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First report of *Aspergillus udagawae* causing feline aspergillosis in New Zealand Focusing a lens on the New Zealand animal health surveillance system



Biosecurity New Zealand Ministry for Primary Industries Manatū Ahu Matua





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Editorial Ministry for Primary Industries helping to control foot-andmouth disease in Southeast Asia

Since 2015, the New Zealand Ministry of Foreign Affairs and Trade (MFAT) has committed considerable funds to the Southeast Asian region (approx. US\$12 million) for control of foot-and-mouth disease (FMD). The Ministry for Primary Industries (MPI) has helped the work programme by providing technical expertise. During 2019, this involved contributing to technical oversight, hosting and training technical staff from Southeast Asia, developing new surveillance methods for FMD, and field-trialling a mobile PCR-testing unit being developed in New Zealand. In previous years, a range of technical inputs to the programme have been provided by staff (**Figure 1**). Some of the activities are described in the literature (e.g. van Andel et al. 2019 a,b; Rawdon et al. 2020), and have also included training government officials in biosecurity, outbreak investigation, geographic information systems (GIS) and surveillance.

In New Zealand, MFAT has a role in providing aid based on its strategic intentions (MFAT 2020), while MPI's core business focuses on prosperity, sustainability and protection of our primary industry (MPI 2020). MPI's involvement in the South East Asia China Foot-and-Mouth Disease (SEACFMD) programme is an example of a government organisation providing aid where this is not part of its direct mandate. One reason for participating in this programme is that focusing control of FMD at source may help mitigate the risk of an incursion in New Zealand. However, control of FMD in Southeast Asia is a very long-term venture given the endemicity of FMD virus in the region. Over and above this, there are other significant benefits for all parties involved: MPI, MFAT, and the country receiving the aid.

New Zealand is free from a number of significant animal disease pathogens (OIE-listed diseases). As a result, our practical experience with programmes to exclude, manage or eradicate these pathogens is limited. However, the present biosecurity response to Mycoplasma bovis has augmented MPI's general disease control experience and in the past we have responded to outbreaks of Theileria orientalis Ikeda; and endemic disease programmes are ongoing to control bovine tuberculosis, enzootic bovine leucosis and leptospirosis. But gaining hands-on experience with managing FMD is problematic, as the disease is not present and has never occurred here. Experience has been gained in the past by MPI staff helping in outbreaks overseas, for example with the UK's response to an FMD incursion in 2001. Staff have also participated in a European Union FMD (EuFMD) training programme to develop front-line capability to investigate suspect cases of FMD. The EuFMD courses are structured around simple epidemiological and clinical investigation of field cases, with a focus on further developing field capacity. However, managing a significant animal disease response requires additional skills, so EuFMD only partially fulfils MPI's need to further develop its capability. Hence the benefits of involving MPI staff in the SEACFMD programme.

These MPI staff that have been enlisted to assist the programme already have a wide range of skills, including experience of managing on-farm investigations of FMD. Many have postgraduate qualifications in epidemiology, immunology, virology and molecular biology; field experience in productionanimal disease and medicine, diagnostic laboratory experience and past experience in biosecurity responses to significant disease events. The SEACFMD programme has offered an avenue by which these skills can be improved and used in a real and practical way to combat diseases of significant importance to New Zealand. Application of these skills provides practical experience as well as theoretical knowledge. In addition, MPI contributors have often been called upon to develop their own skillsets to help solve complex problems. This was particularly apparent with some of the early work on identifying "hotspots" for the purpose of targeting vaccination against FMD.

From the perspective of MFAT, devolving some interest in a programme to another agency also needs justification. At a philosophical level, an advantage to MFAT of MPI's discretionary participation is that there is no vested interest that is likely to influence critical review. As a result MPI has provided free and frank advice, and independent review. With aid investments there is constant pressure on all organisations involved to make a pronouncement of success, regardless of real outcomes. For instance, with donor countries there is political pressure to show that funds provided have had a positive impact. There is also the same pressure on the service provider to show value, thus ensuring a continuation of funding, and on the recipient country to indicate positive impacts in order to continue to receive funds - regardless of any outcomes. Thus, overall there is a bias toward ignoring failures and selectively highlighting examples of success. In addition, positive impacts may be due to factors other than the aid spend. It can be hard to have the level of critical review that would diminish the direct association between a given aid programme and the positive impact observed. Involvement of a non-partisan government organisation for oversight, review and direction offers additional accountability to aid spending.

MPI has not been paid directly for the service it provides. Thus, the client has always been considered to be the recipient country of the aid funding, rather than necessarily the funder. This has meant MPI has been very responsive to meeting the needs of the recipient country of the aid. MPI staff have become involved for a variety of reasons and a key reason (over and above the obvious technical stimulation, learning opportunity and skill development) is that they want to contribute to another country and its culture. The motivation of staff involved does influence attitudes and the way staff are perceived. It is likely that the involvement of MPI staff over many projects has contributed directly to positive relationships with incountry staff and resulted in goodwill from the programme as a whole. This has been one of the most significant benefits to "New Zealand Inc".

Involvement of MPI staff in the New Zealand-funded OIE FMD disease control programme has provided significant opportunities to maintain and develop staff capability. Developing people is an important part of our ability to respond to exotic diseases. Contribution by MPI, a government agency with no direct responsibility for aid, is a new way of doing business and has significant advantages to all parties involved. It is significantly different from past aid programmes and represents a valuable model for future New Zealand aid programmes, but also for other countries that are considering following our model.

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Figure 1: MPI contributors to work on FMD during 2019. From left: Emma Bramley, Kelly Buckle (kneeling), Richard Swainsbury, Nelly Marquetoux, Doug Begg, Rudi Bueno (kneeling), Tom Rawdon, Andy McFadden (New Zealand leader), Barbara Binney. (Absent: Richard Spence, Mary van Andel, Ben Phiri.)

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Obituary: Royce Elliot

Royce Elliott, who died on 19 September 2019, had a distinguished career in the Ministry of Agriculture, where he was a visionary leader for more than 20 years. He led the transformation of the state veterinary service, modernised the regulation of the dairy industry, oversaw the introduction of the fisheries quota management system and led the integration of the Ministry's food safety and agricultural biosecurity systems. He also served on many national and international bodies involved with agricultural and fisheries regulation and trade.

After graduating as a veterinarian in 1956 with honours from Sydney University, Royce practised in Northland, working for 2 years in Ruawai and 4 in Kaitaia before becoming a research scientist at the Wallaceville Animal Research Centre, working on bovine mastitis. He then gained a Veterinary Services scholarship to study at Manchester University, where he gained an MRCVS by examination and a Diploma of Bacteriology. The following year he was awarded a Harkness Fellowship to study brucellosis from 1968–1970 at the Trudeau Medical Research Institute in Saranac Lake, New York. This training left Royce with a profound commitment to science-based decision making and a systematic approach to management.

On his return to New Zealand Royce joined the Animal Health Division of the Department of Agriculture to head the development of the scientific and management infrastructure for New Zealand's world-leading bovine brucellosis eradication programme. This included the expansion of the veterinary diagnostic laboratory network and the establishment of an associated veterinary investigation service.

Royce's vision was for the establishment of a veterinary field and laboratory capability in MAF to "define, protect and improve" animal disease status. This led to his founding *Surveillance* in 1973, modelled on the weekly disease reports of the US Centre for Disease Control, reporting trends and unusual occurrences in animal diseases as well as reports on surveys and epidemiological studies that supported our animal health claims for overseas markets.

From 1985 to 1990 Royce had the challenge of leading a major restructure of MAF. He created MAFQual, which amalgamated the field, laboratory and food inspection services of the Ministry into a single organisation. Out of this eventually grew Biosecurity New Zealand, the Food Safety Authority and AsureQuality, all true to Royce's vision of modern science-based risk management and quality management systems supported by diagnostic and epidemiological expertise. In the process *Surveillance* was expanded to provide support to health and biosecurity status claims for all agricultural and fisheries trade.

During these later years at the Ministry, Royce held a series of senior management positions including 8 years as Assistant Director-General and a year as Acting Director-General, before retiring as Group Director Policy in December 1993. He advised the many Directors-General and Ministers he served without fear or favour – and with great discretion, as is evident from the logbooks of many of those meetings that he left with the National Library.

In the face of the neo-liberal Douglas reforms and the rise of managerialism in the 1990s Royce regretted the declining influence of science on public policy. Throughout his career he had worked to change MAF's culture from one of empirical thinking to one of scientific thinking. In his own words, "Bureaucracy's main concern is to see that certain things don't happen; but there is enormous satisfaction in having a team recognise what a country needs and making it happen!" His motivational leadership style reflected that view and his intellectual curiosity was constantly generating new ideas. He later admitted that "I got my kicks out of innovation and once satisfied that a need could be met, I tended to want to move on."

Royce had an insightful sense of humour that was never barbed and he was a great raconteur. He was wise and self-effacing, never promoting himself but instead supporting and encouraging others. He led by example and, unusually for a senior public servant of that time, kept his desk pushed against the wall so that he would have to turn round and engage his staff and other visitors without that symbol of authority between them.

Royce was not born to privilege but achieved a great deal by personal effort and intellect. He spent his childhood in an orphanage and in 2015 he published "The Glass Jar", the story of that experience.

Royce was one of a select group of veterinarians appointed as a foundation member of the Australian and New Zealand College of Veterinary Scientists. He was also the Harkness Fellowships representative of the Commonwealth Fund of New York for New Zealand from 1984 to 1992. Following his retirement from MAF in 1993 Royce became Executive Director of the Institute of Public Administration NZ for several years and was involved in many community organisations including emergency management and St John New Zealand. For his contributions to agriculture he received a NZ 1990 Medal and an OBE.

Royce had poor health in his later years but remained cheerful and intellectually curious all his life. He was a devoted family man and is survived by Gwenette, his wife of 62 years, and four of his five children and their families.

 Contributed by John Hellström ONZM, BVSc, PhD in consultation with Royce's family and colleagues. John worked under Royce in various roles from 1972 to 1991, including as Chief Veterinary Officer (1987–1991) and subsequently built on Royce's vision for biosecurity as the Chair of the Biosecurity Council, 1997–2002.

ANIMALS

First report of *Aspergillus udagawae* causing feline aspergillosis in New Zealand

Summary

In September 2019 a fungal culture was received by the Ministry for Primary Industries' (MPI's) Animal Health Laboratory (AHL) as part of an exotic disease investigation. The fungus was isolated from a cat with rhinitis in Auckland, and had been reported via the MPI exotic pest and disease hotline by a clinical pathologist from a commercial veterinary laboratory as suspicious for Aspergillus felis, one of the causative agents of invasive aspergillosis (IA) in cats. The fungus was first confirmed as an Aspergillus sp. belonging to section Fumigati and then further identified as A. udagawae using comparative sequence analysis of partial β -tubulin and calmodulin genes. A. udagawae has been emerging as a cause of IA in humans and animals (Sugui et al. 2010) and this is the first report from New Zealand. This agent and its related species are widespread and saprophytic, and A. udagawae is likely to have been present in New Zealand for some time.

Case history

A 5-year-old Domestic Shorthaired cat had been suffering from rhinitis for 2.5 months, with clinical signs of sneezing and unilateral (right) mucopurulent (occasionally haemorrhagic) discharge with nasal deformity. The cat's condition improved somewhat with steroid treatment and worsened once medication ended. A nasal swab was submitted for culture, and the resulting plate was sent to the AHL. Following the diagnosis, the cat was placed on antifungal treatment and its condition had improved at the time of the last report.

Description

Aspergillosis is a sporadic mycosis that occurs worldwide in mammals and birds. In contrast to dogs, in which (nasal) aspergillosis is relatively common, aspergillosis is rare in cats, but considered an emerging infection (Hartmann et al. 2013). The infections are caused by fungal organisms of the genus Aspergillus and are commonly associated with predisposing local or systemic factors. Species in the A. fumigatus complex are responsible for the majority of infections in cats, but A. flavus, A. nidulans, A. niger and A. terreus have also been detected (Hartmann et al. 2013). There are more than 60 closely related species within the section Fumigati and at least seven of these (including A. felis, A. udagawae, A. lentulus, A. fischeri, A. thermomutatus and A. wyomingensis) can cause aspergillosis in cats (Barrs et al. 2013, 2014, 2015; Kano et al. 2008, 2013, 2015; Tamborini et al. 2016; Taylor et al. 2016).

