortions. It is likely that this agent accounted for some of the abortions observed. Exotic disease was excluded and the investigation was stood down.

Aujeszky's disease excluded

A Northland veterinarian called the exotic pest and disease hotline to report a case of suspected Aujeszky's disease in pigs. Pigs were found dead the next day after showing tremors and convulsions and being unable to stand. Two of the 11 pigs on the property were affected, both in the same pen. They underwent post-mortem examination because the combination of neurological signs and sudden death are potentially consistent with Aujeszky's disease (pseudorabies). Aujeszky's disease was the subject of a New Zealand eradication programme in the late 1990's and the national pig herd is now considered disease-free. Tissue samples and heart blood were collected at postmortem and submitted to the AHL for histology, molecular assays and virus isolation. Molecular testing for the viruses that cause Aujeszky's disease, African swine fever, classical swine fever and porcine reproductive and respiratory syndrome gave negative results by qPCR TaqMan. However, tests were positive for porcine circovirus type 2 and a generic herpesvirus by PCR. Virus isolation was carried out with a herpesvirus isolate and Massey University's genome services used a Blastr algorithm to identify it as porcine lymphotrophic herpesvirus type 3. Types 2 and 3 are endemic in New Zealand pigs and have not been associated with the clinical signs reported in this case. Histopathology of the brain tissue confirmed salt poisoning as the likely cause of the presentation. Exotic disease was excluded and the investigation was stood down.

Brucella suis excluded

An official from the Ministry of Health notified MPI that a New Zealand resident had been diagnosed with a *Brucella suis* infection. The affected person had lived in New Zealand for the previous 5 years, but was of British descent and had lived and worked in piggeries in Australia for a number of years. The agent had been cultured from a specimen collected from the pre-patellar bursa of his knee. Pre-patella bursa infection is a rare sequela of chronic infection with *B. suis* (Mawer et al. 2015). Full genomic sequencing of the isolate was carried out and confirmed the diagnosis.

As the affected person had been working on a New Zealand pig farm at the time of diagnosis it was important to exclude New Zealand pigs as a potential source. However, *B. suis* is not known to be present in livestock in New Zealand.

After the affected person was interviewed several times it was determined that the most likely source of the agent was exposure to Australian feral pigs, in which *B. suis* is endemic (Mor et al 2016; Pearson et al 2014). This conclusion was based on the patient's work history in Queensland and a number of potential risk factors enabling exposure to the agent, e.g. carrying out postmortems of dead pigs without personal protective equipment. Notwithstanding these initial findings, it was important to provide additional assurance that infection was not acquired in New Zealand, so a sampling plan was initiated to provide proof of freedom.

On the farm of interest, three separate pig cohorts were tested: the breeding herd (divided into sows and boars) and the grower/finisher mobs. The surveillance carried out in each of these groups was risk-based. For instance, with the sows, 232 vaginal swabs were collected for PCR testing from animals returning to heat and from sows determined to be non-pregnant at a 6-week scan. Thus, these animals were more likely to have experienced early embryonic mortality than a random selection of sows. With the boars, 53 older cull animals were sampled as their age meant there was a greater probability of being exposed if the agent was present in the herd. In boars, the rate of recovery from infection is low (< 50 percent). Thus, once infection occurs there is a reasonable probability of detection, that doesn't rely on testing during the acute phase of infection. Testing of this cohort consisted of harvesting their epididymides (a site where the agent is often found in infected boars) at slaughter and testing them for *B. suis* using PCR.

Blood from 249 grower/finisher animals was collected at slaughter. Timing of the collection still fitted within the window for detection if in-utero infection or recent exposure had occurred. However, testing of this final group was considered to offer the least value, because the sensitivity of detection would be higher in sows and boars if the agent had been present. For each cohort the sampling number was aimed at providing > 95 percent confidence of freedom at a conservative design prevalence (sows and growers 2.5 percent, boars 15 percent). *B. suis* was not detected in any of the blood or tissue swabs tested. Thus the investigation excluded New Zealand pigs as the source of *B. suis* in the human case. Evidence gained from interviews confirmed the likelihood that infection was representative of a chronic infection occurring from exposure to feral pigs in Queensland, Australia. Exotic disease was excluded and the case was stood down.

Contagious equine metritis excluded

A veterinary pathologist at a commercial laboratory called MPI to report vulvovaginitis in a 6-year old mare from Canterbury. The owner had noticed that the mare was in pain when moving, and physical examination showed swelling, ulceration and erosion of the vulva. Bacterial culture showed a mixed bacterial growth. Biopsy specimens submitted for histology showed acute inflammation, but identified no evidence of a causative agent. Causes of vulvovaginitis include endemic equine herpesvirus type 3 associated with coital exanthema, and the exotic agent *Taylorella equigenitalis*, the cause of contagious equine metritis (CEM). An acute ulcerative response is not characteristic of CEM, and the mare had neither travelled overseas nor been bred. Dry swabs and a fresh scraping of the lesions were submitted to the AHL and tested by PCR for the DNA of *T. equigenitalis* and generic equine herpesviruses. All PCR assays were negative. The cause of the vulvitis in this mare was not determined, but CEM and coital exanthema were ruled out.

Equine neurological disease investigated

An equine veterinarian called the exotic pest and disease hotline to report neurological disease in two weanling horses. In the week prior to onset of clinical signs, the two weanlings (a filly and a colt) had been boxed in temporary stables to prepare them for surgery. Both horses presented with nasogastric reflux, muscle weakness, and rapid heart and respiration rates. While the filly was bright and alert, the colt was depressed and showed signs of colic. Both horses were transported to a local veterinary hospital for observation and continued care. The colt was examined by abdominal ultrasound and abdominocentesis. There was an accumulation of gas in the colon, and the presence of purulent material from abdominocentesis indicated peritonitis. Owing to the colt's worsening condition it was euthanased that evening, and the filly was found dead the following morning. At postmortem both horses were found to have gastric dilatation and rupture. Samples were collected for histology, virology and toxicology. A detailed walk-through of the paddock that the weanlings were in identified two weeds that were considered atypical in pasture. Specimens were collected for identification and toxicology. Histology of the brain, heart, liver, lung, kidney was unremarkable. Various sections of the gastrointestinal tract were normal apart from serosal inflammation (and the presence of plant material), which confirmed peritonitis. The plants collected were identified as *Anthemis cotula* (mayweed) and *Datura stramonium* (jimson weed). *D. stramonium*, a member of the nightshade family, is a known toxic plant in New Zealand, with all parts of the plant containing dangerous levels of the alkaloids atropine, hyoscyamine and scopolamine. The clinical and pathological effects of *datura* on horses have been described (Binev et al., 2006), with a key feature being gastric dilatation and rupture. Hyoscyamine inhibits the action of acetylcholine, the main neurotransmitter of the parasympathetic nervous system. It decreases the motility of the gastrointestinal tract and reduces the secretion of acid from the stomach and other fluids from the gut and airways. Inhibition of the parasympathetic fibres of the vagus nerve increases both the heart and respiration rate, and reduces peristalsis. The paralytic ileus that ensues causes constipation and allows the accumulation of intestinal gas, which flows into the stomach where it causes dilation and, in acute cases, stomach rupture. Horses on this farm were the subject of a previous investigation in 2013 into illness characterised by ataxia and weakness, and although a cause was not identified it was considered likely to be the result of ingesting a poison. The earlier cases occurred in the same paddocks where the *Datura* plants were identified, and it seems plausible that the previous event may have also been due to the ingestion of this plant. The clinical signs seen in the horses during the current investigation were considered to be consistent with a parasympathetic block and the result of *Datura* poisoning. Exotic disease

was excluded and the investigation stood down.

African horse sickness excluded

A private veterinarian called the exotic disease hotline to report a significant horse mortality on a Marlborough dairy farm. Four Clydesdale horses died over a 12-hour period, with the remaining three showing signs of respiratory disease (respiratory dyspnoea). The dead horses had blood-tinged respiratory secretions. Necropsies carried out on two of the horses indicated that the most significant finding was pulmonary oedema. These findings were confirmed from histological examination of lung tissue collected at the time. There were no significant findings from serum biochemistry of blood collected from the clinically affected horses. Given the signs of pulmonary oedema, an investigation was opened to exclude African horse sickness (AHS), although this differential diagnosis was considered unlikely given the requirement for an exotic vector and the absence of an introductory pathway that could explain the entry of both the agent and its vector. Lung tissue collected at necropsy and submitted to the Australian Animal Health Laboratory tested negative for AHS by PCR and no virus was detected from virus isolation attempted on the tissue samples. The most likely cause of death and clinical signs of the affected horses was a toxin. A horticultural soil fumigant had been used on the neighbouring property several weeks prior to the mortality event. Exposure to the active ingredients of the fumigant (chloropicrin and 1,3-dichloropropene) was investigated as a potential cause of the acute respiratory signs and mortality. However, despite intensive laboratory testing of a range of tissues collected post-mortem, results were inconclusive. Thus, while exotic disease was excluded, the agent responsible for the event was not determined.

Infectious bursal disease excluded

A veterinary pathologist called the exotic disease hotline to report that tissue samples examined from the bursa of Fabricius of one chicken (of 10 submitted from a flock) had histological lesions that could be consistent with infectious bursal disease (IBD). The lesions were lymphoid depletion with necrosis and heterophilic inflammation. Fresh bursal tissue samples had been submitted to a commercial veterinary laboratory as part of general surveillance at the processing plant. In accordance with standard operating procedure, stored bursal and blood samples from the birds were submitted to the AHL. Bursal samples from all 10 birds were tested for IBD by PCR. In addition, the sera were tested using the IBD virus-neutralisation test (VNT). All bursal samples were negative for IBD virus and there was no evidence of past exposure, evidenced by negative serology (VNT titres < 1:2). Thus IBD was excluded and the investigation was stood down.

Wood pigeon (kererū) mortality investigated

The Department of Conservation called the exotic pest and disease hotline to report an unexpected increase in numbers of kererū (*Hemiphaga novaeseelandiae*) presented to a Northland bird recovery centre. Fourteen birds had been presented, unable to fly and with green diarrhoea, over a 3-day period from within a 30-km radius of the recovery centre. Four of the birds subsequently died. Cloacal swabs were collected from seven affected birds, placed in avian transport medium and submitted to the AHL, where the exotic differentials avian influenza and Newcastle disease were ruled out by PCR testing. A diarrhoea-causing group A rotavirus that has recently been confirmed in New Zealand racing pigeons was also ruled out by PCR testing. The four dead birds were submitted to Wildbase Pathology at Massey University for necropsy. Two were found to have traumatic injuries consistent with flying into windows. The other two birds were juveniles whose deaths were attributed to starvation. Exotic disease was ruled out and the investigation was closed.