Section Fumigati is characterised by the formation of conidiophores with columnar conidial heads consisting of flask-shaped vesicles, uniseriate phialides and long chains of conidia. Conidia are bluish-green to pale green, generally hydrophobic, and 2.5-3.5 µm long (Sugui et al. 2014). Identification of Aspergillus species in section Fumigati to the species level based on phenotypic characteristics is problematic because of the overlapping morphological features of these organisms. Confident identification of cultured Aspergillus isolates requires molecular identification. Although internal transcribed spacer region (ITS) is an official "barcode" for fungi, this may not be enough for identifying some Aspergillus isolates to the species level, and a second identification marker is usually needed (Fletcher & Hanson 2016; Samsun et al. 2014). Additional sequencing of either β-tubulin or calmodulin is recommended by the International Society of Human and Animal Mycology (ISHAM) to identify the actual species (Irinyi et al. 2015). There is also evidence that thermotolerance of different species could be useful in differentiating some species (Sugui et al. 2010; Barrs et al. 2013). Furthermore, matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) gives promising results for rapid and accurate distinction between

A. fumigatus and other *Aspergillus* spp. of the section *Fumigati* in clinical practice (Lamoth 2016).

Culture and identification

The fungus was subcultured onto three different media: Sabouraud dextrose agar (SDA), SDA with chloramphenicol and gentamicin (SDA+), and mycobiotic agar (containing chloramphenicol, gentamicin and cycloheximide), and incubated at 25°C for 2 weeks. Colony morphology and microscopic features of the fungus were examined and recorded at the end of the first week, and later when the fungus started to produce spores (after 10-14 days). Fungal DNA was extracted from mycelia using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research), and ITS PCR and sequencing were performed. The extracted DNA was further amplified for partial calmodulin and β-tubulin genes using cmd5-F & cmd6-R and bt2a-F and bt2b-R primers (Hong et al. 2005; Glass and Donaldson 1995) and sequenced.

Results

After 1 week, colonies grown on SDA (with and without antibiotics) and mycobiotic agar were 50–55 mm in diameter, cream-coloured and velvety with radial wrinkles (**Figure 1**). Under the microscope the fungal conidial head was columnar with uniseriate phialides on the upper two-thirds of the vesicle. The fungus started to produce globose spores after 10–14 days in this condition (**Figures 2 and 3**).

Based on the fungus's morphological characteristics and ITS sequences (100 percent homology of ITS sequences to multiple *Aspergillus* spp.), the isolate was primarily identified as an *Aspergillus* species belonging to the section *Fumigati*. The fungus was further identified as *A. udagawae* using BLAST results of β -tubulin (BenA) and calmodulin (CaM) gene sequences, with 100 percent homology of partial β -tubulin (BenA) and calmodulin (CaM) gene sequences.



Figure 1: Colony morphology on mycobiotic agar and SDA+ after 11 days % $\left(\frac{1}{2} \right) = \left(\frac{1}{2} \right) \left(\frac{1}{2$

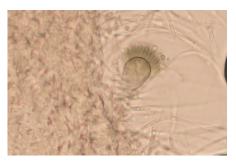


Figure 2: Fungal conidial head:columnar and uniseriate (phialides arranged in one row), ×400



Figure 3: Fungal conidial head, conidiophore and conidia, $\times 400$

Conclusion

Although this represents a new find, it is likely that this organism is not new to New Zealand, since the infection is rare and the technology to differentiate the fungal types has improved in the recent past.

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ANIMALS

Focusing a lens on the New Zealand animal health surveillance system

The Ministry for Primary Industries (MPI) has the legislative mandate to provide biosecurity for the entire animal population in New Zealand under the Biosecurity Act 1993. The biosecurity system has three main components: preborder, border and post-border, and the animal health surveillance system is an essential part of post-border biosecurity. Its principal objectives are:

- to provide early detection of exotic or emerging pests and diseases in both domestic and wild animals;
- to provide assurance of country freedom from specified pests or diseases;
- to support market access requirements of countries importing New Zealand products;
- to describe distribution and occurrence of endemic pests and diseases, for use in developing import health standards and to support the setting of priorities for endemic disease control; and
- to support the fulfilment of New Zealand's international reporting obligations, for example to the OIE (World Organisation for Animal Health) (Tana 2014).

The Animal Health Surveillance System conducts both passive and active surveillance. Surveillance is carried out by dedicated systems and programmes including the exotic pest and disease notification system and regional veterinary diagnostic laboratory data analysis. There are also programmes targeted at specific diseases: apiculture, arbovirus, avian influenza, transmissible spongiform encephalopathies and wildlife diseases.

The OIE Terrestrial Animal Health Code, Chapter 1.4 (OIE, 2019) recommends continuing to provide quality assurance of surveillance systems by periodic auditing. Accordingly, MPI has commissioned a surveillance evaluation framework known as SurF (Muellner et al. 2018). We used this framework, with slight modification, to evaluate the components of the surveillance system. SurF has four components: motivation for the evaluation; scope of the evaluation; evaluation design and implementation; and reporting and communication of evaluation outputs. The evaluation was conducted from 2017 to 2019, and each component is described in further detail below.

Motivation for the evaluation

This component of SurF describes the reasons for conducting an evaluation. Our evaluation was motivated by the desire to:

- assess whether present animal health surveillance objectives have remained relevant, with particular reference to changing stakeholder expectations, organism evolution, modern technology and advancements in laboratory techniques;
- standardise and regularise the evaluation of surveillance activities conducted within MPI;
- assess stakeholder engagement and satisfaction with current surveillance;
- assess the pest and disease notification system with a view to enhancing notifications in a structured manner;
- identify key performance indicators to periodically assess the performance

Functional Group	Attribute
Organisational and management	Programme description Organisation and management Performance indicators and reviews
Processes	Data and information collection Data management and storage Field and laboratory services Resource availability Technical competency and training
Technical implementation	Coverage Data completeness and correctness Timeliness
Output	Historical data Positive predictive value Representativeness and bias Sensitivity Specificity

Table 1: Functional groups and attributesincluded in the evaluation process

of the notification system; and

collate all previous work related to assessing the notification system,

Sector	Representation: Stakeholders external to MPI			
Apiculture	Apiculture New Zealand			
	New Zealand Beekeeping Inc.			
	South North Island Beekeeping Inc.			
Academia and research	Massey University AgResearch			
Equine	Equine Health Association Epi-interactive Ltd			
	New Zealand Veterinary Association			
Commercial	Gribbles Veterinary Laboratories			
laboratories	IDEXX Laboratories SVS Laboratories			
Porcine	New Zealand Pork Pig veterinary practitioners			
Poultry	Poultry Industry Association of			
· outly	New Zealand Poultry veterinary practitioners			
Ruminants	Beef & Lamb New Zealand			
Rummants	Dairy Goat Co-operative			
	Dairy New Zealand			
	Deer Industry New Zealand			
	Landcorp Farming Ltd Livestock Improvement Corporation			
	Meat Industry Association			
	New Zealand Veterinary			
	Association OSPRI			
Wildlife	Wildlife Rehabilitators Network of New Zealand			
	Fish & Game New Zealand			
	New Zealand Centre for			
	Conservation Medicine			
	South Island Wildlife Hospital Wildlife Hospital, Dunedin			
	Wildbase, Massey University			
	Department of Conservation			
	NZVA Wildlife branch			
	Wildlife experts			
	OIE Wildlife Focal Point			
Trada	Stakeholders internal to MPI Market access			
Trade	Animal Imports and Exports			
Preparedness	Readiness and Response Risk Analysis			
Laboratories	Animal Health Laboratory Plant Health and Environment			
	Laboratory			
International	International Standards			
	OIE Animal Disease Notification Focal Point			

Table 2: Stakeholders consulted during the animal health surveillance evaluation process

to identify and address gaps in our understanding of its performance.

These aims may be summarised in the evaluation question: is the current animal health surveillance system fit for purpose?

Scope of the evaluation

The objective was to evaluate all aspects of surveillance except financial aspects such as cost-effectiveness and costefficiency (**Table 1**).

Evaluation of the notification system focused on stakeholder engagement, particularly assessing the timeliness of notifications. The other components of the system, such as the 0800 exotic pest and disease hotline, a legal framework for notification, the network of trained veterinary incursion investigators, and diagnostic laboratory capability (Tana 2014) were only included where it was known that they might affect stakeholder engagement. The evaluations had three objectives: to ascertain whether the animal health surveillance programmes achieved their objectives; to identify the strengths and weaknesses of those programmes; and to recommend improvements where necessary.

Evaluation design and implementation

The evaluations were based on 23 SurFdefined surveillance attributes in five functional groups (**Table 1**). Subsets of 12 and 19 attributes respectively were used to evaluate the notification system and the other surveillance programmes. Scores for each attribute were based on a simple "traffic light" assessment: green (no changes needed); amber (fewer than two-thirds of the attribute's elements needed change); and red (two-thirds or more elements needed change). The scores were summarised in a diagram like the example shown in **Figure 1**.

Attributes were selected for inclusion in the evaluation because they were either relevant to the review question or were classified as core requirements of SurF and recommended for inclusion in any evaluation. Assessment of items under the "organisation and management" heading in **Table 1** provided insights into the non-technical aspects of surveillance programmes, for example how surveillance is overseen and its performance monitored, and the mechanisms in place to ensure programmes are up to date. Assessments under the functional group "processes" aimed at determining how well each surveillance programme was planned, including the type of information to be collected and how it was collected, managed and used. The assessment also aimed to determine whether data-quality controls were in place. We measured how well surveillance activities were carried out in relation to the stated objectives by examining attributes under the functional group "technical implementation".

Reliability of archived information to demonstrate the robustness of surveillance was determined by assessing attributes in the "output" functional group. This included examining the quality of the information, its representativeness, and the ability to detect a specified organism if it were present (or to correctly demonstrate its absence). The benefits of surveillance programmes were assessed under the category of "impact", and here the assessment also provided an indication of how effective the communication strategies for the programmes were.

The evaluation was conducted in two phases. The first phase included

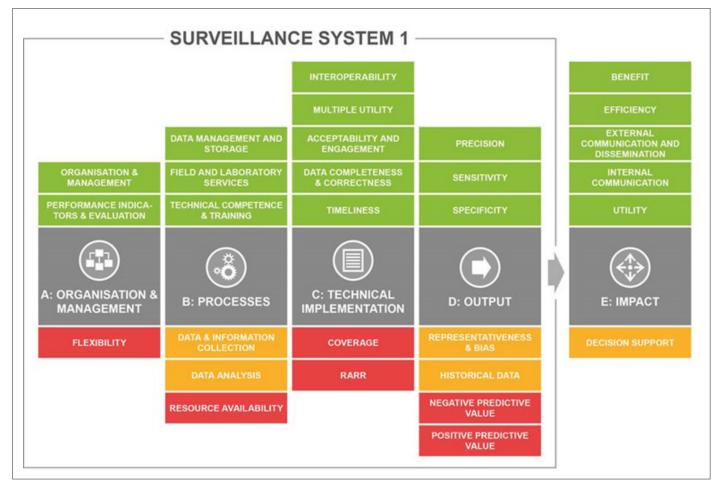


Figure 1: Example of "traffic light" summary used for surveillance attribute assessment using SurF

evaluation of all functional groups except "impact", which was the focus of the second phase. The latter involved consulting both external and internal stakeholders (**Table 2**). More than a hundred stakeholders were engaged, with the aim of determining the level of awareness of existing surveillance, how relevant it was to them and whether it met their expectations. Engagement was by sector rather than by surveillance programme. This was to avoid multiple engagements with some stakeholders that are involved in more than one programme.

Reporting and communicating results

The evaluation report, including stakeholder consultations and feedback, is now undergoing assessment and peer review within MPI's Biosecurity Surveillance and Incursion Investigation (BSII) Animal Health Team. Areas for improvement will be identified, prioritised and appropriate action determined. Findings of the review will be published in due course in *Surveillance*.