Abdominal lesions in hare investigated

An AsureQuality manager based in Wairarapa contacted an MPI incursion investigator regarding a hare with unusual pathology, the perirenal and sub-lumbar fat being an unusual golden or yellow colour and slightly firm. The hare (*Lepus europaeus occidentalis)* had been originally provided to the AsureQuality manager by a hunter. The carcass was submitted to Wildbase Pathology at Massey University, where gross and histopathological examination confirmed a diagnosis of fat necrosis. Focal or diffuse necrosis of mesenteric or other abdominal fat is a common finding at necropsy in domestic animals. The causes and pathogenesis are not fully understood. It can be associated with pancreatitis, there may be a genetic component (as in Channel Island cattle breeds), and diet and trauma may also play a role in its pathogenesis in some species. Exotic or emerging disease was ruled out and the investigation was closed.

Figure 1: The kidney of a hare from Wairarapa with unusual yellow perirenal fat. Photo: Tony Roberts & Cees Groot, AsureQuality

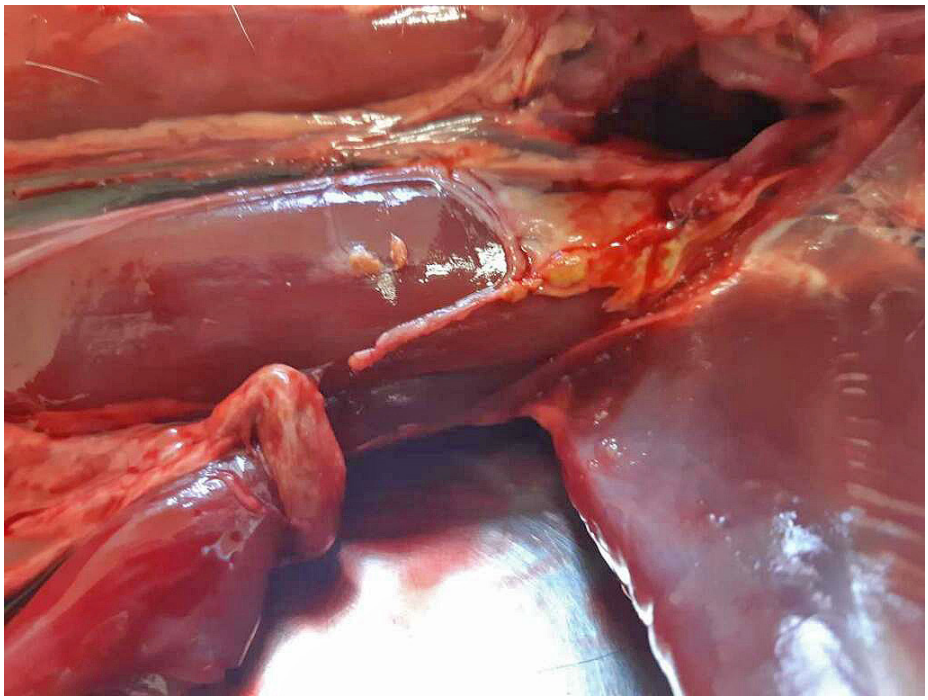

Figure 2: Golden sub-lumbar fat from the hare. Photo: Tony Roberts & Cees Groot, AsureQuality

Brucella canis excluded

A veterinarian called the exotic pest and disease hotline about a 10-year-old Labrador dog with unilateral orchitis/epididymitis. The dog had been unwell with a swollen testicle but had responded well to anti-inflammatory and antibiotic treatment. The owner of the dog wanted to use it for stud so did not want it castrated. There were no direct pathways for an exotic disease incursion because the dog was New Zealand-born and had not yet been used for stud. A blood sample was collected and submitted to the AHL where the *Brucella canis* card test returned a negative result. The dog went on to make a full recovery. Exotic disease was excluded and the investigation was stood down.

A veterinarian reported a male dog showing signs of orchitis and epididymitis, where canine brucellosis was a differential diagnosis. The dog was a 12-year-old Labrador born and bred in New Zealand and it had not been used as a stud dog. The affected testis was surgically removed and submitted to the AHL, where affected tissues were determined to be negative by PCR for *Brucella canis*. Histology identified a focally extensive necrotising, pyogranulomatous and fibrosing orchitis with intra-lesional gram-negative bacilli. Exotic disease was excluded and the investigation was closed.

Rabies excluded

A veterinarian called the exotic pest and disease hotline to report an 11-year-old New Zealand-born British Blue cat that had been behaving abnormally, showing significant aggression towards its owners. After a few days of ataxia and restlessness, the cat had suddenly become aggressive, biting its owners and subsequently the veterinarian. The cat was normally very friendly. On veterinary examination, an ear infection was identified but the eardrum was intact and the infection looked to be minor. Given the unusual behaviour of the animal, the veterinarian considered rabies as a remote but potential differential diagnosis and called the MPI hotline. At the time of the report only limited specimens were available for further diagnostic work as the cat had been euthanased and the cadaver was stored in a freezer. Under the direction of an Incursion Investigator, the brain was removed for histological examination, but no significant lesions were identified and no lesions consistent with rabies were seen. This did not confirm a cause for the aggressive behaviour, but excluded rabies as a potential aetiology. It was considered that pain associated with the ear infection could have caused the cat to behave unusually and show aggression. Exotic disease was excluded and the investigation was stood down.

Canine distemper virus excluded

In late June, a veterinarian in Auckland called the exotic pest and disease hotline on two consecutive days to report possible cases of canine distemper (CD) virus in two dogs from separate households, both with incomplete CD vaccination histories, and both from a part of Auckland where dogs are largely unvaccinated against CD. The first case was in a 3-year-old unneutered female Rottweiler with conjunctivitis, coughing, fever and bilateral nasal discharge. The second affected dog brought to the clinic a day later was a 4-year-old unneutered Staffordshire Terrier cross, which presented with a fever, cough and bilateral nasal discharge. CD is notifiable in New Zealand. Although rare, isolated cases have been investigated and confirmed, none have been associated with outbreaks as is seen in wild-type disease. The Rottweiler's condition worsened and it was euthanased, with an autopsy, sample collection and histology carried out. PCR for CDV and parvovirus was performed on swabs (nasal exudate, mouth, and rectum) and faeces. Tests were negative for CD RNA, allowing CDV to be excluded as a cause of disease in this dog. Given the dog's poor vaccination record, samples were sent for CDV and parvovirus testing by immunohistochemistry (IHC). Samples were negative on both IHC and PCR for CDV and parvovirus.

The Staffordshire dog had been rescued and re-homed a week before the onset of disease. Specimens were collected from the dog, including a nasal swab, urine, blood and serum. After sampling, but prior to results being received, the dog was surrendered to a shelter, which then was urged to keep the dog in isolation pending laboratory results. PCR assays for CDV and parvovirus were performed, with negative results. Although a connection between the two dogs was suspected initially, when further information was obtained about the timing of clinical signs and the locations of the dogs, it became apparent that the cases were separate events. Although a cause was not established in either case, distemper virus was excluded in both dogs and the investigation was closed. Over the past decade, sporadic cases of CD have been detected, which are either

positive by PCR (when fresh tissues are available) or IHC (when only fixed tissues are available). Isolated cases have been confirmed from both the North and South Islands. In each case there has been no confirmed spread to other dogs. The cases are suspected to be either a vaccine-associated strain or an atypical CD virus. Reporting of all possible CD cases helps build a better understanding of the epidemiology of CD in New Zealand, and we appreciate hearing from clinicians and pathologists who see possible cases.

Bacillus anthracis excluded

A commercial laboratory called MPI about a bacterial culture that could not be differentiated by classical culture methods from *Bacillus anthracis*, the agent of anthrax. The bacterial culture was part of routine quality-assurance testing for a small food company, and was intended to test for *B. cereus*, a potential agent of food-borne illness. The cultured agent was sent to the AHL, where PCR testing ruled out *B. anthracis*. The bacterial culture was analysed by matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectrometry, a technique that analyses the "fingerprint" of an organism and identifies it from a digital library. MALDI-TOF analysis identified the bacterium as *B. subtilis*, an organism that is ubiquitous in soil, hay and the digestive tract of humans and ruminants. Anthrax was excluded and the investigation was closed.

European foulbrood excluded

An Apicultural Officer from AsureQuality called the exotic pest and disease hotline to report beehives showing signs consistent with European foulbrood, which is caused by the exotic agent *Melissococcus plutonius*. Several beehives at each of the beekeeper's eight apiary sites were affected. Although the beekeeper had been undertaking varroa mite control, it appeared to the Apicultural Officer that there were signs of sacbrood and chalkbrood, pointing to parasitic mite syndrome, and there were also C-shaped and discoloured larvae. PCR testing at the AHL was negative for *Melissococcus plutonius* (and for *Paenibacillus larvae*, the bacterium responsible for American foulbrood disease, which is endemic). Advice was provided to the beekeeper by the AsureQuality staff to improve varroa mite control in the hives. Exotic disease was excluded and the investigation stood down.

Bee mortality investigated

A commercial beekeeper called the exotic pest and disease hotline to report a significant bee mortality event. The beekeeper had been carrying out his first spring health check and had observed about a 63 percent mortality rate (55 out of 88 colonies) at the first few apiaries he inspected. The history (including dead colony observations, live colony observations and colony management) obtained by the AsureQuality Apiary Officer (AAO) indicated that a treatment failure for *Varroa destructor* was the most likely cause of mortality. This was particularly so since oxalic acid had been used as an autumn treatment (instead of synthetic miticide compounds such as pyrethroids or amitraz) and many failures had been observed this season with oxalic acid. The AAO considered that the only exotic differential diagnosis likely was external bee mites, or an exotic bee incursion such as *Apis mellifera scutellata*. Composite samples of bees were collected from the surviving hives in each of the three or four apiaries that had elevated mortality rates. These samples were submitted to MPI's Plant Health and Environment Laboratory (Tamaki) for examination. External bee washings identified *Varroa destructor* mites and the species of bee was confirmed to be *Apis mellifera*. Thus exotic species of bee and bee mites were excluded and the investigation was stood down.

Moku virus identified in wasps

A government scientist emailed MPI to enquire about documenting a new to New Zealand virus, Moku virus, found in wasps (*Vespula vulgaris*) as part of a metatranscriptomic study (Quinn et al., 2018). Moku virus is a member of the iflavirus family, a family of RNA viruses that infect insects and includes deformed wing virus (DWV). The wasps in the study had been collected from Canterbury for research into the presence of pathogens. Moku virus was among a number of pathogens that were detected. This virus was originally discovered in Hawai'ian wasps, but has also been detected in honey bees (*Apis mellifera*) and varroa mites (*Varroa destructor*) (Mordecai et al., 2016). Although its presence in varroa mites suggests Moku virus might be transmissible to bees by that route, its pathogenicity in bees is not known. The detection of this virus is thought to be due to improved sensitivity of surveillance and the use of new tools such as metatranscriptomics, rather than the recent introduction of this agent. The purpose of this description is to provide a searchable record of the discovery of Moku virus, a potential honey bee pathogen, in New Zealand wasps.