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ANIMALS Quarterly report of diagnostic cases

Gribbles Veterinary Pathology Bovine

From a South Canterbury farm, samples were received from a 2-month-old calf that was part of a group in which several had diarrhoea. The calves had recently been treated with anthelmintic, and faecal worm egg counts were negative. Neither Salmonella nor Yersinia bacteria were recovered from the intestinal contents. Histopathological examination of the small intestine revealed blunting of villi, neutrophils within crypts, and bacteria attached to enterocytes along the surface of the villi, with scalloping of the surface of the epithelial cells. These changes were typical of an attaching and effacing Escherichia coli infection.

Eight calves from a group of 50 on a South Canterbury farm became blind and ataxic. Six had already died before the attending veterinarian arrived. Samples were collected from one of the calves. Histopathological examination revealed no lesions in the brain and no acidfast inclusions were seen in the tubular epithelial cells of the kidney. However, analysis of a heparinised blood sample showed a blood lead concentration of 2.28 mg/L (toxic level > 0.35), consistent with a diagnosis of lead toxicity. The source was lead-based paint from an old house.

A 15-month-old Jersey heifer from Northland became acutely recumbent, hypothermic, dyspnoeic, apparently blind and had bloody faeces. At necropsy the large intestine and caecum appeared haemorrhagic with intestinal congestion. Histological examination revealed abomasitis and enteritis with haemorrhage, necrosis and villous atrophy with nematode parasites and endothelial intranuclear inclusion bodies. Along with a weakly positive reaction to a bovine viral diarrhoea (BVD) antigen ELISA test, these findings suggested a combination of bovine adenovirus infection, nematode parasitism and BVD virus infection leading to the unusual finding of adenoviral lesions with haemorrhagic enteritis in an adult

animal (most cases of adenoviral enteritis are seen in calves under 12 months old).

A yearling beef heifer was found recumbent on a Southland sheep-andbeef farm. Its rectal temperature was subnormal and the coronary bands on all four feet were very red and congested. The udder also had red and congested teats. A blood sample showed low serum albumin (15 g/L; reference range 27–42). Ovine herpesvirus-2 (the cause of malignant catarrhal fever, MCF) was detected in the serum by PCR. Unusually, there were no signs of ocular or nasal discharge or corneal oedema in this case.

Seven 2-month-old calves from a group of 200 on a Southland dairy farm were found dull and recumbent, and two others had died. Over the next 3 days ten more calves died. Histopathologic examination showed a severe nonsuppurative encephalitis consistent with a diagnosis of sporadic bovine encephalomyelitis (SBE), a *Chlamydia pecorum* infection. PCR testing of brain tissue was positive for *C. pecorum*, further supporting this diagnosis.

In late November there were a number of sporadic deaths in a group of calves on a Southland dairy farm. A large proportion of the group were coughing and most were thin, with evidence of diarrhoea (faecal staining around the hindquarters). The calves had been treated with a broad-spectrum anthelmintic about 5 weeks previously. Necropsy of one calf revealed consolidation of about 75 percent of both lungs. There were abundant adult lungworms in the larger airways. Bacterial culture of the lung was unrewarding but histopathological examination revealed a severe acute bacterial bronchopneumonia and pleuritis. Histophilus somni was identified by PCR in the fresh lung tissue. The respiratory syndrome associated with this infection occurs most often in young cattle, especially those kept in large groups or on feedlots.

During the spring there were multiple outbreaks of *Salmonella* **Brandenburg** infection in young dairy calves on several properties in Canterbury and Westland. The calves were generally 1–4 weeks old and displayed diarrhoea, lethargy and weakness. Some deaths occurred. Diarrhoeal disease in calves is now a very common manifestation of this infection in these regions, although diarrhoea, abortion and sometimes fatal sepsis in adult sheep and cattle are also seen.

Other Bovidae

Several water buffalo (Bubalus bubalis) calves from Franklin died suddenly. Histopathological examination revealed lymphocytic vasculitis in the abomasum, heart, kidney, lung and liver, but not the brain. This was considered highly suspicious for malignant catarrhal fever virus (MCF) infection. The property had no sheep, but did have goats. PCR for ovine herpesvirus-2 (OvHV-2), the cause of MCF, was negative on frozen spleen and lymph node samples, but positive on formalin-fixed tissues, which was unusual. Additional PCR tests for caprine herpesvirus-2 (CpHV-2) were performed, as there have been reports of possible caprine-associated MCF in domestic water buffalo, but results were negative. MCF is usually sporadic, but outbreaks can occur. High stocking density and stress are suspected to be predisposing factors. Sources of infection can include deer and sheep. Disease is the result of cross-species infection by viruses from the MCF group of ruminant rhadinoviruses, which are highly cell-associated lymphotropic herpesviruses. Mortality in susceptible species approaches 100 percent, although there are rare recorded cases of chronic infection or recovery, especially in infected goats, bison, cattle and pigs. Although the agent is transmissible, the disease does not appear to be contagious among cattle or bison by direct contact. Sheep-associated MCF occurs where bovids or deer have an association with sheep, though direct or close contact is not necessarily required. There is considerable variation in the susceptibility of various ruminant species to sheep-associated MCF. Domestic cattle (Bos taurus and B. indicus) appear to require high levels of exposure to induce disease. In contrast, Bali

cattle or banteng (*Bos javanicus*), the domestic water buffalo (*Bubalus bubalis*), American bison (*Bison bison*) and most species of deer, with the exception of fallow deer (*Dama dama*), seem to be highly susceptible.

A 6-year-old bison (Bison bison) in a zoo had been unwell and inappetent for 96 hours and died shortly before it was to be sedated for examination. Necropsy revealed that the animal was in good body condition. There was reddening of the abomasal mucosa and parts of the intestinal mucosa. The most notable lesions seen on histopathological examination were in the liver. The portal areas were expanded with abundant large lymphocytes and there was a similar cellular infiltration of the walls of the portal veins. These findings were suspicious for malignant catarrhal fever. Subsequent PCR testing for ovine herpesvirus-2 on a mesenteric lymph node was positive, confirming this diagnosis.

Ovine

On a large Southland sheep farm with 5,000 ewes, six 1-month-old lambs were found dead over a period of a few days. One lamb was necropsied by the attending veterinarian, who found large subcutaneous areas of haemorrhage over one side of the chest and the sternum, along with a thick layer of fibrin covering the congested lungs. Histological examination showed a severe fibrinous pleuritis and alveolar collapse. Sections from the affected area of skin on the chest showed a severe necrotising dermatitis and panniculitis with large areas of haemorrhage and occasional large colonies of bacilli. Mannheimia *haemolytica* was isolated from the pleural lesion. This is a common cause of bronchopneumonia in sheep, particularly in young animals around weaning age, and septicaemia may sometimes occur.

On a large Otago sheep farm 2,300 hoggets were yarded for shearing. About 24 hours later, while still in the yards, they were treated with a combination drench containing three different anthelmintics, including levamisole. Drenching treatment was halted after 26 hoggets died within a few minutes of being treated. Two days later the remainder of the group were drenched. There were no further immediate deaths but the next morning eight more animals were found dead. Two sick hoggets recovered. On investigation it was found that the dose of anthelmintic used was 2–3 times higher than recommended. Necropsy of one animal showed only a mild, patchy consolidation of the lungs, and no significant lesions were found on histopathological examination of a range of tissues. The circumstantial evidence in this case strongly suggested that the deaths were the result of **levamisole toxicity**.

Three Arapawa lambs died suddenly on a Canterbury property at 2–3 months of age. The lambs had been treated with anthelmintic a month previously. They had not been vaccinated against clostridial diseases but the ewes had been vaccinated prior to lambing. Necropsy revealed excessive pericardial fluid. The brain showed no obvious fluorescence under ultraviolet light. Histopathological examination showed focal proteinaceous oedema in the meninges of the cerebellum and around blood vessels in the cerebellum and corona radiata. There was vacuolation of the cerebral cortex, with scattered proteinaceous perivascular droplets and proteinaceous fluid in the connective tissue around some arterioles in the inner cortex of the kidney. The lesions in the brain were considered typical of enterotoxaemia caused by *Clostridium perfringens* type D. The perivascular oedema in the kidney was unusual but was also attributed to the effects of the epsilon toxin responsible for this disease.

Caprine

Eight Angora goats from a herd of 300 near Warkworth had an acute onset of neurological signs (circling). Three died. They had been fed mouldy baleage. Histopathological examination of the brain from one animal showed a moderate, multifocal, subacute meningoencephalitis with moderate numbers of gram-positive, short bacilli in the areas of inflammation, consistent with a diagnosis of listeriosis. Bacterial encephalitis caused by Listeria monocytogenes usually occurs in winter and spring in adult ruminants. The bacteria reside in the environment (especially in silage and soil) and in ruminant faeces. It is thought that initial infection in the nasal or oral cavity spreads by local invasion to the cranial nerves and then to the brain. Listeriosis can be sporadic, but outbreaks can be associated with feeding silage or baleage.

Porcine

An 11-year-old kunekune sow from Auckland was euthanased after a mass in the uterus was discovered. Histopathological examination revealed that the mass was a uterine adenocarcinoma with intravascular invasion, necrosis and mineralisation. The lungs, heart, kidneys, adrenal glands and abdominal lymph nodes were all found to contain multiple metastatic adenocarcinomas. Uterine (endometrial) carcinoma is rare in domestic animals but there have been several reports from pigs, including in studies of pet pigs. This type of pig seems to have a tendency to develop hyperplastic and neoplastic uterine lesions, especially in older sows. The tumours generally metastasise to the lungs and regional lymph nodes.

In Canterbury, skin swabs were collected from two pigs that were showing clinical signs typical of greasy pig disease (brown, greasy areas of the skin with scales and scabs). *Staphylococcus hyicus* was isolated from both, supporting the clinical diagnosis. The isolates were sensitive to amoxicillin with clavulanic acid, trimethoprim with sulphamethoxazole, and ceftiofur, neomycin, erythromycin, and marbofloxacin, but resistant to penicillin, ampicillin and tetracycline.

Camelid

An alpaca of unspecified age from a property in Wairarapa was lethargic and had pale mucous membranes. Examination of a blood sample showed changes consistent with iron-deficiency anaemia, with haematocrit 0.09 (reference range 0.24–0.40), marked hypochromasia, microcytosis and significant numbers of dacryocytes and fusiform cells. A faecal egg count showed 15,700 strongyle eggs per gram (epg) of faeces. In alpaca, counts over 300-400 epg are considered high and haemonchosis should be a consideration in these circumstances. The serum copper concentration was 1.0 umol/L (reference range 5.2–16), likely signifying a copper deficiency that had arisen secondary to the parasitism and which may have been contributing to the anaemia, as copper is required for the utilisation of iron and its absorption from the gastrointestinal tract. The anaemia was likely the result of combined parasitism and copper deficiency.

Three alpaca from Northland were reported to be very sick and one died before the veterinarian arrived. The other two animals were found to be in shock, with severe dehydration, tachycardia, hypothermia and haematuria. The owner had placed clippings of oleander (Nerium oleander) on a bonfire 2 days previously. Necropsy of one alpaca revealed large ecchymotic haemorrhages in the heart, along with reddened mucous membranes and regions of reddening in the small intestine. Histopathological examination showed multifocal myocardial necrosis and haemorrhage, consistent with oleander toxicity. Oleander contains a cardiac glycoside (oleandrin) with a structure similar to digoxin.

Equine

A veterinarian was called to examine a 2-year-old Standardbred horse from Canterbury that had a fleshy mass protruding from the rectum. Histopathological examination of sampled tissue revealed this to be a **rectal adenoma**. Rectal adenomas are commonly recognised in dogs but rarely seen in horses.

A 19-year-old Clydesdale cross horse from Mid Canterbury developed a small mass on the edge of the third eyelid. Histopathological examination showed that this was a **squamous cell carcinoma**. The surrounding tissue had solar elastosis. It is likely that this tumour was induced by ultraviolet solar radiation.

A 14-year-old Thoroughbred horse from Northland had bilateral uveitis characterised by blepharospasm, epiphora and miosis. There was no corneal ulceration. Clinical response to topical corticosteroids in combination with systemic non-steroidal antiinflammatory drugs was slow. Serum haematology and biochemistry revealed only minor changes, with eosinophils 0.4 x 10⁹/L (reference range 0–0.03 x 10⁹), creatinine 80 µmol/L (reference range 81-164), phosphate 1.05 mmol/L (reference range 1.2–2.2), albumin 33 g/L (reference range 34–41), bilirubin 13.2 µmol/L (reference range 40–100) and creatine kinase 392 IU/L (reference range 0–410). Serological testing revealed a 1:1,600 titre to *Leptospira* serovar **Pomona** in the microscopic agglutination test, suggesting the horse could have leptospirosis-associated uveitis. The syndrome of "equine recurrent uveitis" has been linked to leptospirosis infection,

possibly with some component of autoimmunity in its pathogenesis.