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Thomas Rawdon
Team Manager
Surveillance and Incursion Investigation (Animal Health)
Diagnostic and Surveillance Services
Ministry for Primary Industries
thomas.rawdon@mpi.govt.nz

Quarterly report of investigations of suspected exotic marine and freshwater pests and diseases: July to September 2019

Exotic marine and freshwater pest and aquatic disease investigations are managed and reported by MPI Diagnostics & Surveillance Directorate, Wallaceville. The following is a summary of investigations of suspected exotic marine and freshwater diseases and pests during the period from July to September 2019.

New to New Zealand ascidian, Great Barrier Island

MPI was notified about prolific numbers of an unknown ascidian in Smokehouse Bay, Great Barrier Island. The notifier had never seen the ascidian before. Recreational vessels often frequent Smokehouse Bay to clean biofouling from boats. The ascidians were found in habitats varying from rocky reef to soft gravel. They were also found growing on mangrove seedlings and on algae. NIWA's Marine Invasives Taxonomic Service (MITS) identified the colonial ascidian, *Clavelina oblonga* (Ascidiacea: Clavelinidae) and MPI initiated a response to assess and manage any biosecurity risk.

New to New Zealand nudibranch, Wellington

MPI was informed that a nudibranch had been found among red seaweeds collected from the inter-tidal zone at Moa Point, Wellington. As the collection of seaweeds was a mixture it was not possible to determine which seaweed it was associated with. MITS identified the nudibranch from photos and video as *Melibe australis* (Gastropoda: Tethydidae), a species not previously recorded from New Zealand waters. *M. australis* is native to temperate Australian waters, ranging from Western Australia to New South Wales, as well as Tasmania. Given its native range, this species may establish here, or have been present for some time. *M. australis* is not known to have any invasive characteristics. As this was a single detection, and the biosecurity risk to New Zealand appears to be low, the investigation was stood down.

Aquatic pathogen at commercial fish hatchery

A commercial laboratory contacted MPI to report the isolation of the pathogen *Yersinia ruckeri* from salmon samples collected from a commercial hatchery. *Y. ruckeri* serotype O1b is the only serotype considered endemic in New Zealand. Exotic serotypes of *Y. ruckeri* are unwanted organisms under the Biosecurity Act 1993.

Two isolates of *Y. ruckeri* were sent to the MPI AHL, checked for purity and sent for serotyping at the Australian Animal Health Laboratory. The two isolates were confirmed as serotype O1b. There is no biosecurity risk as this serotype is endemic to New Zealand, so the investigation was stood down.

Mediterranean fanworm range extension, Dunedin

The exotic Mediterranean fan worm, *Sabella spallanzanii* (Polychaeta: Sabellidae), was found in Port Otago for the first time this year during MPI's Marine High Risk Site Surveillance programme. *S. spallanzanii* was first detected in New Zealand in Lyttelton Harbour in 2008 and attempts were made to suppress this population. It was later found to be widespread and established in Auckland, and has also been detected in Whangarei, Nelson, Tauranga, Gulf Harbour, Picton, Tutukaka, Coromandel, Wellington, Gisborne, Thames, Tarakohe and Opua.

First report of a digenean parasite in New Zealand

A university researcher notified MPI that they had identified the digenean parasite *Transversotrema patialense* (Trematoda: Transversotrematidae) for the first time in New Zealand. Cercaria (an intermediate life stage) were first found in water associated with a non-indigenous thiarid snail, *Melanoides tuberculata*, which had been had been purchased from an online aquarium supplier. A survey was being undertaken of *M. tuberculata* specimens from aquaria and "wild" populations in geothermal springs. *T. patialense* was only found in the snails from the aquaria. Transversotrematids are unusual among digeneans in that they only require two hosts rather than the usual three to complete their life cycle. *T. patialense* infects a number of different aquarium fish species as its definitive host but is highly specific with respect to its intermediate host: *M. tuberculata* is the only known intermediate host species in New Zealand. For this reason, it is likely that *T. patialense* can only establish in natural waterways in New Zealand where *M. tuberculata*, is present, i.e in geothermal waters (owing to its minimum temperature requirements). Should *T. patialense* establish in natural waterways in New Zealand, its effects on native fish species are unknown. However, as native fish populations are unlikely to overlap with *M. tuberculata* populations (owing to their different temperature tolerances), the impacts are likely to be minimal and restricted to aquarium populations of fish. The biosecurity risk was considered low and the investigation was stood down.

Eudistoma elongatum range extension, Kaipara Harbour

NIWA notified MPI that the Australian droplet tunicate *Eudistoma elongatum* (Ascidiacea: Polycitoridae) was found on a marine farm during a targeted marine pest survey of Kaipara Harbour, conducted by NIWA on behalf of Auckland Council in partnership with Northland Regional Council and MPI. *E. elongatum* was first reported from the Bay of Islands in 2005, and has since been found in a number of locations on the east coast of the North Island, from Waitemata Harbour to Rangaunu Harbour. However, this is the first time this species has been found on the west coast of the North Island.

Scallop mortality in Queen Charlotte Sound

A commercial diver contacted a Fisheries New Zealand staff member after noticing that a tubeworm had suddenly appeared in vast numbers in Queen Charlotte Sound. In the same area, the diver had observed dead and apparently dying scallops, *Pecten novaezelandiae* (Bivalvia: Pectinidae), in areas where the tubeworm was growing. Samples of worms and scallops were submitted to MITS and the MPI Animal Health Laboratory (AHL) respectively, to determine whether the worm was an exotic species and evaluate the health of the scallops.

The worms were identified as the parchment worm *Chaetopterus chaetopterus*-B (Polychaeta: Chaetopteridae). This is a cryptogenic species (unknown whether native or introduced) that is known to be present in Marlborough.

To further evaluate the health of the scallops, five individuals were taken from within the parchment worm bed ("affected") and five from outside the bed ("controls"). Histology samples were taken, and the pathologist reported an inflammatory response of the connective tissues in "affected" and "control" scallops, but particularly in the former. It is uncertain whether this inflammatory response was the result of an infectious or a degenerative process. No infectious agents were found to be associated with the inflammatory response.

There was evidence of virus-like particles within the digestive glands of both the "affected" and "control" scallops. These are frequently reported in New Zealand scallops but it is unknown whether they have significant health effects. Although the parasite *Perkinsus olseni* has previously been found in scallops from Queen Charlotte Sound, it was not detected via histology or culture. Overall, there was no clear cause of the reported scallop mortality or ill-health. The main difference between the "affected" and "control" scallops was the more marked inflammatory response. However, there was not enough information to find a definitive association between the parchment worm bed samples and the pathology of the scallops. These results were communicated to Fisheries New Zealand and the commercial diver, and the investigation was closed.

Bonamia sp. in wild oysters, Foveaux Strait

While conducting histology on a New Zealand flat oyster, *Ostrea chilensis* (Bivalvia: Ostreidae), a pathologist at a research laboratory detected the parasite *Bonamia* sp. The oyster sample had been collected from Foveaux Strait, where *B. exitiosa* is endemic but *B. ostreae* has not been detected. While both species do occur in New Zealand, *B. ostreae* is a notifiable and unwanted organism and is subject to movement controls to protect flat oysters in Foveaux Strait. Additionally both *Bonamia* species are "listed" by OIE (the World Animal Health Organisation). As a member country, New Zealand reports on its status of "listed" diseases. For reporting purposes it was necessary to determine which species of *Bonamia* was present. The histology block was sent to MPI's AHL for speciation. After DNA was extracted, the sample tested positive for *B. exitiosa* and negative for *B. ostreae*. As *B. exitiosa* is endemic, the investigation was stood down.

Polychaetes in biofouling sample, Coromandel Peninsula

MITS notified MPI after receiving some polychaete worm specimens directly from the Pauanui Waterways development on Coromandel Peninsula, where they had been found growing on a chair dumped in the waterway. They were identified as a parchment worm, *Chaetopterus chaetopterus*-A (Polychaeta: Chaeptoridae), and *Acromegalomma suspiciens* (Polychaeta: Sabellidae). *A. suspiciens* is a native species while *C. chaetopterus*-A is cryptogenic and has been recorded from Whangarei, Auckland, Tauranga, Bay of Islands, Opua and Coromandel Peninsula. Another cryptogenic parchment worm, *C. chaetopterus*-B, is also present in New Zealand, but has only been recorded from the Marlborough region. The results were communicated to Pauanui Waterways and the investigation was stood down.

Suspect exotic mussel in Whangarei Harbour

MPI was contacted by a marine scientist after a number of specimens of the suspected exotic mussel *Mytella guyanensis* (Bivalvia: Mytilidae) were found in sieve samples collected in Whangarei Harbour. The preserved specimens were sent to MITS, and were identified by molecular analysis as *Xenostrobus securis* (Bivalvia: Mytilidae), widely distributed native species. As there was no biosecurity risk the investigation was stood down.

Skin lesions on grey mullet, Port Waikato

MPI was contacted by a member of the public who was net-fishing at Port Waikato and caught a grey mullet, *Mugil cephalus* (Actinopterygii: Mugilidae), with chalky scales and lesions underneath. The fish was lethargic and separate from other healthy fish in the net.

The fish was sent to the MPI AHL for diagnostic testing to rule out the exotic pathogen *Aeromonas salmonicida* and to test for the endemic parasite *Myxobolus* sp. DNA of *A. salmonicida* was not detected in this fish but *Myxobolus* DNA was identified from the skin and gills by PCR. Nucleotide sequencing identified this as *M. episquamalis*, which has been recorded overseas as affecting mullet and was first reported from New Zealand in 2015 from mullet in the Mahurangi River. The present case is the first report of *M. episquamalis* from the west coast of the North Island. As this is not a new to New Zealand detection there is no biosecurity risk and the investigation was stood down.

Rock oyster mortality, Tasman Bay

A member of the public contacted MPI to report the apparent mass die-off of what appeared to be Pacific oysters, *Crassostrea gigas* (Bivalvia: Ostreidae) in Tasman Bay, near Split Apple Rock and Mosquito Bay. Photos sent to the investigator showed large numbers of empty oyster shells attached to semi-submerged rocks. The oysters all appeared to be missing their top shell and many showed the beginning of algal growth on the remaining one. The photos suggested the mortality was not recent. The notifier attempted to collect live oysters for disease testing but none could be found. Owing to the absence of live oysters for testing, the investigation was stood down.

Pipi mortality, Bay of Plenty

A member of the public contacted MPI to report a mass mortality of pipi, *Paphies*

australis (Bivalvia: Mesodesmatidae) at a location known as The Landing, Waiotahi. The notifier is a local resident who lives a few hundred metres from the affected location and regularly collects pipi for eating. The notifier agreed to collect samples to send to the MPI AHL for testing; but then became unavailable owing to personal circumstances. As several weeks had passed since the mortality event had taken place, it was decided that any samples collected might no longer be representative, so the investigation was stood down. The notifier was asked to contact MPI again if they notice further mortalities in the future.

The traceback enigma: A case study featuring a tropical species of carpenter ant

It has long been known that shipping containers are an important entry pathway for insects of significant biosecurity risk to New Zealand. The Ministry for Primary Industries (MPI) undertakes a comprehensive range of programmes to counter this, including risk analyses and profiling of shipping containers as they arrive in New Zealand. Recently an incursion of structure-infesting ants found in a shipping container illustrated the complexity of this risk-analysis and profiling process.

In mid-June 2019, MPI was notified by AsureQuality of a large live ant (**Figures 1, 2**) found in an empty shipping container at a Taranaki exporting business. Provisional identification based on photographs was *Camponotus* sp. (carpenter ant, not present in New Zealand). The photos were forwarded to MPI Plant Health Incursion Investigators, who arranged for the specimen to be sent to Plant Health & Environment Laboratory (PHEL) entomologists, who confirmed the identification. As the species of *Camponotus* are difficult to differentiate, molecular analysis was initiated at this stage. Meanwhile, an urgent measures response was launched by the investigator. The shipping container was quickly isolated and the area around it treated with insecticide, prior to methyl bromide fumigation of the container and further insecticide treatment of the location where it had sat. One live and several dead ants were found inside the container, but the live specimen was in a poor state of health. It appeared that an infestation of carpenter ants was located somewhere below the timber flooring of the shipping container. Other response work included determining the port of origin of the container, investigating its previous travel history and identifying the cargo.

This traceback work revealed a remarkable sequence of events. The shipping container had originally been loaded with bulk raw plastic beads in Houston, Texas, in early April, transported by rail to Long Beach, California, and shipped in late April to the Port of Tauranga, arriving in May 2019. From there the container was trans-shipped to Hamilton and then Palmerston North, before arriving at the importer's premises in New Plymouth in late May 2019, whereupon the contents were devanned. The empty container was then moved and stored at a transport yard in New Plymouth until mid-June, when it was sent to the exporter. The exporter spotted the first live suspect ant during a routine inspection just before the container was to be loaded. This traceback work also identified two further sites in Taranaki that required follow-up work to ensure the carpenter ants had not become established elsewhere, undetected.

Following these operational activities, PHEL entomologists provided results of the molecular diagnostic work that identified the ant as *Camponotus conspicuus*, a tropical species of carpenter ant originating in Central America and the Caribbean region.

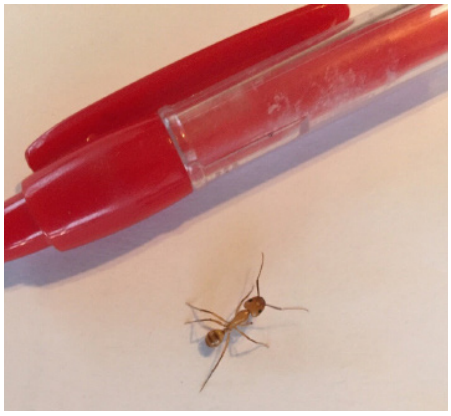

Figure 1: Carpenter ant, *Camponotus* sp. Photo supplied by AsureQuality

Figure 2: *Camponotus conspicuus*. Photo: ww.AntWeb.org. The text says that this is the species identified by PHEL so no need to repeat.

The obvious issue quickly identified was that the port of origin of the shipment (Houston) was not a location where this ant species was known to be present. This raised the question of where the infestation of the shipping container had occurred. It seemed unlikely that the ants were hitchhikers on the actual cargo of plastic beads, as this cargo did not come from an area where this species has been recorded. The most likely scenario was that the shipping container itself had become contaminated before it was loaded and shipped from Houston. With the assistance of the shipping line that owned the container, further traceback determined that the container had indeed been located in Honduras from February to March 2019, before being loaded with cargo, shipped to Houston, emptied and then reloaded with the plastic bead consignment for New Zealand in April 2019. *C. conspicuus* is native to Honduras.

This sequence of events shows the remarkable tenacity of ants, in that this infestation of a large tropical ant species had survived some 4 months in the floor of a shipping container that had been repeatedly loaded, unloaded and moved on trucks, trains and ships before finally ending up in Taranaki. It also illustrates the difficulties in risk-profiling of both cargo and shipping containers, and the speed at which a shipping container can be moved from location to location around the globe. Such rapid movement makes it difficult to trace dispersal pathways such as these for biosecurity risks like ants and other insects.

Fortunately in this instance the ants were detected, thanks to the diligence of the exporter in Taranaki and an AsureQuality staff member present at the time, and this allowed follow-up measures to be undertaken. It is also fortunate that this ant species is tropical, meaning that it is unlikely to become established in New Zealand's temperate environment.

Paul Craddock
Flybusters/Antiants
Private Bag 92905
Onehunga, Auckland
paul@flybusters.co.nz

Graham Burnip
Incursion Investigator
Plant Health Biosecurity Surveillance & Incursion Investigation
Diagnostic & Surveillance Services Directorate
Biosecurity New Zealand
Ministry for Primary Industries - Manatū Ahu Matua

Plant health surveillance and incursion investigation report: July to September 2019

The Ministry for Primary Industries (MPI) Incursion Investigation team and Plant Health and Environment Laboratory (PHEL) investigate and diagnose suspect exotic pests and diseases in the plant and environment sectors. Incursion Investigators and scientists are based in Auckland, Wellington, Rotorua and Christchurch. These teams provide field investigation, diagnostic testing and technical expertise to detect and report new pest and diseases affecting plants and the environment. They support surveillance and response functions, including carrying out research and development for better diagnostic tools and processes to manage biosecurity risks.

The third quarter of the 2019 year saw a 25 percent increase in notifications compared with the same quarter in 2018 (**Figure 1**). Of the 251 notifications, investigators immediately stood down 102 cases because the presence of biological risk was ruled out; in comparison, 61 were stood down in the same period last year. The number of cases that required further investigation (139) in this quarter increased compared with 2018 (125). However, in both quarters, 48 investigations required urgent measures to contain and manage the biological risk.

Investigations referred to Compliance Services Directorate

One of the primary functions of the Compliance Services Directorate is the investigation of potential breaches of MPI legislation. Many of these potential breaches are identified by other groups within MPI, including the Incursion Investigation Team. The investigators provide a referral to this team whenever they identify a potential breach of legislation that requires a more comprehensive investigation and may result in a prosecution.

Rose of Jericho plants discussed on social media

Rose of Jericho, *Selaginella lepidophylla* (Selaginellales: Selaginellaceae) plants were discussed by a group of Facebook subscribers. It was suspected that one Facebook user had illegally imported the plant in 2018 and the plant was likely still in her possession. Facebook updates showed the user attempting to revive the plant in a bowl of water, even though others in the forum had told her that the plant was a prohibited import. An investigation was undertaken alongside MPI's Compliance Team and a search warrant to enter the residence was gained. Two suspect Rose of Jericho plants were seized, plus several fragments in water, and 29 packets of unauthorised seeds were seized. All were sent to PHEL for identification. *Selaginella lepidophylla*, a suspect new to New Zealand organism, was identified, along with a wide variety of seed including some subject to very strict biosecurity requirements: *Citrus* sp., *Vitis* sp., and *Prunus* sp. Other seeds included *Rosa* sp., *Amaranthus* sp., *Descurainia* sp., *Cucumis* sp., *Echinacea purpurea*, *Carica papaya*, *Acacia* sp., *Oxalis* sp., *Setaria italica* and *Lupinus* sp. The seeds were destroyed and the owner was issued with a formal warning letter from MPI Compliance.

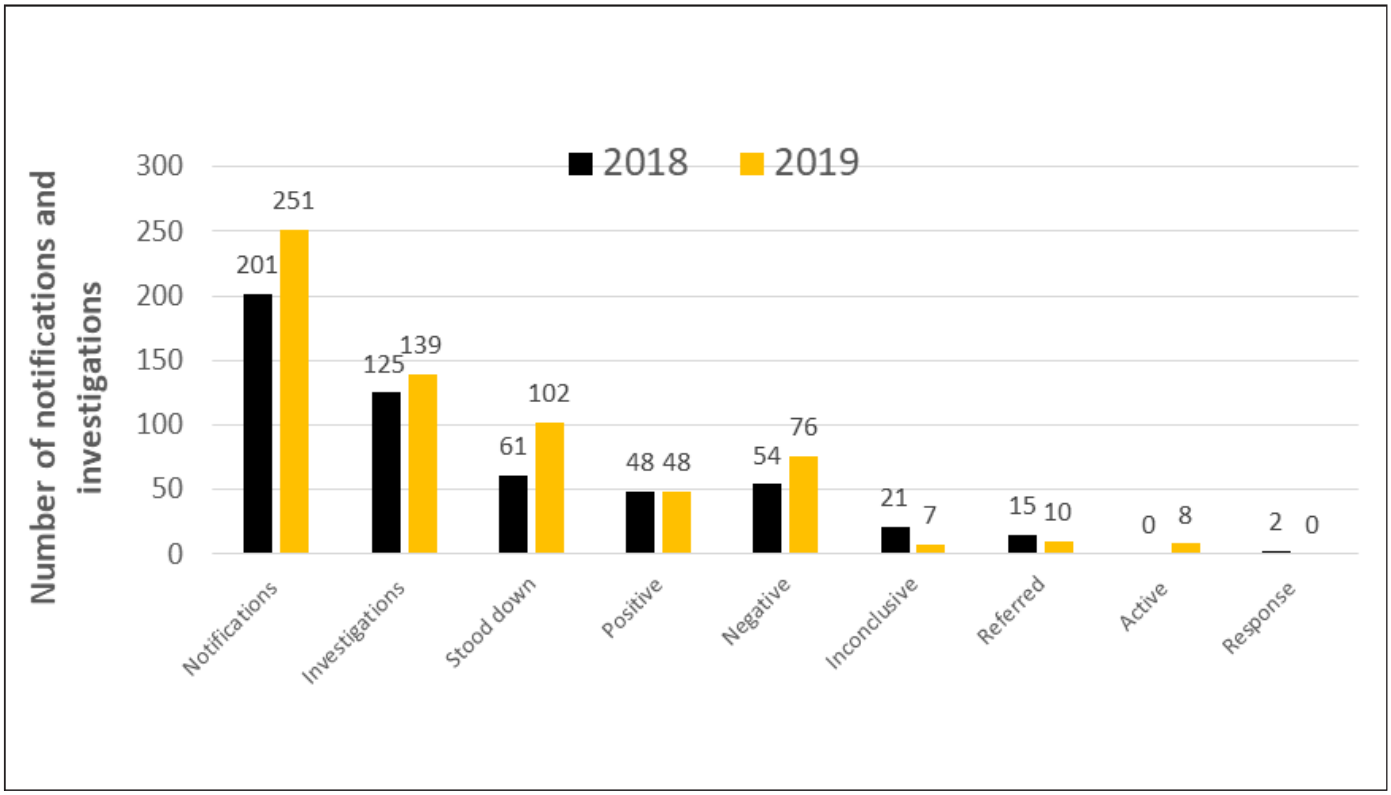

Figure 1: Plant health notifications, investigations and other outcomes, July–September 2018 and 2019

Positive; referred from MPI responsibility

Suspect *Salvinia natans* for sale on Trademe

Freshwater ferns not known to be present in New Zealand were offered for sale on Trademe, identified by the seller as *Salvinia natans*. A detailed sales history, including contact details of both seller and buyers, was obtained by the MPI intelligence team. An investigator purchased a plant from the Trademe listing for identification by PHEL. After consultation with two external experts, the plant was confirmed as Kariba weed, *S.* x *molesta* (Salviniales: Salvinaceae), an unwanted and notifiable organism. Kariba weed forms extensive floating mats that can double in size within 10 days, smothering waterways and affecting water quality. The weed mats kill off native plants, attract breeding mosquitoes, block dams and irrigation systems and remove oxygen from the water. Furthermore, by obscuring a water body with its green covering, Kariba weed creates a drowning risk for humans and animals. As *S. x molesta* is one of the species managed by the National Interest Pest Response programme, the case was referred to MPI's Long Term Management Team to follow up.