A Thoroughbred foal from Auckland had purulent fluid aspirated from a distal radial physis. Culture of a swab produced a heavy growth of *Salmonella* **Typhimurium phage type 56** variant, consistent with *Salmonella* osteomyelitis. *Salmonella* spp. can cause osteomyelitis in this location secondary to septicaemia in foals, typically when they are less than 4 months of age. Other bacteria that may cause this type of lesion include *Escherichia coli, Streptococcus* spp., *Klebsiella* spp. and *Rhodococcus equi*.

Three foals less than a week old on a large horse-breeding establishment in Otago became unwell and developed a severe diarrhoea. The first two affected animals died despite intensive supportive treatment. The first foal had been sick for 5 days before it died. Necropsy of this foal revealed a severe enterocolitis. No significant enteric bacteria were isolated on routine culture but histopathological examination revealed severe coagulative necrosis of the intestinal mucosa. The luminal surface of the affected mucosa was lined with abundant large bluntended gram-positive bacilli resembling a *Clostridium* species. The second foal had been sick for only 24 hours when it died at 2 days of age. Histopathological examination revealed similar changes in the intestinal mucosa, and routine bacterial culture was again negative. However, PCR tests of the intestinal contents for *Clostridium difficile* toxins A and B were positive for both foals, supporting a diagnosis of *Clostridium*associated enterocolitis. A third sick foal was treated with metronidazole and a broad-spectrum antibiotic, using a stomach tube, and it recovered rapidly. It is unusual to see outbreaks of Clostridium-associated enterocolitis. It was of interest that in this case the mares and foals were living in a muddy paddock with large puddles, as there had been a lot of rain over a short period. The mares and remaining foals were moved to drier paddocks and no more cases were reported.

Avian

A 7-month-old domestic chicken from Auckland exhibited weakness of the legs. It was euthanased and samples of sciatic nerve, adipose tissue, duodenum and pancreas were submitted to the laboratory. Histopathological examination showed infiltration of all the tissues with sheets of neoplastic large lymphocytes, consistent with lymphoma. In birds, lymphoma can be induced by the viruses that cause **Marek's disease** or lymphoid leukosis. These two diseases are difficult to differentiate histologically, but finding neoplastic lymphoid infiltrates in the peripheral nerves (as in this bird), or in the iris or brain, supports a diagnosis of Marek's disease. This disease is common in New Zealand and is caused by an alpha-herpesvirus. It primarily affects chickens (Fletcher & Abdul- Aziz, 2008).

An 11-month-old scarlet macaw (Ara macao) from Marlborough was found dead. The bird was in poor body condition. Histopathological examination revealed a multifocal necrotising heterophilic and granulomatous hepatitis, splenitis and pneumonia. The lesions were moderate to severe and subacute to chronic. Gram-negative bacteria suspicious of Yersinia, Salmonella or Escherichia coli were seen within the lesions. This severe bacteraemia was considered to be the cause of death. The lesions were at least several days old. The poor body condition must have developed over a period of weeks rather than days, and this may have been a predisposing factor, but chronic infection was also a possible cause. There was no histopathological evidence of underlying viral or chlamydial infection.

A female kahu/harrier hawk (*Circus approximans*) in a raptor rescue facility suddenly became very weak and began convulsing and vomiting. The concentration of lead in a blood sample was found to be very high at 5.46 mg/L, confirming **lead toxicity**. No reference range was available for this species, but in general a blood lead concentration of 0.2 mg/L or more in birds suggests acute lead toxicity if there are supportive clinical signs. The source of the lead could not be determined.

A group of pateke/brown teal (*Anas chlorotis*) hatched at a captive breeding facility in Canterbury were transferred into an aviary of their own at 7 weeks of age. Their weights were within expectations for their age and they appeared clinically normal despite a recent influx of mice in the parent aviary. Routine screening of faeces showed no evidence of worms but *Yersinia kristensensii* was isolated from the faeces.

This organism is not usually considered to be a pathogen but its epidemiology is unclear. It has been reported previously in kiwi creches but we are not aware of any reports from pateke.

Canine

A swab was taken from the left ear canal of a 13-year-old desexed female Schnauzer from Christchurch. The dog had a history of left otitis externa and otitis media with rupture of the tympanic membrane. There were severe ulcerative lesions of the ear canal and pinna, with discharge from the middle ear. Facial nerve paralysis was also evident. Previous culture had found Pseudomonas aeruginosa and the dog had been treated with enrofloxacin. No bacteria were cultured from the new swab, but there was a pure growth of *Aspergillus fumigatus*, confirming mycotic otitis externa and otitis media. This condition is uncommon but is often associated with immune suppression, otic foreign bodies and, commonly, a history of prior antibiotic use (Goodale, 2016).

A 2-year-old male German shepherd from Canterbury had recurrence of a painful subcutaneous mass on the left thorax, which had initially been successfully treated with amoxicillin/ clavulanic acid 6 weeks previously. Cytology performed at the clinic at that time was reported to show infectious cells (presumed to indicate neutrophils). A swab was collected from the lesion and bacterial culture revealed scant growth of an Actinomyces species most closely resembling Actinomyces *hordeovulneris*. Methods of identifying this organism included matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) at a referral laboratory. This supported a diagnosis of **subcutaneous** actinomycosis. The organism was sensitive to most commonly used antibiotics when tested by the disc diffusion method, including amoxicillin and clavulanic acid, which had been used previously with some clinical success. Actinomyces infection commonly results in pyogranulomatous inflammation and is frequently associated with migrating grass awns that have been inhaled or ingested, or that have penetrated the skin. This can result in abscesses within body cavities, internal organs, the central nervous system and also subcutaneous tissues (Sykes, 2012). It is likely that

the presence of a grass awn at this site resulted in the recurrence of the lesion in this case.

Feline

A 10-month-old neutered male Persian cat from Queenstown was presented for veterinary examination with chronic mucoid, bloody diarrhoea. The veterinarian noted that the cat was small for its age. It had been treated previously with metronidazole and spiramycin antimicrobials and the broad-spectrum anthelmintic fenbendazole. There was no reported improvement following this treatment, but a subsequent change of diet did appear to result in some improvement. PCR testing of a faecal sample did not detect any Tritrichomas fetus genetic material, and ELISA tests for Giardia and Cryptosporidium were negative, but a faecal egg count showed 250 ascarid eggs per gram of faeces, which was surprising given the recent anthelmintic treatment. There was also a heavy growth of a Salmonella species. These findings supported a diagnosis of salmonellosis with concurrent ascarid parasitism.

A 15-year-old tiger from a zoological collection was anorexic and lethargic for 2 months. The clinical signs appeared to respond to non-steroidal and steroidal anti-inflammatory drugs. Serial serum biochemistry and haematology were unremarkable apart from hyperglobulinaemia, with globulin 60 g/L (reference range 21-45). A splenic mass was found on abdominal ultrasound. Biopsies were collected from the mass and the ileocaecal lymph node for histological examination, along with fineneedle aspirate samples from peripheral nodes for cytological examination. Results of both examinations were similar and revealed the presence of neoplastic intermediate to large lymphocytes, consistent with a diagnosis of lymphoma. Non-domestic felids are prone to many of the same neoplastic diseases as domestic cats, with lymphoma reported in African lions and more rarely in cheetahs or cougars. In lions, lymphoma occurs most often in the spleen and a T-cell origin is commonly reported.

Marsupial

Four sick Australian brushtail possums (*Trichosurus vulpecula*) in poor body condition were found on an Otago farm over a 2-month period. One,

which was found emaciated and dull with neurological signs, was killed and necropsied. There were no gross changes at necropsy but histopathological examination of a range of tissues revealed changes consistent with wobbly possum disease in the liver and the brain. The characteristic histopathological feature of this disease is perivascular infiltration with mononuclear cells in multiple organs including the liver, spleen, brain and kidney. This disease was first reported in captive possums in 1995 and later also found in wild animals. A novel nidovirus was identified in affected animals in 2011. The virus is probably endemic in possums in New Zealand. It only occasionally results in clinical signs and death. It is suspected that disease is more likely to be manifest if the possum population is under some type of stress.

Otariidae

A wild New Zealand fur seal/kekeno (Arctocephalus forsteri) was euthanased after being found on an Auckland beach with severe wounds on the hind flippers. There was concern that members of the public might recently have inflicted trauma. Histopathological examination showed that the subcutis in the region of the left groin contained multiple granulomas with intralesional cestodes (presumptive *Diphyllobothrium* and/or Phyllobothrium species). The stomach had a moderate multifocal subacute ulcerative gastritis with numerous luminal nematode parasites presumed to be ascarids (Contracaecum and/or Anisakis species). The airways of the lungs contained numerous nematodes with larvae (presumptive *Parafilaroides* species). The large intestine contained numerous luminal cestodes. Infection with multiple parasites is common in this species. The right flipper had a severe regionally extensive subacute laceration with dermatitis, cellulitis, myositis, granulation tissue and superficial mixed bacteria. The flipper wound was estimated to be at least 3-5 days old, which meant that it had occurred before the seal was found on the beach. The origin of the wounds could not be determined. Natural predators of this species include great white and sevengill sharks, sea lions, orca and leopard seals.

Reptilian

A male grand skink (*Oligosoma* grande) from Auckland had a history

of chronic weight loss and infection of the hemipenes (paired intromittent hemipenis organs characteristic of squamates), and was euthanased. Histopathological examination showed that multiple organs including the liver, kidney, testis, lung, coelom and epicardium contained chronic multifocal active granulomatous inflammation with short acid-fast bacilli (presumptive Mycobacterium species) identified in Zeihl-Neelsen-stained sections. The skeletal muscle (especially in the tail, pelvic and head regions) and kidney had large foci of inflammation with circular cytoplasmic structures (skeletal myofibers and macrophages) of 6-20 round organisms about 2 mm in diameter (presumptive microsporidia). In the hemipenes region there was a bilateral dermatitis with numerous mixed bacteria. These included gram-negative short rods, gram-positive cocci and short rods, and mixed fungal hyphae that were narrow to wide, even to uneven, walled, branching and septate. It was thought likely that the chronic mycobacterial infection resulted in immunosuppression with secondary bacterial, microsporidial and hyphate fungal infections. The skeletal muscles of the pelvic area and tail would have been severely affected, making movement difficult.

Piscine

A school shark (*Galeorhinus galeus*) in an aquarium was euthanased after a bite wound on its tail developed a green colouration and was found to contain a malodorous, gelatinous fluid. Histopathological examination of the lesion revealed severe acute regionally extensive ulceration with haemorrhage, necrosis, cellulitis and myositis, and short curved gram-negative bacilli were present. Photobacterium damselae ssp. damselae (formerly Vibrio damsela) was cultured from the wound. This marine halophilic bacterium causes infections and fatal disease in a wide range of marine animals and in humans. Initially isolated in 1981 as the causative agent of skin ulcers in damselfish, it is a primary pathogen causing ulcers and haemorrhagic septicemia in a number of marine animals including sharks, dolphins and shrimps, as well as in wild and cultivated fish. Most of the reported infections in humans originate in wounds inflicted while handling fish, or are associated with exposure to seawater and marine animals or eating raw seafood.

New Zealand Veterinary Pathology Bovine

On a Waikato property, four out of a mob of 45 Murray Grey cows aged 2 years presented with very light body condition and loose faeces. Faecal samples from three of the animals revealed 50–1,050 strongyle eggs per gram, with the most severely affected animal having the highest egg count. Serology on all four cows was negative for bovine viral diarrhoea virus antigen by ELISA but there was a medium pooled result for *Fasciola hepatica* ELISA, with an estimated 20-50 percent of animals infected. All four affected animals had low serum copper levels (3.1–7.7 µmol/L; reference range 8.0-22.0). A diagnosis of copper deficiency was made, which may have been due to low copper intake or induced deficiency as a result of excessive molybdenum and sulphur intake. This was complicated by **endoparasitism** in these animals.