Investigation positive; establishment prevented through urgent measures

These investigations found organisms that were not known to be present in New Zealand, under circumstances that enabled treatments to be applied and biosecurity mitigation confirmed. They typically involved imported goods and containers.

Brown marmorated stink bug (BMSB) cases

There were 17 investigations of suspected BMSB, *Halyomorpha halys* (Hemiptera: Pentatomidae). Two were positive for dead BMSB, three other insects were identified as yellow spotted stink bug (YSSB), *Erthesina fullo* (Hemiptera: Pentatomidae), and 11 of the investigations involved stink bugs already present in New Zealand: green vegetable bug, *Nezara viridula* (9) and brown soldier bug, *Cermatulus nasalis* (2). Another suspect BMSB was ruled out by PHEL entomologists from photos, but without the specimen the identification was inconclusive.

A dead stink bug was found inside a medicine cabinet purchased on Trademe. The specimen was submitted to PHEL and identified as a female BMSB. Dead stink bugs are not considered a biosecurity risk but an investigation was opened to trace the pathway of the product into New Zealand and communicate with all parties involved, from the exporter to the end user. The cabinet had been imported from China along with other furniture. A fumigation certificate accompanying the consignment stated the goods were treated with methyl bromide at 80g/m^3 prior to shipping. Upon arrival in New Zealand the furniture was stored in Auckland. MPI fact sheets on BMSB were sent to the importer, the storage facility operator and the Chinese exporter to distribute among their staff.

Exotic spiders

There were 13 investigations into suspected exotic spiders (or webbings) associated with imported vehicles, grapes and a wooden toy: six red-back spiders, *Latrodectus hasseltii* (Araneae: Theridiidae), one brown widow spider, *Latrodectus geometricus* (Araneae: Theridiidae) and one black widow spider, *Latrodectus* sp. The remaining five spiders were either dead or were identified as established in New Zealand. Urgent measures were undertaken to prevent establishment, including an area search, fumigation and insecticide applications. MPI Quarantine Officers (QOs) often assist with inspections, and provide specimens to PHEL for identification. Other spiders found and notified to MPI included *Badumna* sp. (Araneae: Desidae) and the Natal daddy-long-legs, *Smeringopus natalensis* (Araneae: Pholcidae). The MPI Facilities and Pathways Team is advised of any non-compliance by importers.

Exotic ant detections

In this quarter, four exotic ant species of ants (Hymenoptera: Formicidae) were reported via the MPI pest and disease hotline: *Trichomyrmex destructor* in cartons of plastic tubing from Singapore, *Tapinoma melanocephalum* in raw sugar cane and nut oils from Fiji, *Camponotus modoc* in sawn timber from Canada, *Camponotus conspicuous* in an empty sea container received by a Waitara company ready to load for export. Urgent measures for preventing establishment were implemented, depending on the case, and included site inspection and heat or methyl bromide treatment. Ant species and localities were added to the 2020 National Invasive Ant Surveillance programme (NIAS) for monitoring during summer.

Four other investigations were negative for exotic ant species. Three of these were identified as already established in New Zealand (*Technomyrmex jocosus*, *Iridomyrmex suchieri* and *Ochetellus glaber*) and one case was closed as inconclusive, as no photo or specimen was provided by the notifier.

Exotic plants and seeds

Of the 23 investigations of plants and seeds only three were inconclusive, owing to difficulties in identifying the seeds, incomplete information or circumstances preventing determination of a biosecurity issue. In any case, the risk was mitigated by destruction of the seeds by the notifier or MPI staff. Illegal or incidental seed importation continues to create a significant workload for the investigators.

E-commerce (via Wish, VOVA, AliExpress) was the most common way of importing seeds. Often customers placed their online orders thinking the seeds or plants would be sourced from New Zealand-suppliers, but on arrival, seed packages proved to be posted from overseas. Five investigations were of plants or seeds on sale from New Zealand providers (via Trademe). All seeds non-compliant with relevant MPI import health standards (IHSs) were sent for destruction in quarantine waste. Online retailers have been reported to the MPI Intelligence & Targeting Team to monitor repeat offending.

There were other notable investigations of exotic plants that posed potential biosecurity risks. A commercial nursery reported finding a suspect exotic weed growing in plant pots. Plants submitted to PHEL and Auckland War Memorial Museum were validated as Egyptian grass, *Dactyloctenium aegyptium* (Poales: Gramineae). The weeds were recovered from pots containing cabbage trees *Cordyline australis* (Asparagales: Asparagaceae) and oioi, *Apodasmia similis* (Poales: Restionaceae) that had been planted in November and December 2018. This period coincided

with the peak potting season, when more than one load of potting media (coco fibre, peat and bark mix) was delivered each week. The suppliers of potting media were notified of the find. Copies of a booklet on weeds found in coco fibre (MAF 2008) and an awareness flyer (designed during the investigation) were distributed among the staff to assist inspections for suspicious exotic weeds at their sites. The suppliers responded that they maintained a robust quality-assurance programme that included weed assays of each imported coco fibre shipment. They had not received any other reports of suspect exotic weed contamination in their soil mixes. All parties were asked to inform their customers about the detection.

A seed packet containing a specifically named cultivar of sunflowers (imported from the Netherlands) was found to contain seeds of Johnson grass, *Sorghum halepense* (Poales: Poaceae), a regulated, unwanted and notifiable organism. Johnson grass is known internationally as a serious weed of arable land. About half the sunflower seeds had already been sold in small packets, either directly to home gardeners or to retailers supplying home gardeners. No seed was sold to commercial producers. The remaining 1 kg of seed held by the importer as packets and in bulk was recalled. The packaged seed was destroyed, and the bulk seed was examined for the presence of Johnson grass seed. The contamination of Johnson grass seeds was determined to be below 1 percent, which could explain the lack of detection of the contaminant at the border during MPI inspection and also by the importer during dressing and handling procedures. Within 48 hours of the notification the importer contacted all retail distributors and advised them to immediately remove these packets from sale. An e-newsletter asked gardeners to contact MPI if they had purchased seeds of the sunflower cultivar. Although only a small number of gardeners did contact MPI it was considered likely that if any Johnson grass seed had been inadvertently planted, and if the seeds germinated, they would likely be weeded out by gardeners as the seedlings were so different to sunflowers. The overall risk that Johnson grass might establish in New Zealand as a result of this incident was estimated to be low to very low.

Ten overseas students arrived in New Zealand to attend a school in Southland. One of them brought in undeclared plant material as a gift. Photos of the plant material identified dried calabashes (gourds) containing devitalised seeds. The translator accompanying the group was contacted and determined that four calabashes had been sourced from the student's backyard in China, and as such did not meet IHSs. The translator was asked to tell the group of students to declare any plant or plant material they had brought into New Zealand. No other plant material was reported as having been brought in by the students. The four calabashes were sent to MPI for destruction.

Scientists from the School of Environmental and Animal sciences at Unitec reported a suspect Himalayan wineberry (also known as yellow Himalayan raspberry), *Rubus ellipticus* (Rosales: Rosaceae), in Auckland. Botanists at PHEL and the Allan Herbarium at Manaaki Whenua – Landcare Research (MWLR) validated the identification as *R. ellipticus* var. *obcordatus,* listed as Entry Prohibited in the Plant Biosecurity Index and included in MPI's Schedule of Regulated Weed Seeds. The Department of Conservation (DOC) and Auckland Council were notified of the find. Himalayan wineberry is considered one of the world's 100 worst invasive alien species. It can reproduce by both vegetative means (root-suckering) and seed, which is dispersed by avian and mammalian vectors via consumed fruit. Owing to its invasive characteristics and tolerance of cold, it could potentially establish in some parts of New Zealand, but its impacts on native flora may be limited to some extent by climate. Investigation revealed that *R. ellipticus* was well established in the area of detection, and may have been present for 5–10 years, based on the size of the canes. More plants were discovered on properties close to the original observations. The investigation results were conveyed to MPI's Response Team for its consideration. A delimitation survey within a 3-km radius of the initial detection was initiated. This survey included all parks and reserves, as well as riparian areas, but excluded private properties. The Response Team is now considering options with Auckland Council for future management of this plant. A fact sheet containing key diagnostic information was prepared to help DOC and Auckland Council field staff identify the plant for passive surveillance purposes.

Wood borers

There were eight investigations of suspect exotic borer beetles associated with wood material, of which three were positive for *Heterobostrychus* spp. (Coleoptera: Bostrichidae) from China: *H. hamatipennis* in a wooden mirror frame and in spiderwood (natural driftwood from the root zone of a tree), and *H. aequalis* in a coat hanger. Investigators managed the risk by fumigation, verification of phytosanitary certificates and ongoing monitoring of detection sites. The other five investigations established no biosecurity issue: in each case beetles found were not associated with the wood material, the species was already present in New Zealand, or live specimens were not found.

Live wasps on machinery from Mexico

A suspect exotic wasp nest, including live larvae, was found in an Oamaru store, attached to the frame of a used four-wheeled motorbike originating from Mexico and imported via Australia. The Initial Investigating Officer couriered a bio-bottle to the store to enable secure transport of the mud nest to PHEL, where scientists confirmed it was the Australian hornet, *Abispa ephippium* (Hymenoptera: Vespidae), a species previously detected but not established in New Zealand.

Grapes released incorrectly from the US

A container of grapes from the US was released but later found to have been non-compliant as the thermograph did not meet the IHS. Part of the consignment that had been sent from Wellington to retailers in Nelson, Christchurch and Dunedin was removed from sale, shrink-wrapped and fumigated in accordance with the IHS for grapes from the US. No live insects were reported after fumigation.

Investigation positive; urgent measures limit harm

These investigations resulted in detection of organisms that were not known to

be present in New Zealand, and in circumstances where treatments could be applied to all retrievable items (usually recent imports). There may be some residual risk associated with items that could not be retrieved.

Live insects in rice from India

Live insects were found in brown basmati rice imported from India. The notifier was uncertain as to when or where the rice had been purchased. He was advised to freeze the rice and send specimens for identification, but MPI did not receive any specimens after 2 months. No follow-up was undertaken because the overall risk from stored product pests in rice is low.

Live insects in confectionery from Australia

Live insects were found by a Queenstown confectionery retailer in peanut brittle imported from Australia. The risk product had been withdrawn from sale at the time of notification to MPI. Photos of the insects showed larvae consistent with Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), a stored-product pest of global distribution including Australia and New Zealand. The importer advised the Australian supplier, who had already discovered the issue at their facility and taken steps to manage the pest.

Moth on cargo from Hong Kong

A dead moth was found under an air-can that had recently arrived from Hong Kong. The moth was identified as *Eudocima* sp. (Lepidoptera: Erebidae), a genus not present in New Zealand. The air-can was shrinkwrapped and sprayed with insecticide. The submission also contained a freshly dead weevil (body still pliable) identified as *Hypera postica* (Coleoptera: Curculionidae), an unwanted organism.

Snails on machinery from Israel

Snails were found on diesel generators imported from Israel, which had arrived in-store about a month prior to the notification. In the interim, some of the generators had been distributed to clients. An Incursion Investigator visited the site and found three snails on one generator. The snails were identified as the brown-lipped snail, *Cepaea nemoralis* (Gastropoda: Helicidae), a snail previously detected on goods imported from the Mediterranean region and considered a low biosecurity risk.

New to New Zealand butterfly found at Lincoln University

A Lincoln University scientist reported a suspect new to New Zealand butterfly that had flown into his office on campus. It was identified as the small tortoiseshell butterfly, *Aglais urticae* (Lepidoptera: Nymphalidae), a regulated and unwanted organism that is widespread in the northern hemisphere, including most of temperate Europe and Asia. The host plants for caterpillars are the common stinging nettle (*Urtica dioica*) and dwarf stinging nettle (*Urtica urens*), both weed species found in New Zealand where the former is a host for the native red admiral (*Vanessa gonerilla*) and yellow admiral (*V. itea*) butterflies. If *A. urticae* was to establish there is the possibility of resource competition with the New Zealand admiral butterflies. A site inspection at the Lincoln University campus by two Incursion Investigators and a PHEL entomologist found no more *A. urticae* and only a small number of common stinging nettle plants. MPI has detected this species several times before at the border, including individuals found on clothing from Europe and used vehicles from Asia. Given the number of international students and visitors to Lincoln University, it is suspected the individual butterfly was a solitary hitch-hiker that arrived on luggage, clothing or equipment. Lincoln University released a news story about the butterfly detection and asked people on campus to contact MPI if *A. urticae* were found. No further sightings have been reported.

Dead wasps on outdoor furniture from China

Six dead wasps were found in cardboard packaging of outdoor furniture in Rotorua. Further inspection of the consignment revealed a mud-wasp nest in a couch. The container of furniture had not been fumigated on arrival into New Zealand so there could still be live larvae in the nest. A local pest controller destroyed the nest and treated the couch with insecticide. Staff at the distribution centre inspected other furniture in the container but found no more wasps.

Investigation positive; no action taken

These investigations revealed organisms that were not previously known to be present in New Zealand, but no action was taken. Typically, they included cases where a risk assessment indicated that a potentially new to New Zealand organism (or a newly described indigenous organism) had become well established and was considered unlikely to damage economic, environmental, social and cultural values. Alternatively, the organism may have already been established and been under management by MPI and/or local authorities.

It is worth noting that only the Environmental Protection Authority (EPA) can officially determine (under s26 of the Hazardous Substances and New Organisms Act 1996) that an organism is present in New Zealand and therefore not a new to New Zealand organism. If the EPA does not made such a determination, MPI does not have the legal mandate to determine whether an organism is new to New Zealand or not. In that situation, MPI can only state that its status is "undetermined".

"Undetermined" new to New Zealand organisms

New to science *Diaporthe* species

Scion identified a suspect new to New Zealand fungus on a magnolia leaf collected during MPI's High Risk Site Surveillance (HRSS) programme. Scion had identified the fungus by morphological and molecular techniques as *Diaporthe sackstonii* (Diaporthales: Diaporthaceae), which is not recorded from New Zealand. Cultures and sequence data submitted to PHEL for validation suggested the culture was not *D. sackstonii* but a closely related *Diaporthe* sp. That is new to science (and commonly found on a variety of plants). The *Diaporthe* sp. was not associated with anything imported, so it may have been present in New Zealand for some time. It was determined not to be a biosecurity risk.

Phytophthora and *Pythium* spp., Northland

A Scion research programme analysing soil samples from Northland identified five potentially new to New Zealand fungi. However, PHEL mycologists determined that they all had previously been reported from New Zealand and posed no biosecurity threat. PHEL consulted with Scion, who conferred the isolates as *Phytophthora multivora*

(two submissions), *Phytophthora* taxon *personii*, *Pythium* sp., and *Pythium macrosporum*.

Phytophthora hybrid from kauri tree roots

A Scion pathologist isolated a new *Phytophthora* species from the soil near a dead kauri tree, *Agathis australis* (Pinales: Araucariaceae), in Rotorua. Analysis of the phytophthora genome indicated it was a hybrid of *P. cactorum* (maternal parent) and *P. hedraiandra* (paternal parent), and it has been named *P.* x *serendipita*. Historic sequences indicate that both *P. cactorum* and *P. hedraiandra* are established in New Zealand, and there are taxonomic opinions that *P. hedraiandra* may be synonymous with *P. cactorum*. Therefore, *P.* x *serendipita* does not pose a biosecurity risk. It is not known whether this species caused the kauri decline seen, as the tree had experienced prolonged drought stress.

Suspect new fungal DNA sequence from grapevine trunk

A Plant & Food Research (PFR) scientist working on a project using Next Generation Sequencing of DNA from grapevines in Marlborough identified three sequences as indicators of possible new to New Zealand species. PHEL mycologists analysed the results and did not support the initial species identifications, but instead confirmed that all DNA sequences were of species already present in New Zealand.

Curtobacterium flaccumfaciens on whau tree, Auckland

During a BioBlitz at Whatipu, near Auckland, MWLR scientists isolated 86 bacteria from the soil, water and plant surfaces. Eighty-five of the environmental bacteria were of no biosecurity concern, but one culture, deposited in the International Collection of Microorganisms from Plants (ICMP) as ICMP 22320, was a suspect new to New Zealand plant pathogenic bacterium, *Curtobacterium flaccumfaciens*. It was isolated from the native whau tree, *Entelea arborescens* (Malvales: Malvaceae). DNA sequence analysis using reference sequences of the type isolate in GenBank gave conflicting results owing to inaccuracy of the reference data. The analysis confirmed that the specimen is *Corynebacterium ilicis* (syn. *Curtobacterium flaccumfaciens* pv. *ilicis*). There is little published information on the impact of this bacterium, suggesting it may not be a significant pathogen.

Pine needle cast, Mangawhai

A property owner reported pine trees showing signs of red needle cast, which is normally caused by *Phytophthora pluvialis*. Tests at PHEL were negative for that species, but found *Lophodermium australe* (Rhytismatales: Rhytismataceae), an unwanted organism not previously reported in New Zealand. This is an endophytic fungus associated with healthy foliage of pine, and a saprophyte of fallen needles; there is no scientific evidence that it is a pathogen. A revocation letter will be submitted to MPI's Chief Technical Officer for removal from the unwanted organism register. Other organisms isolated from the sample were *Sydowia polyspora* (Dothideales: Dothideomycetidae), a fungus reported as an epiphyte or endophyte of conifers and previously isolated from *Pinus radiata* (Pinales: Pinaceae) in New Zealand; *Sphaeropsis sapinea* (Botryosphaeriaceae: Botryosphaeriales), the causal agent of *Sphaeropsis* blight or tip blight, a disease that affects but typically does not kill trees; and *Lophodermium conigenum* (Rhytismatales: Rhytismataceae), a fungus commonly found on *P. radiata* litter. The cause of the symptoms was likely a combination of the identified organisms and abiotic factors.

Non-compliant grapes from the US

An MPI pre-shipment inspection of grapes in the US established no known biosecurity risk organisms. However, during inspection for integrity of the consignment on arrival into New Zealand, the consignment was found not to conform to quarantine procedures, as the pallets had been divided up in order to secure the load within the shipping container. A Biosecurity Authority/Clearance Sheet instruction to destroy the product was issued but 180 cartons from each of two containers were released without authorisation of the distribution staff at the Transitional Facility. This was identified as an operational transgression by the operator of the facility, for which MPI Quarantine issued a Corrective Action after considering an infringement. As the operator had no previous history of non-compliance, this was delivered with a warning that an infringement may be incurred for any further transgressions now that an incident of this type of non-compliance has been recorded. No known biosecurity risk organisms were associated with the grape consignment, so it was not recalled for re-shipment or destruction. This investigation will be re-opened if any biosecurity risk is subsequently identified.

Suspect new to New Zealand fungus on kiwifruit

A PFR scientist reported a suspect new to New Zealand fungus, *Ilyonectria lusitanica* (Hypocreales: Nectriaceae). It was isolated from kiwifruit (*Actinidia* sp.) trunk and root samples collected from two sites in Auckland (2006) and Te Puke (2017) and were tested at PFR. Sequence validation by PHEL did not confirm the detection of *I. lusitanica*. The isolates represent an undescribed species within the *I. radicicola* species complex. This complex has previously been found on at least 40 species from different families of host plants in New Zealand and is likely to contain a number of cryptic species. Because the samples are from multiple sites and were taken over a significant period of time, the biosecurity risk appears to be low.

New to New Zealand mealy bug and thrips on crocus bulbs

Live mealy bugs were found on crocus bulbs purchased by a Senior Quarantine Officer (SQO) to conduct a training exercise for new QOs. PHEL identified *Phenacoccus* sp. (Hemiptera: Pseudococcidae), morphologically close to *Phenacoccus avenae*, a regulated pest. Molecular analysis was inconsistent with any species known to be present in New Zealand. To determine the level of contamination across the country, further bulbs were purchased from an Auckland store of the same chain. No more mealy bugs were found, but larvae of the tobacco thrips, *Frankliniella fusca* (Thysanoptera: Thripidae), an unwanted organism and regulated pest, were found. The producer of the crocus bulbs and the wholesaler in the North Island both import bulbs from countries where *F. fusca* is recorded as being present. The producer has an integrated pest management programme and a comprehensive decontamination programme, which covers the bulb storage cells, bulb storage crates and

machinery. The crocus bulbs had been stored in a tulip bulb storage cell, but tulip bulbs imported in 2018 had been planted immediately and not been harvested before the crocus were in transit to the North Island wholesaler. The wholesaler informed that they receive about six containers of bulbs each year, all of which are fumigated with methyl bromide on arrival, except for anemone, *Anemone coronaria* (Ranunculales: Ranunculaceae), from Israel. Therefore there were always other bulbs in proximity to the crocus bulbs over that period. At the time there would have been hyacinths, *Hyacinthus orientalis* (Liliales: Liliaceae) and daffodils, *Narcissus* sp. (Asparagales: Amaryllidaceae), both local or imported from the Netherlands, as well as freesias, *Freesia* sp. (Asparagales: Iridaceae) and Dutch irises, *Iris* × *hollandica* (Asparagales: Iridaceae) imported from the Netherlands. With the mixture of imported and locally grown bulbs in the supply chain, it is unclear whether the mealy bugs and thrips originated from cross-contamination from imported bulbs or they were from locally grown bulbs.