A first-calving heifer from Waipa presented with a fractured leg. A fresh liver sample was submitted for copper analysis, with a result of 41 μ mol/kg (reference range 95–2,000). **Copper deficiency** is a risk factor for fractures, often of the humerus, in the absence of a history of trauma. Copper is required for the enzyme lysyl oxidase, which is involved in forming collagen crosslinkages. If this process is compromised, bone strength may be decreased.

On a property in South Taranaki, two rising-1-year-old cattle from a mob of 140 presented with lethargy and clinical signs of anaemia. EDTA blood samples from both animals revealed *Theileria orientalis* Ikeda by PCR, leading to a diagnosis of theileriosis.

Two out of four Lowline Angus cows aborted on a lifestyle property in Kāpiti. Examination of one aborted fetus revealed that it was moderately autolysed, with an estimated gestational age of 120 days. No gross lesions were present, but histology revealed foci of necrosis and chronic inflammation in the brain and heart. A presumptive diagnosis of abortion caused by Neospora caninum infection was made. Outcomes of congenital infection with N. caninum range from early embryonic death to abortion to congenital defects to production of a normal calf, depending on the gestational age at infection.

A middle-aged Friesian cross cow from a property in New Plymouth presented with disseminated, variably alopecic and sometimes ulcerated cutaneous nodules 1-4 cm in diameter. Over a period of 4-6 weeks the nodules had spread to cover the entire body. A wedge biopsy from a nodule was submitted for histopathology, revealing effacement of the dermis by closely packed sheets of medium to large lymphocytes with a high mitotic rate (60 figures per ten 400x fields). Neoplastic cells extensively infiltrated the epidermis and follicular epithelium, leading to a diagnosis of epitheliotropic lymphoma. This is a form of **T-cell lymphoma** uncommonly reported in cattle and considered to be a sporadic disease. Advanced disease may progress to involve the lymph nodes and viscera.

Two dairy heifers in Northland were found dead, less than 500 m apart. The animals had breached a farm fence and entered an area containing sodium fluoroacetate (1080) bait stations, which were found to be disrupted and empty. The carcasses were at least 4 days old when skeletal muscle and rumen contents were submitted for 1080 analysis. Although 1080 is highly labile and may not be detected 24-48 hours after death, it was found in all samples in varying levels, up to $0.54 \,\mu\text{g/g}$ in the rumen contents from one cow. These results implicated 1080 toxicosis as the cause of death.

Increased numbers of mature Friesian/Jersey cross cows on a dairy property in Taupo were presenting with mastitis and not responding to treatment. Milk culture from an infected animal revealed moderate growths of both Corynebacterium sp. and a Pseudomonas sp. (not P. aeruginosa). Corynebacterium spp. (including C. bovis) are considered to be opportunistic mammary pathogens that may be associated with suppurative lymphoplasmacytic or mixed inflammatory infiltrates in mammary tissue (Bianchi et al., 2019), but can also colonise the distal teat canal without inducing mastitis. The contribution from the Pseudomonas sp. isolate is uncertain, but some species (especially P. aeruginosa) can form biofilms that reduce antibiotic efficacy (Park et al., 2014). In addition, Pseudomonas spp. can survive in chlorhexidine-based products, and

infections have been caused by using contaminated dry cow therapy tubes and contaminated hot water.

An adult beef cow from a property in Hauraki presented with a very large swelling in the back of the throat, about three times the size of a tennis ball. A biopsy taken for histopathology revealed coalescing pyogranulomatous inflammation centred on aggregates of bacteria forming club colonies. Gram stain revealed indistinct gramnegative bacteria within the club colonies. A presumptive diagnosis of **actinobacillosis** was made.

A 4-month-old Friesian cross heifer calf from Waikato presented with lethargy, pale mucous membranes, anorexia and haemorrhagic scouring. A faecal sample revealed moderate to high numbers of coccidial oocysts, leading to a diagnosis of **coccidiosis**. Coccidiosis primarily causes clinical disease in cattle aged 3–8 months, although younger animals and adults can also be affected.

A 17-month-old dairy heifer from New Plymouth presented with ill-thrift and diarrhoea. A faecal egg count was negative for parasites, and an ELISA test for BVD antigen was negative. Faecal culture revealed a heavy growth of *Yersinia pseudotuberculosis*. **Yersiniosis** is most commonly diagnosed in weaned calves, yearlings and young adults. Clinical disease is more common in winter and spring, with environmental or management stress factors increasing the risk.

A property in Waikato had recurrent problems with pinkeye or conjunctivitis in Friesian cows. Three ocular swabs were submitted for culture, and *Moraxella bovoculi* was isolated from two (identified by the Bruker Maldi biotyping method). While *M. bovis* is historically considered to be the primary agent involved in **infectious bovine keratoconjunctivitis**, *M. bovoculi* and *M. ovis* can also be involved in clinical cases.

Ovine

A submission of mastitic milk from a Waikato property yielded a heavy growth of *Mannheimia haemolytica*. This organism has the potential to cause severe peracute **mastitis** and gangrenous necrosis of the udder, with potential for toxaemia and death. Teat lesions may increase the risk of infection. Six Texel/Suffolk cross lambs were born in Napier with tremors and hindquarter ataxia. The affected lambs showed normal appetite and weight gain, with no progression of neurological signs. A ram lamb was sacrificed at 1 month of age for further investigation. Histology of the central nervous system showed hypomyelinogenesis in white-matter tracts of the cerebellum and spinal cord, consistent with hairy shaker/ border disease, although PCR was not performed to confirm a diagnosis. This disorder is due to congenital infection with **border disease virus**, a pestivirus closely related to BVD virus. Fetal lambs infected after 85 days' gestation are usually born normal, but prior to 85 days there is insufficient development of the fetal immune system and infection typically results in abortion or birth of live lambs with some degree of damage to the nervous system and skin (known as "hairy shakers"). These lambs are immunotolerant to the virus and remain persistently infected.

A property in Taupo had lambs under 12 months of age presenting with clinical signs described as "swayback". Fresh liver from an affected lamb submitted for copper analysis had 53 µmol/kg (reference range 95–2,000), confirming copper deficiency. Swayback or enzootic ataxia is associated with copper deficiency, which decreases the activity of multiple enzymes including cytochrome oxidase. This enzyme is involved in phospholipid synthesis and needed to develop normal myelin sheaths around nerve fibres. Copper deficiency prior to parturition can induce congenital disease, while a deficiency at or after the time of birth results in delayed onset of clinical signs.

Equine

A 9-year-old Thoroughbred mare in Palmerston North presented with clinical signs of toxaemia. The mare had recently foaled. Abdominocentesis revealed opaque, red-to-brown fluid with marginally increased nucleated cells $(1.8 \times 10^9/L;$ reference range $0-1 \times 10^9$), increased RBC $(0.8 \times 10^{12}/L;$ reference 0), and elevated fluid protein (45 g/L; reference range 0-25). Despite the low cellularity, cytology revealed **septic suppurative inflammation** with mixed bacteria. Additionally, there were several large ovoid ciliate organisms characterised by a dense ovoid eccentrically placed macronucleus, multiple internal granules and vacuoles, and bipolar tufts of cilia (**Figure 1**). Based on morphology, these organisms were suspected to be *Polymorphella* sp., and the presence within the abdominal fluid with concurrent septic inflammation was strongly suggestive of **large intestinal** *rupture*. Post-mortem examination confirmed rupture of the colon, consistent with a foaling injury.

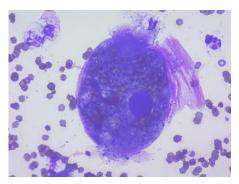


Figure 1: Ciliate organism found on cytology of peritoneal fluid. Its morphology suggests *Polymorphella* (Diff-Quik stain, 500x magnification).

A yearling Thoroughbred colt presented in Auckland with a history of severe weight loss over a period of 4 months, followed by acute colic. On post-mortem examination an abscess-like lesion was identified surrounding part of the jejunum. Histopathology revealed that the lesion was composed of sheets of neoplastic lymphoid cells, which also infiltrated the mucosa, submucosa and muscularis of the small intestine. Scattered epithelioid and multinucleated cells were also noted. Ziehl-Neelsen staining was performed to rule out concurrent mycobacterial infection, and was negative. A diagnosis of intestinal lymphoma was made.

Caprine

A single 4-year-old wether from Kapiti presented with bilaterally swollen carpal joints. Serology was positive for **caprine arthritis encephalitis virus** by antibody ELISA test. This infection is present in many herds within New Zealand, and most commonly leads to chronic arthritis in mature animals. Disease is exacerbated by stress.

Lagomorph

A captive and partially spayed (ovaries retained) 7-year-old female Flemish Giant rabbit from Horowhenua presented with an 8 x 10-cm ulcerated ventral abdominal mass with a small satellite nodule. Histopathology of both lesions revealed a poorly differentiated carcinoma with production of myxoid matrix, presumed to be poorly differentiated **mammary carcinoma**. Mammary neoplasms are relatively uncommon in rabbits, but have been diagnosed in conjunction with uterine adenocarcinoma. It is unknown whether the partial-spaying procedure in this animal had been performed in response to uterine neoplasia. Most mammary tumours diagnosed in rabbits are malignant.

Avian

An adult bantam hen from a backyard situation in Tauranga presented with weight loss; another had died suddenly without clinical signs. A smallintestinal mucosal swab and a piece of fresh liver were submitted for culture and Salmonella Typhimurium was isolated from both samples. This is an important zoonotic infection and is often subclinical in poultry. It is important to maintain good hygiene practices with backyard poultry, including handwashing after working with the birds or their environment, preventing birds from entering the house (especially where food is prepared) and supervising children under the age of 5 to prevent facial contact and ensure hand-washing.

A 1-year-old male little spotted kiwi (*Apteryx owenii*) presented with persistent neurological signs consisting of ataxia and hypermetric gait. Paired serology for *Toxoplasma* was performed, with the first sample yielding an increased titre of 1:1,024, and a second sample 3 weeks later yielding a higher titre of 1:4,096. These results indicated recent *Toxoplasma gondii* infection, which likely explained the clinical signs. *T. gondii* can cause fatal disease in a number of native New Zealand birds (Howe et al., 2014).

A captive male monal (*Lophophora* sp.) in Wellington presented with a prior history of a galvanised intestinal foreign body. Follow-up serum zinc analysis was performed, with a result of 54.6 μ mol/L; levels > 30 are considered diagnostic for **zinc toxicity**. Zinc toxicity in birds can present with a wide range of clinical signs, including weakness and neurological signs, urinary signs, gastrointestinal disease, feather-picking and death.

SVS Laboratories Bovine

Mastitic milk samples from two cows on the same farm in Hauraki were submitted to the laboratory for culture. *Nocardia* sp. was isolated in pure culture from both samples. *Nocardia* mastitis often appears as mastitis that is refractory to treatment. It is often the result of soil contamination of teat tips, udders or milking equipment, or using intramammary preparations.

A heifer from a Waipa farm was euthanased owing to a **fractured humerus**. This was the third heifer in the group to have a broken humerus. The copper level in a liver sample collected at post-mortem was below the detection limit of 50 umol/kg (reference range 95–2,000), confirming **copper deficiency**.

A rising-2-year-old heifer died after being seen straining to defecate the previous day. Two weeks previously, two heifers from the same group had been seen with similar clinical signs and one of them had died. Ruminal acidosis was suspected clinically and a range of fixed tissues were sent to the lab for histopathology. There was neutrophilic rumenitis and reticulitis with ulcerations, which may be seen with ruminal acidosis but also with infectious diseases. Three weeks later a third animal from the same group was dehydrated, with decreased rumination and multiple oral ulcerations. Haematology revealed a marked inflammatory leukogram, with neutrophils $0.7 \ge 10^9$ /L (reference range 1.0-4.6 x 10⁹), band neutrophils 4.2 x 10⁹/L (reference range $0.0-0.3 \ge 10^9$), metamyelocytes $0.6 \ge 10^{9}$ /L (reference level 0.0) and monocytes 3.3 x 10⁹/L (reference range $0.0-1.0 \ge 10^9$). The fibrinogen was also increased, at 12.0 g/L (reference range 2.0-8.0). There was haemoconcentration, with haematocrit 0.52 (reference range 0.26-0.48). Biochemistry revealed a mild azotaemia, with creatinine 164 umol/L (reference range 55–130) and urea 16.5 mmol/L (reference range 2.7-12.3), and albumin was mildly increased at 42 g/L (reference range 25-40), consistent with dehydration. A PCR test on serum detected **bovine** viral diarrhoea virus nucleic acids and a presumptive diagnosis of **mucosal** disease was made. Unfortunately the animal died a few days later and could not be re-tested to confirm persistent infection. No fresh tissues were available

for testing from the animals that had died previously.

Ovine

An aged ram in Waikato was examined for multiple ulcerated skin masses. The ram had been treated with antibiotics but did not respond and the lesions continued to progress. One of the dermal masses was excised and submitted for histopathology. The mass consisted of a round-cell neoplasm infiltrating the dermis and extending into the subcutis. A presumptive diagnosis of **cutaneous lymphoma** was made, but immunohistochemistry to confirm the diagnosis was not performed.

Equine

Uterine lavage fluid from a mare in Waikato was submitted to the laboratory for cytology and culture. No history was provided with the submission. Cytology revealed large numbers of degenerate neutrophils and bacteria, and culture revealed a heavy growth of Nocardia sp., consistent with Nocardia endometritis. This agent can be isolated from the genital tract of healthy mares but is also sporadically associated with infertility, endometritis, placentitis and abortions. A veterinarian submitted blood samples from a 3-day-old filly that had slightly discoloured corneas. The foal was a twin, but the larger twin had died. The foal had not been feeding from the mare but had been drinking well from a bottle. Serum biochemistry showed low globulins (9 g/L; reference range 24-44) and the IgG level was 126 mg/dL (> 800 indicates adequate passive transfer; < 400 indicates failure of passive transfer). Serum amyloid A was 1,708 mg/L (reference range 0-8). A CBC demonstrated a neutropenia (neutrophils 1.2 x 10⁹/L; reference range 4.3-11 x 109) and a left shift with band neutrophils at 0.8 x 10⁹/L (reference range $0.0-0.4 \ge 10^9$), consistent with inflammation and a concern for sepsis. Three weeks later the leukogram had returned to within the reference range. A section of skin had sloughed off from a superficial wound, which was thought to be the portal of entry for sepsis, and the wound was healing well.

Poultry

In early November a veterinarian was consulted regarding increased mortality in a flock of 4-week-old Hyline Brown layer hens, with 0.7–0.8 percent deaths per week over the previous 5 weeks. At postmortem the animals showed signs of septicaemia, with enlarged spleens, pinpoint areas of hepatic necrosis and lesions of valvular endocarditis. Spleens from two animals were sent to the laboratory for culture and **Pasteurella multocida** was cultured from both. This is the causative agent of **fowl cholera**, which causes septicaemia and manifests in flocks as increased mortality, often without previous clinical signs. Subclinical carriers are often the source of infection, but wild birds or mammals can also occasionally carry the organism and introduce the disease in a flock.

Canine

A Border Terrier of unspecified age from Hawke's Bay was brought to the veterinarian with anorexia, dehydration, lethargy and mucoid diarrhoea. The dog had lost weight since the previous visit, from 8.8 to 6.4 kg. In-house chemistry showed hyponatraemia and low creatinine. A faecal sample submitted to the laboratory had 54,950 Trichuris eggs per gram of faeces and *Campylobacter* coli was cultured. In dogs, trichiuriasis, also called whipworm infection, is caused by Trichuris vulpis. The parasites live in the caecum and colon and cause inflammation that can lead to weight loss and diarrhoea, sometimes with fresh blood in cases of heavy infection. Campylobacter is commonly isolated from normal dogs as well as dogs with diarrhoea, and therefore is not always clinically significant, but C. coli is a zoonotic organism that can cause diarrhoea in humans.

Reptilian

Faeces from a bearded dragon (*Pogona* sp.) were submitted for routine parasitology screen and very large numbers of coccidia were found. **Coccidiosis** in reptiles often causes non-specific signs such as poor growth, weight loss and depression, as well as diarrhoea and melena. As coccidia in bearded dragons have a direct cycle, and oocysts can survive for long periods in the environment, these can quickly accumulate if enclosures are not meticulously cleaned regularly.

Zoo animal

A female Siamang gibbon (*Symphalangus syndactylus*) was lethargic and had a tender abdomen. Abundant diarrhoeic faeces were present in the enclosure. Culture of faeces yielded a moderate

growth of *Campylobacter jejuni*. This organism can cause enteritis in nonhuman primates, but can also be cultured from healthy animals. It is zoonotic and a common cause of foodborne illness in humans.

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Quarterly report of investigations of suspected exotic diseases: October to December 2019

Enzootic bovine leukosis excluded

A Southland veterinarian called the MPI exotic pest and disease hotline to report a 4-year-old dairy cow with suspected enzootic bovine leukosis (EBL), after two large masses were identified in the cranioventral neck region. EBL is caused by bovine leukaemia virus (BLV), an agent thought to be absent from New Zealand's dairy herd. A biopsy of each mass was obtained and submitted to MPI's Animal Health Laboratory (AHL) (Wallaceville). Histology identified pyogranulomatous lesions consistent with Actinobacillis ligneiresii infection. Tissue samples were submitted to the Elizabeth MacArthur Agriculture Institute in New South Wales, where they tested negative by PCR for BLV. Serum also tested negative in an EBL ELISA test carried out at the Livestock Improvement Corporation. BLV was excluded and the investigation closed.

In another case, an AsureQuality laboratory manager called the MPI exotic pest and disease hotline to report a seropositive result in a routine pre-export serology test for BLV. The serum was submitted to the Livestock Improvement Corporation for testing with the gp51 ELISA test and found to be negative. EBL was excluded and the investigation closed.

Vesicular disease excluded

A Waikato veterinarian called the MPI exotic pest and disease hotline to report a 4-year-old dairy cow with unusual tongue lesions. There were multiple red lesions on both the dorsal and ventral aspects of the tongue, mostly on the dorsal aspect. Towards the tip and lateral margins of the tongue the lesions were irregular, granulating ulcers, and towards the midline dorsal aspect of the tongue were many circular raised red pustular lesions (Figures 1 to 3). The tongue did not have the characteristic firmness that is typically evident with "woody tongue" (Actinobacillus ligneiresii) infection of cattle. Clinical and epidemiological findings, including photographs, were reviewed and were not considered

Exotic disease investigations are managed and reported by the Diagnostic and Surveillance Services Directorate, Wallaceville. The following is a summary of investigations of suspected exotic and emerging diseases from October to December 2019.

consistent with an exotic vesicular presentation. The cow was sedated and two biopsies were collected from affected areas of the tongue. Fresh and fixed samples were submitted for bacterial culture and histological examination. Histological findings were consistent with *A. ligneiresii* infection, and included multifocal pyogranulomas characterised by aggregates of neutrophils surrounded







Figures 1-3: Irregular, granulating ulcers on the tongue of a Waikato cow. Photos: Suzanne Poland, Vetora Te Awamutu.

by numerous macrophages, including multinucleated cells, and small lymphocytes, and plasma cells. On aerobic culture an Acinetobacter species was isolated, but specific media for the isolation of Actinobacillus spp. was not used. Given that the histological pattern was more consistent with *A. ligneiresii*, while *Acinetobacter* was recovered from affected tissue, findings indicated a mixed infection that likely accounted for the atypical presentation in this case. Response to therapy with parenteral streptomycin was good, with improvement in 3 days and full recovery over 2 weeks. Exotic disease was excluded and the investigation stood down.

A Waikato veterinarian called MPI via the exotic pest and disease hotline after examining a 5-year-old Friesian cross cow with oral lesions, because the signs were potentially consistent with footand-mouth disease (FMD). The cow had presented 5 days prior with upper respiratory tract noise and bright red bilaterally ulcerated nasal mucosa with fibrin present (Figure 4), and ulcers of various sizes limited to the underside of the tongue (Figure 5). No vesicles or vesicular flaps were evident. The cow had been producing milk in normal quantities throughout the illness. Antibiotics had not resulted in clinical improvement. There was no lameness, nor any foot lesions of any kind, and the remaining cows in the mob had no signs of disease. The epidemiological features of this case, combined with the clinical findings and distribution of lesions, enabled initial exclusion of exotic vesicular disease. Possible endemic causes of oral and nasal ulceration in cows include malignant catarrhal fever (MCF), infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD) and hypersensitivities. Serum and whole

blood were collected but a complete blood count and biochemistry panel were both unremarkable. Molecular tests for BVD and MCF virus were negative, but IBR antibodies were detected. However, many dairy cows have antibodies to IBR as the result of bovine herpesvirus-1, so the significance of this is not clear. When the lesions failed to resolve with additional antibiotic treatment, further investigation to identify an endemic cause was conducted. Histopathology of lesion biopsies revealed ulceration with many eosinophils present, indicating that the cause of the rhinitis and stomatitis condition was most likely to be a hypersensitivity reaction. Allergic rhinitis is a sporadic condition caused by reaction to environmental allergens (e.g. pollen or fungal spores), although stomatitis is not commonly reported as part of this syndrome. An exotic cause of the oral and nasal ulcerations was excluded and the investigation was closed.



Figure 4: Nasal ulceration and fibrin exudate from a Waikato cow, which also had oral ulceration. Photo: P. Taylor



Figure 5: Multifocal oral ulcerations in a Waikato cow. Ulcers are well demarcated and confined to the sublingual region, and there is blunting of nearby papillae. Photo: P. Taylor

A Northland veterinarian called the MPI exotic pest and disease hotline to report oral lesions after clinical examination of a 4-year-old Jersey cow that had been losing weight over 3 days. Discrete ulcers were present on the caudal hard palate, dorsal surface of the tongue and on the cheek mucosa. Only one cow in the herd of 300 was affected and there were no other clinical signs consistent with a vesicular disease. A somewhat similar presentation has reportedly been seen sporadically in mature beef cattle in Northland, involving diffuse ulceration of the hard palate. The veterinarian and Incursion Investigator (II) were satisfied that an exotic disease could be excluded, and an endemic aetiology was pursued. At the request of the II, the veterinarian submitted hard palate and tongue biopsies for histology. Histopathological examination revealed pyogranulomatous and eosinophilic inflammation with club colonies affecting both the tongue and hard palate, consistent with Actinobacillus lignieresi infection. While this infection classically involves the tongue ("woody tongue"), it can affect other tissues in the oral cavity and occasionally other sites such as the nares, integument and oesophageal groove (Malmo et al. 2010). The cow responded to treatment. Exotic disease was excluded and the investigation closed.

Pneumonia associated with Mannheimia haemolytica serovar 6

A commercial laboratory pathologist called the MPI exotic pest and disease hotline to report an unusual pneumonic syndrome leading to death in dairy cows, associated with the bacterial pathogen Mannheimia haemolytica. Within the previous 2 weeks, about 15 cows had died on the large dairy farm. A full postmortem was performed on one cow, which had severe fibrinous pneumonia, and M. haemolytica was cultured from the lesions. A previous outbreak of a similar nature had been seen on a *Mycoplasma bovis*-positive farm a few months prior to discovery of M. bovis on that farm.. It is possible for *M. bovis* and M. haemolytica to facilitate disease, in that *M. bovis* is known to compromise respiratory clearance, and M. haemolytica requires entry into the deep lung to be facilitated by viruses or other bacteria. The aim of this investigation was to identify the M. haemolytica serotype

involved, and to attempt to understand whether *M. bovis* might also be involved in the presentation. Sections of affected lung were negative by molecular test for *M. bovis*, but this result was considered inconclusive since it is known that *M. bovis* is not evenly distributed in the lungs. Typing of the *M. haemolytica* showed it to be serovar 6, one of the two most common serovars associated with clinical disease in other countries (Klima et al. 2017). This is the first *M. haemolytica* serovar typing from a New Zealand case that we are aware of, and adds to our knowledge about this syndrome in New Zealand. At the time of this investigation, the *M. bovis* status of this farm was not further determined. Further M. bovis surveillance rounds have been scheduled as part of MPI's programme of work on this pathogen. The relationship between *M. bovis* and M. haemolytica in the pathogenesis of acute pneumonia in this herd is unclear and will be examined with interest in further cases. The investigation was closed.