Suspect new to New Zealand psyllid

During the Christchurch Port Hills City Nature Challenge, an exotic psyllid was collected from sticky boards placed near broom plants. It was identified as the broom psyllid, *Arytaina genistae* (Hemiptera: Psyllidae), which is not previously reported in New Zealand. Also found was another broom psyllid species, *Arytainilla spartiophila*, which was introduced to New Zealand in the mid-1990s as a biocontrol agent for scotch broom, *Cytisus scoparius* (Fabales: Fabaceae). In the mid-1980s, *A. genistae* was assessed as a biocontrol agent but rejected because it can breed on tree lucerne, *Chamaecytisus palmensis* (Fabales: Leguminosae), a possible fodder crop for livestock. Further site inspections are planned to better understand the distribution of these species.

New to New Zealand fungi identified from giant buttercup

MWLR reported 17 new to New Zealand fungi identified from giant buttercup, *Ranunculus acris* (Ranunculales: Ranunculaceae), an introduced weed in pastoral systems. The samples were collected in 2013 from Taranaki and Nelson. PHEL reviewed the information and determined that several species were misidentified or required additional gene sequencing before identifying to species level. Six of the records were confirmed as new to New Zealand: *Nigrospora musae* (Xylariales: Apiosporaceae), *Epicoccum italicum* (Pleosporales: Didymellaceae), *Penicillium attenuatum* (Eurotiales: Aspergillaceae), *Neoascochyta tardicrescens* (Pleosporales: Didymellaceae), *Papiliotrema frias* (Tremellales: Tremellomycetidae) and *Diaporthe lusitanicae* (Diaporthales: Diaporthaceae). None of the newly detected species are considered likely to pose a biosecurity risk.

New to New Zealand *Halophytophthora* spp., Waiwera River

MWLR has obtained isolates of *Halophytophthora* spp. (Peronosporales: Peronosporaceae), a genus of fungus-like organisms not previously reported for New Zealand. They came from a MWLR researcher who was conducting a study to detect and isolate *Halophytophtora* by leaving detached leaves (oak, mangrove, pine, *Pittosporum*) in plastic cages suspended in water for a week. When sequences of 24 isolates were submitted to PHEL for validation, six isolates were confirmed as *H. avicenniae* but the rest could not be identified to species level. *Halophytophthora* spp. are mostly known as leaf-decomposers in forests of mangroves, *Avicennia marina* var. *marina* (Lamiales: Acanthaceae), but have also been found in freshwater habitats. Although some studies suggest that this genus might be pathogenic towards salt marsh plants, to date there is no evidence for this hypothesis. No disease signs had been observed on plants growing in the area.

Trogoderma variabile in NZ milk powder

A consignment of New Zealand milk powder was returned by the Chinese importer after a solitary live beetle was found. The specimen was identified as the warehouse beetle, *Trogoderma variabile* (Coleoptera: Dermestidae), a regulated pest. China maintains that it is free of *T. variabile*, but PHEL has validated three interceptions of this species from China. The origin of the beetle and how it ended up in the can of milk powder is not clear, although it is unlikely the beetle would have survived the production process in New Zealand. No other milk product was provided for testing and since the species is not present in New Zealand the investigation was closed.

Suspect unwanted fungus in exported bottled water

A suspect new to New Zealand fungus was identified from bottled water exported to the US. One bottle of water was couriered back to New Zealand and tested at two independent laboratories, which identified the contaminant as *Leptosphaeria ogilviensis* (Pleosporales: Leptosphaeriaceae). The culture was then sent to PHEL for validation, and additional sequencing determined that the isolate is *Septoriella hollandica* (Pleosporales: Phaeosphaeriaceae), a recently described species isolated from *Phragmites australis* (Poales: Gramineae) in the Netherlands. No information is available about the symptoms *S. hollandica* caused on that host, but it may be a plant pathogen.

The water came from a Hauraki Plains bottling plant that follows a quality-assurance programme including microfiltration, UV irradiation (effective against many bacteria and viruses) and ozonation (disinfection with ozone gas, which remains active for 24–28 hours). The investigation concluded that this left very little opportunity for contamination. Retained samples from the bottling process and two further unopened bottles that had been retained by the US agent were sent for testing at PHEL but were negative for *S. hollandica* or any other fungi. As a precaution, destruction of all bottles from the questionable shipment was ordered.

New to New Zealand disease on eucalyptus trees, Pauatahanui

Samples of *Eucalyptus* spp. showing signs of decline were analysed and several fungi and fungal-like organisms were detected, notably the new to New Zealand fungi *Valsa fabianae* (Diaporthales: Valsaceae) and *Readeriella dendritica* (Capnodiales: Teratosphaeriaceae). *V. fabianae* is an unwanted and regulated organism. However, it has been detected a number of times (under its synonym *Cytospora eucalypticola*) on a range of different eucalyptus species in various regions of the North Island. In addition,

C. eucalypticola was detected in *Vitis vinifera* cv. *Arneis* in Auckland in 2005, suggesting it is already established in New Zealand. *Eucalyptus* spp. are susceptible to a wide range of fungi that cause leaf spots, including *R. dendritica*. It has previously been recorded from *E. deanei* in New South Wales, Australia. Tracing information indicated this was not a recent infection, suggesting the fungus had been present but unreported for some time.

Investigation for high impact pests; negative

Thirteen investigations resulted from reports of suspected high-impact pests or diseases such as fruit flies, ants, termites and others. All initially reported organisms were proven not to be present in New Zealand, or investigations established that the pests and diseases were already in the country.

Suspect new disease of pine trees

An Auckland pine plantation owner reported dying radiata pine trees, *Pinus radiata* (Pinales: Pinaceae). A contractor experienced in forest health-related issues was engaged to conduct a field visit. PHEL analysed the samples and extracted a fungus belonging to the family Coricaiaceae. Genetically this fungus is closest to an undescribed species that had been recorded growing on a decaying stump of silver beech, *Nothofagus menziesii* (Fagales: Nothofagaceae) in New Zealand. It is likely to be a wood-decaying species that does not pose a biosecurity risk. Isolations of fungi from the branch and needle samples were identified as *Pestalotiopsis disseminata* (Xylariales: Sporocadaceae). This fungus is a pathogen of various host plants and common in New Zealand although it is not recognised as a pathogen of concern on radiata pine and does not pose a biosecurity risk. No pathogenic fungi were isolated from the root and soil samples.

New to New Zealand fungus *Colletotrichum kinghornii* on *Phormium tenax*

Leaves of native flax, *Phormium tenax* (Asparagales: Asphodelaceae), displaying leaf spot symptoms were collected during routine sampling as part of HRSS, from Birkenhead Domain, Auckland. PHEL isolated the fungus *Colletotrichum kinghornii* (Glomerellales: Glomerellaceae) for the first time in New Zealand. This fungus has also being isolated from *P. tenax* in the UK. A recent study assigned *C. kinghornii* as a new species to the *Colletotrichum acutatum* species complex, which have been recorded as pathogens on a wide range of host plants. The same study suggested a close relationship between *C. kinghornii* and *C. phormii,* an indigenous species known to be widely distributed on flax in New Zealand. PHEL conducted a sequence comparison of the isolated fungus and over 20 historic *C. phormii* isolates from the ICMP collection and found a low level of genetic variations among the *C. kinghornii* and *C. phormii* isolates. This was considered a natural intraspecific population variation so therefore both species may be synonymised in the future. Since the host plant is endemic to New Zealand, it is likely that *C. kinghornii* is also endemic and has been introduced into the UK. This investigation found no evidence of any new disease or change of disease status associated with *C. kinghornii*.

Suspect new *Neopestalotiopsis* sp. on feijoa, Tauranga

Fruit of feijoa, *Acca sellowiana* (Myrtales: Myrtaceae) displaying rot symptoms were collected from a Tauranga home garden. PHEL isolated a number of fungal species from the fruit but none were known pathogens. Two saprobic species were isolated that had not previously been found on feijoa in New Zealand: *Neoconiothyrium hakeae* (Pleosporales: Coniothyrideae), a species present in New Zealand and considered a common plant endophyte that can become a secondary pathogen when plants are stressed; and *Neopestalotiopsis* sp. (Xylariales: Amphisphaericeae), an undescribed species. The genus *Neopestalotiopsis* was separated from *Pestalotiopsis* in 2014. Species of *Pestalotiopsis* are typically saprobes, endophytes and opportunistic pathogens on a wide range of plants in New Zealand. *Neopestalotiopsis* sp., genetically identical to the Tauranga isolate, has been previously isolated from diseased feijoa fruit in a Whangarei home garden. However, it was not consistently isolated from the symptomatic tissues and therefore was not considered the primary pathogen. There was no evidence to suggest it was a newly arrived exotic species, so it is considered unlikely to pose a biosecurity threat. Although no further action was undertaken, the gene sequences that describe this apparent new species are now on record and will be updated in future if new published information helps to clarify its taxonomic status.

Suspect *Thrips palmi* on exported capsicums

The Australian Department of Agriculture and Water Resources identified two quarantine pests on a consignment of fresh capsicum (*Capsicum* sp.) fruits exported from New Zealand: *Thrips palmi* (Thysanoptera: Thripidae), a highly significant horticultural pest, and *Coleosoma floridanum* (Araneae: Theridiidae), a cobweb spider. Both species have wide global distribution and are absent from New Zealand, but *T. palmi* is an unwanted organism that would have considerable trade implications for New Zealand. A biosecurity investigation was undertaken to rule out or confirm the presence of *T. palmi* and *C. floridanum* in New Zealand. The export consignment originated from a producer growing capsicums in greenhouses on three properties with a production area totalling over 23 ha. As part of an integrated pest management plan, yellow sticky boards were used to manage western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). As these traps were also highly attractive to *T. palmi,* it was assumed that if *T. palmi* were present in the greenhouses it would be seen on the yellow sticky boards. However, none were seen out of 27,314 thrips examined so it was considered more likely the detections were post-export contaminants, rather than representing New Zealand populations. This conclusion was supported by further information collected during the review of the pre-export treatment process, export-chain events to Australia and other international markets, and monitoring at the producer's site. No non-conformity with procedures was established and no further thrips were reported.