Bacillus anthracis excluded

A veterinarian in Southland called the MPI exotic pest and disease hotline to report sudden death in two dairy cows. The cows were first-calving heifers (rising-3-year-olds) in a herd of 450. They were found dead with frank blood from the eyes, vulva and anus. Bacillus anthracis, which causes the disease anthrax, is a cause of sudden death with haemorrhage, while other causes can include septicaemic conditions and conditions causing decreased production or increased consumption of clotting factors. Although once present in New Zealand, B. anthracis is now considered exotic. The veterinarian took blood smears from both cows. Initial triage of the case was facilitated by examining photographs of Diff-Quickstained blood smears, which revealed long rod-shaped bacteria consistent with Clostridium spp., but there was no evidence of the abundant blunt-ended rods that would be consistent with B. anthracis septicaemia. All followup samples submitted to the AHL for B. anthracis PCR testing were negative. Cultures of the blood and spleen were unrewarding, with mixed growth of species consistent with environmental contamination and post-mortem overgrowth. No causative factor was found in this case. Comprehensive

examination of cases of sudden death with frank haemorrhage from the eyes and orifices is often complicated by the necessary exclusion testing for anthrax prior to exclusion of other possible causes. This syndrome in New Zealand is often idiopathic, although a comparable syndrome representing a New Zealand case series with an apparent immunemediated aetiology has been published (Rawdon et al. 2017). Exotic disease was excluded and the investigation closed.

Exotic causes of bovine abortion excluded

A commercial veterinary pathologist called MPI via the exotic pest and disease hotline to report an unusually severe abortion storm among dairy heifers in Southland. A total of 283 Friesian/ Jersey cross heifers had been grazing in two equal-sized mobs at a dairy-support property. Over a 3-week period, 39 of them had aborted from both mobs. The heifers were then moved to their home dairy platform, where the abortions continued. At the time of notification, 56 had aborted. Up to this time the heifers aborted without assistance and there were no retained placentas or illness associated with the abortions. All but three of the heifers came into milk even though the abortions were up to 2 months before the planned start of calving. Aborted fetuses ranged from decomposed to fresh. Endemic causes of abortion are many, but it is uncommon to see an abortion storm on this scale in New Zealand. Prior to notifying MPI, the local veterinarians working with the support property and the dairy platform had conducted postmortems on multiple fetuses and submitted fresh and fixed tissues to a commercial laboratory to investigate endemic causes of bovine abortion. No lesions were observed grossly or on histology. Neospora, bovine viral diarrhoea virus, Leptospira spp., fungal agents, Listeria monocytogenes, Salmonella spp. and Ureaplasm diversum were ruled out by serological, molecular and culture methods. Possible exotic causes of abortion include Brucella abortus, Coxiella burnetti, Mycoplasma spp. and Chlamydia spp. Samples were submitted to the AHL (Wallaceville) and these agents were ruled out by pathology and molecular diagnostic techniques. Abortions and stillbirths continued in the heifers until and after the planned start of calving, with some of the heifers

needing assistance. Plant toxicity is still being investigated as a possible factor in this abortion outbreak. Exotic disease was ruled out and the investigation was closed.

Calf mortalities investigated

A Massey University pathologist contacted MPI via the exotic pest and disease hotline to report an outbreak of mortalities in 2-3-week-old mixedbreed calves. Fifteen of 45 calves had died within a 3-week period and many also had scouring. Post-mortem examinations and histology had been carried out on six calves, with no obvious cause of disease identified. However, several calves had unusual histological lesions consisting of cells resembling syncytial cells, along with small-intestinal crypt necrosis. Syncytial cells are created by fusion of cells, often the result of viral infection whose causes reportedly include herpesviruses, adenoviruses, pestiviruses and parvoviruses. Tissue samples were submitted to the AHL where screening for all of these except parvoviruses was performed via available molecular assays, but nothing significant was found. Virus isolation using multiple cell-lines was also undertaken, and did not yield any suspect viruses. The cause of this outbreak was not determined, but an exotic agent was ruled out and the investigation closed.

An Otago farm veterinarian called the MPI exotic pest and disease hotline to report arthritis in a mob of calves. Fifteen out of the mob of 50 had developed non-responsive pneumonia and swollen joints, progressing to death. Other mobs of calves on the farm had no disease. Causes of non-responsive pneumonia and arthritis are many, but one of the major diseases is Mycoplasma bovis, for which New Zealand is currently operating an eradication programme. No other major exotic agents commonly cause this combination of lesions. Before calling MPI the veterinarian had submitted samples for *M. bovis* testing, with negative results. Under the direction of an Incursion Investigator, two postmortems were carried out, with sampling of multiple organs. Synovium and joint taps collected from multiple affected joints all tested negative for M. bovis by PCR. Histopathology showed severe inflammation of the joints sampled, but lesions did not reveal any particular agent and all bacterial cultures

were negative. When a third calf that became sick 2 months after the beginning of the outbreak was autopsied, PCR for *M. bovis* again was negative and no cause was discovered, despite gross and histological evidence of arthritis. Exotic disease and diseases of concern including *M. bovis* were ruled out in this case. Further sampling during the next calving season is planned in case of a recurrence.

Chlamydia pecorum ewe abortions investigated

A commercial veterinary pathologist contacted MPI to report an abortion storm in a mob of ewes where molecular screening at a commercial laboratory had made a preliminary identification of Chlamydia pecorum. This bacterium has been isolated in New Zealand (Mackereth & Stanislawek 2002) and is associated worldwide with sporadic bovine encephalomyleitis (SBE) (Buckle & Ha 2015). C. pecorum is also recognised as a cause of sporadic abortions in sheep, goats and cattle (Schlafer & Foster 2016), and in New Zealand is receiving attention as an emerging cause of sheep abortion (Bingham 2018; Harvey 2019). Exotic causes of abortion in sheep include Chlamydia abortus and Coxiella burnetii; the latter was ruled out histologically by the absence of its characteristic placental lesions. Histologically, the lambs had variable severity of mononuclear encephalitis and myocarditis. Placental lesions included mild multifocal neutrophilic infiltration and small amounts of necrotic debris, although some placentas had no lesions in the submitted section. Molecular testing of lamb brain and stomach contents was negative for Chlamydia abortus, but sequences were highly homologous with Chlamydia pecorum. Paraffin-embedded tissues sent to the University of Sydney were positive by immunohistochemistry for Chlamydia pecorum. This is one of several cases of Chlamydia pecorumassociated sheep abortion seen in the last few years that are being further investigated to develop an understanding of the presentation, epidemiology and impacts. Exotic disease was excluded and the investigation stood down.

Bluetongue virus excluded

A veterinary pathologist notified MPI via the exotic pest and disease hotline of a mortality and reproductive loss event in Northland lambs that had histological evidence of vasculitis. An Incursion Investigator visited the farm to examine the flock, collect records and understand the management of sheep at the property. A significant loss of lambs in the two-tooth ewes in the 2019 season was apparent. Scanning of the two-tooth ewes had found 53 percent with dead lambs. Also, in the two-tooths retained as in-lamb after scanning there was significant loss of lambs between scanning and weaning.

The exotic disease considered of prime concern was bluetongue, caused by the bluetongue virus (BTV). This agent causes vasculitis with high fever, salivation, nasal stertor, swelling of the lips and tongue, coronitis and lameness, and can result in chronic or latent infection in some animals, providing a means of over-wintering. BTV may also be transmitted from mother to fetus, resulting in abortion or stillbirth if incurred in early gestation. On examination of the farm records it was found that poor weaning results were not uncommon and this year had been particularly poor, with 69 percent scanning-to-weaning. Endemic diseases that cause abortion and stillbirth in sheep - toxoplasmosis and Campylobacter fetus - had been poorly managed, with incomplete vaccination of both age cohorts. A high level of potential exposure to feral pigs was noted, with about 250 feral pigs shot on the farm in the current year. Pigs are the reservoir of Leptospira interrogans Pomona, an endemic cause of fetal loss in sheep (Ridler et al. 2015), and they may also prey on lambs.

On examination of the lambs remaining in the flock, there was evidence of facial swelling and disfigurement in a minority of animals. After clinical examination of affected lambs, the cause was identified as parapox (contagious pustular dermatitis, orf, scabby mouth) infection. A number of the ewes also had orf lesions on their teats. Parapox infection can cause vasculitis in sheep, but did not explain the reproductive losses observed at scanning. Two of the worst-affected lambs were euthanased and underwent post-mortem examination, with blood and tissues collected for histology and virology. Bluetongue was excluded on histology, and by competitive ELISA testing and virus isolation from affected lambs. Serology for leptospirosis was carried

out because of the history of exposure to feral pigs. High titres consistent with exposure to *Leptospira interrogans* Pomona were found in both dams and surviving lambs. These findings indicated a significant burden of leptospirosis in the flock, with implications for ewe reproductive performance, lamb mortality and zoonotic risk to farm staff (Ridler et al. 2015). Exotic disease was ruled out and the investigation closed.

Equine infectious anaemia excluded

A Massey University veterinarian called the MPI exotic pest and disease hotline to report a 16-year-old gelding with possible equine infectious anaemia. The horse was icteric, had splenomegaly, was febrile, and had suffered recurrent bouts of anaemia that had responded to intravenous dexamethasone, but to no other treatment. The horse was eventually euthanased owing to the cost of the ongoing intravenous steroid therapy. Blood samples submitted to the AHL (Wallaceville) tested negative for equine infectious anaemia in the agar-gel immunodiffusion test. Equine infectious anaemia was excluded and an immune-mediated cause of anaemia and splenomegaly was suspected. The investigation was closed.

Newcastle disease excluded

A commercial laboratory called the MPI exotic pest and disease hotline to report low-level positive antibody test results for avian paramyxovirus-1 (APMV-1) among birds in a commercial broiler breeder farm. The APMV-1 serotype includes virulent strains responsible for the exotic Newcastle disease (ND), an important OIE-listed disease. The ELISA antibody test used detected suspicious low-level antibodies in two of eight samples. The samples were from a breeder flock in a high-biosecurity facility and there were no indications of disease that could be attributed to ND virus. Repeat sera were tested by haemagglutinin inhibition assay, which is thought to be equally sensitive but more specific than the ELISA test, but it did not detect antibodies in any of the samples. These results, combined with lack of disease in this flock, confirmed that the original ELISA results were false positives and the investigation was closed.

A poultry laboratory manager called the exotic pest and disease hotline

to report seropositive test results in a commercial ELISA test for APMV-1. Sera collected from a laying flock of 70-week-old red shaver birds experiencing respiratory disease and egg-drop were tested in an ELISA panel for various respiratory pathogens. Two of 16 returned seropositive results for APMV-1. Oropharyngeal and cloacal swabs from 20 birds in the affected shed were collected and submitted to the AHL (Wallaceville) for PCR testing. All swabs were negative for APMV-1 and the sera were negative by HI assay. High titres to infectious bronchitis virus (IBV) on ELISA indicated a recent cycle of IBV infection, while co-infection with Mycoplasma gallisepticum was also apparent on serology. The respiratory disease and egg drop observed in the affected shed was subsequently attributed to IBV, with mycoplasmal infection a likely contributor. Exotic disease was excluded and the investigation closed.

Infectious bursal disease excluded

A pathologist called the exotic pest and disease hotline to report histopathological lesions in chicken bursae submitted from a commercial broiler flock. Surveillance for infectious bursal disease (IBD) has been enhanced owing to the discovery in June 2019 of IBD virus in the South Island (Mulqueen 2019). As part of surveillance efforts focused on broiler operations, samples of bursal tissue are collected when bursal changes are seen at processing. Mild histopathological lesions were observed in two of the 10 birds sampled. Fresh bursal tissue samples from these birds had been stored frozen, awaiting the findings of the histological examination, and were submitted to the AHL, where they tested negative in the IBD virus PCR test. IBD was excluded and the investigation closed.