Plants from seeds imported from Australia

A consignment of parsnip seeds *Pastinaca sativa* (Apiales: Apiaceae) imported from Australia had been

given biosecurity clearance without the required testing to rule out the presence of *Candidatus* Liberibacter solanacearum (Lso) haplotypes C, D and E. The seed lot was imported in October 2018 and most had already been planted out in Ohakune and Pukekohe. Some of the resulting parsnips had been harvested and sold on the domestic fresh produce market. The parsnip seed grower was contacted by the importer and advised that nothing of relevance had been observed in the crop. An initial Investigator and a PHEL virologist conducted a site inspection at the remaining farms and took a number of samples. Only 5,430 seeds remained of the original seed lot that could be tested, 4,570 short of the 10,000 seed sample required. Nevertheless, the 5,430 seeds and some field plant samples were sent to PHEL. All tested negative for Lso. In the absence of a positive detection of Lso haplotypes, the investigation was closed and the MPI Plant Imports team was made aware of the investigation.

Negative – Other

These investigations were negative for the presence of any biological risk.

Range extension of two insects from the Kermadec Islands

New distribution records were collected for two insect species known to occur at the Kermadec Islands and now confirmed for the North Island. *Psylliodes brettinghami* (Coleoptera: Chrysomelidae) was recorded on poroporo, *Solanum* sp. (Solanales: Solanaceae), in Waikato. This beetle is native to New Zealand, but only known to occur at the Kermadecs, in Australia and in several Pacific Islands. Specimens were collected by SPS Biosecurity for PHEL Entomology to confirm the identification. *P. brettinghami* has been recorded from a number of Solanaceae including *S. nigrum* (black nightshade), *Physalis* sp. (cape gooseberry) and *Datura stramonium* (datura). There are no host records from *S. lycopersicon* (tomato), *Capsicum annuum* (capsicum/chilli) or *S. tuberosum* (potato). A site visit to a local park was carried out, but no specimens were found. A further detection, likely to be of the same insect on poroporo, was uploaded to iNaturalist from Kawau Island in April 2019.

PHEL entomologists, in conjunction with Auckland War Memorial Museum and international experts, reported the range expansion of a green lacewing, *Mallada basalis* (Neuroptera: Chrysopidae). This species was first reported from the Kermadec Islands in 1908, and has also been found on offshore islands around New Zealand. However, in the last 2 years, *M. basalis* has been documented as becoming more abundant, widespread and established on the mainland, with many prominent entomologists recording its presence. Lacewing insect larvae are predatory and often beneficial in gardens as biocontrol agents.

Fresh produce

Nine investigations followed notifications of possible illegal imports of fresh produce, or reports of live pests or disease symptoms on fresh produce such as pears, strawberries, lettuce, bananas, durian and apples. Three investigations were closed with the symptoms attributed to possible physiological and/or storage issues. Two investigations were for IHS compliance and in both cases no breach had occurred. The other four cases involved three organisms already present in New Zealand and therefore not a biological risk.

Stored products

There were 15 investigations of organisms associated with imported stored products such as walnuts, food containers, animal feed, dates, rice, fennel seeds, sesame seeds, almonds, tea, beans and dried grains. All investigations found common pests of stored products that are already established in New Zealand and not considered a biosecurity risk.

Pests associated with inanimate hosts

Seven cases investigated invertebrates associated with imported inanimate hosts including a watering can, floor, glasshouse, kitset garden shed, picture frames, plastic resin and a coat worn by an overseas traveller. None of the investigations established any biosecurity issue because all the species found were already present in New Zealand.

Cockroaches

Three investigations dealt with cockroaches found in personal belongings of travellers coming from overseas or in imported goods. All identifications were of species already established in New Zealand.

Plant diseases

A photograph posted on the New Zealand Flower Gardeners Facebook page showed unusual symptoms on an agave plant and the dry rot pathogen *Phyllosticta concava* (Botryosphaeriales: Botryosphaeriaceae) was suspected. This pathogen is known to cause disease overseas but has never been reported from New Zealand. A sample submitted to PHEL was identified as *Paraphaeosphaeria recurvifoliae* (Pleosporales: Didymosphaeriaceae), a fungus that causes leaf spot and necrosis on perennial shrubs or trees that belong to the family Agavaceae. It is present in New Zealand.

A forestry consultant reported dieback of plantation-grown ponderosa pine trees, *Pinus ponderosa* (Pinales: Pinaceae), in the Mackenzie Basin, South Island. A site inspection by an experienced forest-health expert was carried out and samples from four locations were sent to PHEL where a mycologist identified *Diplodia sapinea* (Botryosphaeriales: Botryosphaeriaceae) a common pathogen of pine trees in New Zealand. It causes tip dieback and crown wilt in stressed trees. There was no biosecurity issue and the investigation was stood down.

Scion Research reported suspected European larch canker disease, *Lachnellula willkommii* (Helotiales: Lachnaceae), from radiata pine, *Pinus radiata* (Pinales: Pinaceae), in a Bay of Plenty forest. Fungal cultures and DNA sequencing were provided to PHEL and were identified as an undescribed *Lachnellula* species. A number of species of this genus are reported from New Zealand, most of which are saprophytes. The photos showed physical or mechanical damage to the tree, which may have enabled the fungus to colonise the damaged tissue and produce fruiting bodies on the bark around the wounds. The notifier has been asked to keep monitoring the site and report if similar symptoms are seen on trees in this forest, as *Lachnellula* species are associated with canker disease, growing exclusively on bark more rarely wood or resin of conifers.

Other investigations of note

Examples from the rest of the investigations are presented below. They involve hosts or incursion pathways that are not commonly investigated and may

present potential biosecurity issues.

A PFR scientist reported the presence of unknown visible structures (UVSs) observed by scanning electron microscopy on antennal sensilla of samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae), an organism imported into PFR's Auckland containment facility but absent from the New Zealand environment. *T. japonicus* is an egg parasitoid of brown marmorated stink bug and was imported into containment as a readiness initiative, only to be released if BMSB becomes established in New Zealand. The presence of the UVSs was notified because of the risk that they might be live microorganisms. However, an investigation concluded that regardless of what the UVSs were, there was no evidence of a biological risk outside the MPI-approved containment facility. All biosecurity issues relating to *T. japonicus* are managed within MPI-approved facilities.

A pillow imported from China was described as being contaminated with insects but no photos or samples were submitted. The investigation concluded this was a possible case of delusional parasitosis. The notifier was advised to seek medical attention.

A live wasp was found inside a packet of pre-packaged bananas from the Philippines. From a photo the wasp was identified by a PHEL entomologist as the Asian paper wasp, *Polistes chinensis* (Hymenoptera: Vespidae), established in New Zealand and not a biosecurity issue. Wasps are unlikely to survive the chilled conditions under which export bananas are transported; it is likely the wasp entered through holes in the packaging after importation.

A souvenir carved cow skull purchased in Bali was found to be infested with invertebrates on return to Christchurch. The skull had been inspected by QOs at the border and released to the passenger. The notifier submitted the skull to PHEL for testing. Two organisms were identified: adults and larvae of red-legged ham beetle, *Necrobia rufipes* (Coleoptera: Cleridae), established in New Zealand; and empty fly pupal cases (Diptera). Photos were submitted to the Border Team Managers for raising awareness among the QOs. The skull was placed in the freezer for another week and taken to the Biosecurity Control Area at Christchurch Airport, where an attempt was made to clear out the pupal cases using high air pressure. Not all cases were removed successfully. The notifier was informed about the measures taken and the skull was returned.

A biosecurity investigation aimed at establishing whether biological samples had been submitted with a microscope purchased from China. The notifier was concerned that shrimps and orchid diseases might have been introduced with the microscope. The investigation established no evidence of biological samples submitted with the microscope.
An auction on Trademe advertised "Chicken of the woods fungus *Laetiporus sulphurous*" (Basidiomycota: Fomitopsidaceae). This wood-rot fungus from the US is an unwanted organism in New Zealand. As the trader appeared to be a commercial mushroom grower advertising several varieties of mushroom for culture, the initial investigator referred this investigation to the MPI Compliance Team. Compliance officers paid a visit to the trader and collected samples for identification. These samples were identified as *Laetiporus squalidus,* a species known to be present in New Zealand. Other material collected from the trader's property suggested the source material may have been purchased from the MWLR fungal herbarium, ICMP.

Fungi were found growing on a "beechwood" toilet brush imported from Germany. Beechwood is a general term which may refer to several species so it was unclear which variety of timber the brush handle was made from. Photos of the fungal fruiting bodies were consistent with a polypore fungus common on untreated wood in damp environments. It is possible that these are local fungi.

Inconclusive

Bugs found in dog food from Australia

Many dead and live insects were found in a packet of dog food imported from Australia. The notifier had killed the live insects with fly spray but wanted to identify them. A specimen was requested since the insects could not be identified from the photos submitted, but no specimen was received at PHEL. Biosecurity risk was mitigated by the method of treatment.

Live insect on imported cut flowers from Netherlands

A live weevil was found by a Timaru florist on flowers of lisianthus, *Eustoma* sp. (Gentianales: Gentianaceae), imported from the Netherlands. The solitary weevil was not identifiable from the photo supplied. A request was made to send the weevil to MPI for identification but the specimen was inadvertently disposed of. No further action was considered necessary given the overall low risk posed by a solitary invertebrate.

Suspect risk pest species in container floor

Mud-tunnelling was found on two empty shipping containers sent for treatment at a Port Nelson fumigation facility. Termites or wood-boring insects were suspected to be present in and beneath the floors of both containers. The treatment was done at high rate (80g/m^3 MeBr for 24 hr) to ensure efficacy against live insects within the floor. Cleaning and 5-sided inspection of the containers were arranged after fumigation. No specimens were found. The presence of live insects prior to fumigation was not confirmed.

Suspect exotic wasp in Auckland

An unusual wasp was found in a shipping container storage yard at Wiri, Auckland. PHEL identified the exotic yellow paper wasp *Polistes olivaceus* (Hymenoptera: Vespidae) by photo. The specimen was requested for a formal identification but the wasp had been stood on and killed and disposed of. There have been several instances of multiple queens hibernating together and many lone intercepts of *P. olivaceus* recorded for New Zealand. At the time of notification, with the advance of cold winter temperatures, no wasp activity or even survival was expected, negating the point of conducting surveillance.

Reference

MAF (2008). *New to New Zealand Weeds. A guide to Weeds Found in imported Coco Peat.* Ministry of Agriculture and Forestry, 35 pp.

VETERINARY DIAGNOSTIC LABORATORY

Gribbles Veterinary Pathology

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

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NEW ZEALAND VETERINARY PATHOLOGY

- HAMILTON
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- HAMILTON
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  Hamilton 3241
  Ph: 0800 SVS LABS (0800 787 522) or 07 444 5101
  Fax: 07 444 5102
  Email: info@svslabs.nz

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
231 Morrin Road
St Johns
Auckland
Tel: 09 909 3568

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

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