Campylobacter hepaticus confirmed

An academic poultry veterinarian called the exotic pest and disease hotline to report a suspected finding of a new-to-New Zealand organism, *Campylobacter hepaticus*, from a chicken with a condition known colloquially as "spotty liver". Spotty liver has been recognised worldwide since the 1950s and until recently was considered idiopathic (Van et al. 2017). *C. hepaticus* is now recognised as the causative agent and is increasingly being identified in free-range flocks worldwide, including in Australia (Crawshaw 2019; Van et al. 2017). Spotty liver is a bacterial septicaemia resulting grossly in a miliary hepatitis and frequently in death, and has not previously been reported in New Zealand. The current notification was based on isolation of a bacterium that was consistent with C. hepaticus on culture, and was also positive by PCR for C. hepaticus. Based on this and the historical assumption that spotty liver has been present for decades, this detection was attributed to enhanced culture and molecular techniques. The pathogen is likely to be of worldwide distribution and infection seems to be related to husbandry and environmental factors. This is considered a baseline surveillance find and the investigation was closed.

Backyard chicken mortality investigated

A member of the public in Waikato called the exotic pest and disease hotline to report respiratory disease and death in her flock of backyard chickens. Over two and a half weeks 15 birds in the flock of 29 had died, one or two every couple of days, mostly adults but also some younger birds. While the epidemiological picture did not fit Newcastle disease or high-pathogenicity avian influenza, these are two exotic differentials for respiratory distress in chickens. On the day of notification four chickens had died and these were submitted for post-mortem examination at a commercial veterinary laboratory. Postmortem and parasitology findings were not consistent with any exotic disease. A parasitology panel on intestinal contents from two birds showed that both contained coccidia, albeit in low numbers, and intestinal damage was seen on histology in all four birds. Given that these chickens were not being fed a coccidiostat, and the presence of coccidial infection in all birds, it was concluded that coccidiosis was likely to be the cause of death in this flock. Exotic disease was ruled out and the investigation closed.

Exotic ticks excluded

A Hamilton dog owner who called the exotic pest and disease hotline was concerned that ticks she had found on her 18-month-old New Zealand-born Cocker Spaniel could be the exotic brown dog tick, Rhipicephalus sanguineus. She had spent the weekend in rural Waikato and had noticed the ticks after walking the dog through a paddock. The dog's history and location suggested that it had come in contact with the endemic New Zealand cattle tick (Haemaphysalis longicornis) rather than the exotic brown dog tick. Nevertheless the duty Incursion Investigator had the ticks submitted to PHEL (Tamaki). The submission consisted of nymphs that could only be identified to the generic level, as Haemaphysalis sp. (Figures 6 and 7), but this ruled out the genus Rhipicephalus. Molecular testing confirmed the nymphs to be those of H. longicornis. The investigation was closed.



Figure 6: One of several *Haemaphysalis longicornis* nymphs found on a Waikato dog (scale bar = 1 mm). Photo: Emma Scheltema, PHEL Tamaki



Figure 7: *Haemaphysalis longicornis* nymphs found on a Waikato dog (scale bar = 1 mm). Photo: Emma Scheltema, PHEL Tamaki

Canine distemper virus excluded

A veterinary pathologist called the exotic pest and disease hotline to report possible exotic canine distemper virus (CDV) infection in a 6-month-old Huntaway puppy from a Canterbury farm. The puppy had presented to a veterinary clinic with bilateral mucopurulent ocular discharge and hyperkeratosis of all four footpads. Initial serology showed a positive titre for CDV, prompting the call to MPI. Suspicions of CDV were worrying, especially, because the puppy mixed on-farm with a large number of unvaccinated dogs that would be susceptible to infection. At first the puppy's vaccination history was not clear but follow-up questioning of a previous owner produced a history of at least one vaccination. Whole blood and serum were submitted to the AHL, where serology was subcontracted to an overseas laboratory. Antibody titres were >/= 1:80 on ELISA testing, and > 320 in the virus-neutralisation test, both indicating high antibody levels and consistent with recent vaccination and/or natural infection. The high titres precluded the use of repeat testing and rising titre levels to point towards recent exposure. PCR testing at the AHL did not detect CDV virus and the puppy improved slowly. Other in-contact, unvaccinated dogs did not reportedly develop clinical signs of disease, an epidemiological picture inconsistent with CDV infection. Although the results were not clear-cut, the evidence suggested that this dog was not infected with wild-type CDV and the investigation was closed.

Gastrointestinal disease in imported dog investigated

A veterinarian called the exotic pest and disease hotline to report vomiting and diarrhoea in a 10-week-old American bulldog puppy imported a day previously from Australia. The puppy had been vaccinated twice, most recently within 48 hours of travel. A second littermate shipped with the puppy remained clinically healthy. Import Health Standards for importation from Australia do not require quarantine, and the puppy was taken to a local veterinarian by the concerned owner. The vet recognised the potential risk of exotic disease and called MPI. Although the clinical signs observed were not specifically indicative of exotic disease, many infectious agents can cause enteric disease. Enteric clinical signs are not uncommon in puppies, and a workup was initiated to rule out endemic causes of vomiting and diarrhoea. An enteric panel and routine blood tests were completed. Molecular assays on faeces were positive for *Cryptosporidium* spp, Salmonella spp, Campylobacter jejuni and Campylobacter coli. Any one of these agents could explain the presentation, and all are present in New Zealand. The puppy was treated symptomatically with supportive therapy, and recovered. Exotic disease was ruled out and the investigation was closed.

Feline rhinitis associated with Aspergillus udagawae

A veterinary pathologist at a commercial laboratory emailed MPI investigators to report isolating an unusual fungus from a 4-year-old male Domestic Shorthair cat with rhinitis. The fungus was cultured at the commercial laboratory and was determined to be most closely related to Aspergillus felis, an emerging fungal pathogen. A subculture was sent to the AHL where part of the genetic code was amplified by polymerase chain reaction (PCR) and subsequently sequenced. The sequence most closely matched a different and unusual fungal pathogen, Aspergillus udagawae (also known as Neosartorya udagawae). A. felis and A. udagawae are closely related and appear to fill the same clinical niche in causing sinonasal and sino-orbital infections in cats and dogs, although A. udagawae is less common in the cases reported (Barrs et al. 2012). In this case, the veterinary clinician began antifungal treatment and the cat's condition was improving at the time of last report. To our knowledge this is the first confirmation of A. udagawae in New Zealand. This agent and similar related saprophytic fungi are widespread in the environment and this detection is considered to represent a baseline surveillance find for New Zealand. For further details of the case see pages 6-7 in this issue.

Tularaemia excluded

A Massey University pathologist called the exotic pest and disease hotline to report suspected tularaemia in a 1.5-year-old Flemish Giant rabbit from Horowhenua. The rabbit had a 3-4-month history of weight loss, and was euthanased after signs progressed to lethargy, respiratory distress and pale gums. On post-mortem examination, about a third of the spleen was swollen, with pale, pinpoint, raised foci forming larger coalescing areas of necrosis. In a tularaemia-endemic area these lesions would be consistent with infection. Tularaemia, caused by the exotic bacterium Francisella tularensis, affects rabbits but also other species including companion animals and humans, and can be fatal. Rabbits with tularaemia often show multifocal necrotising splenitis and hepatitis, often leading to death. In New Zealand the agents Yersinia pseudotuberculosis

and Y. enterocolitica can cause similar lesions in rabbits and other species, and are considered the main endemic differentials for tularaemia. Yersinia infection is often characterised histologically by the presence of vast numbers of finely granular "pools" of organisms. Microscopically, in this case the splenic lesions did not show this change, and therefore tularaemia was considered a potential differential diagnosis. Bacterial culture of the frozen lesion at a commercial laboratory yielded a low growth of *E. coli*, excluding the involvement of Yersinia and Francisella. E. coli was a surprising find but considered potentially consistent with septicaemia. Exotic disease was excluded and the investigation was closed.

A Canterbury veterinarian called the MPI exotic pest and disease hotline to report sudden death in two pet Flemish Giant rabbits that were vaccinated against rabbit haemorrhagic disease (RHD) virus. The call was made initially because the veterinarian thought there must have been a failure of efficacy of the vaccine against RHD. Post-mortem examination of the rabbits was conducted and they were found to have multiple confluent cream-coloured areas 4-8 mm in size in the spleen, with similar but smaller areas in the mesenteric lymph nodes, and cream-coloured 1-mm nodules in the distal small intestine. These lesions suggested a bacterial aetiology. Culture of the spleen yielded Yersinia pseudotuberculosis, a known pathogen in rabbits, and this was considered the likely cause of the deaths. Having two separate cases submitted independently to the same veterinary practice just a few days apart was likely due to the seasonal nature of versiniosis in some species. An exotic and unwanted organism aetiology of sudden death in rabbits, such as Francisella tularensis, was ruled out on the basis of results from postmortem, histopathology and bacterial culture.

Avian influenza and Newcastle disease in wild birds excluded

An eastern Bay of Plenty lifestyle block owner called the exotic pest and disease hotline to report a mortality event involving wild birds, including sparrows, mynahs, blackbirds, thrushes, collared doves and spotted doves, that were fed daily on her property. Up to 200 birds could be on the property at a time and it was not unusual to find the occasional dead one, but not on the scale reported. Over the previous 10 days, nine Malay spotted doves (Streptopelia chinensis) and one thrush had died. Birds would be observed hunched in the grass and next day found dead. Choanal and cloacal swabs from two doves were submitted to the AHL, where the exotic differentials avian influenza and Newcastle disease were ruled out by PCR testing. Postmortem examinations of two spotted doves were conducted at Wildbase Pathology at Massey University. On gross postmortem both birds were assessed to be in poor to moderate body condition, with one having a slightly enlarged liver and a moderately enlarged spleen. Histology of liver, spleen and intestinal serosa in both birds showed largely non-suppurative inflammation along with the presence of numerous intralesional organisms consistent with Chlamydophila. PCR testing on a dry swab of the liver at a commercial laboratory confirmed Chlamydophila, and specific PCR testing conducted at the AHL confirmed the presence of Chlamydophila psittaci. Birds are the primary hosts of C. psittaci, and associated disease (avian chlamydiosis) has been identified in many wild bird species but particularly in columbiforms, including doves (Beckman et al. 2014). A previous outbreak in spotted doves in New Zealand has been described (Rawdon et al. 2010).

Possum neurological disease investigated

A private veterinary practitioner called the exotic pest and disease hotline to report unusual neurological signs in several feral possums (Trichosurus vulpecula) on an Otago farm. Affected possums were found dazed on the ground, in daylight, and were ataxic and unable to escape when approached. Post-mortem examination of an affected possum was conducted and samples were collected for histology and toxicology. Histopathological examination revealed lesions of mononuclear inflammation in the brain, myocardium, kidney and lung, consistent with chronic wobbly possum disease. This disease is caused by a nidovirus and is considered endemic in possums in New Zealand. It was first identified and reported in caged possums at Invermay in 1995 (Dunowska et al.

2013; Giles et al. 2016). Exotic disease was excluded and the case closed.

Exotic bee mites excluded

An AsureQuality Apiculture Technical Adviser contacted MPI via the exotic pest and disease hotline regarding a private beekeeper's report of a bee with unusual mites on the abdomen. The exotic Tropilaelaps mites (T. mercedesae and *T. clareae*), are an emerging threat to European honeybees, and were considered as potential differentials (Chantawannakul et al. 2018). Mites submitted to entomologists at the PHEL (Tamaki) were identified as Neocypholaelaps novaehollandiae, a common species first reported in New Zealand in 1963. They feed on nectar and honey and use insects as a means of moving between flowers. The specimens were all adult females, which is the common dispersal stage for this family of mites. Exotic Tropilaelaps spp. were ruled out and the investigation stood down.

A bee scientist called the exotic pest and disease hotline to report a mortality event involving a single hobbyist colony in Wellington. A sample of bees was collected and submitted to the PHEL (Tamaki) for examination for *Acarapis woodi* (tracheal mite) by tracheal dissection. No tracheal mites were identified and an exotic component to the presentation was excluded. The colony gradually recovered, although the cause of the mortality event was not determined.

Israeli acute paralysis virus excluded

An AsureQuality Apiary Officer called the exotic pest and disease hotline to report mortality affecting multiple hives in Northland. Colony deaths were not attributable to starvation or reproductive failure but were considered potentially consistent with the exotic agent Israeli acute paralysis virus (IAPV). The commercial beekeeper was a very experienced operator with a low post-winter mortality (< 2 percent of hives) but this winter had experienced significant death of bees and queens in 18 percent of wintered hives. A sample of bees was collected and submitted to the AHL for exclusion of IAPV. Samples were negative on PCR testing for IAPV, but positive for Nosema ceranae. The presence of N. ceranae in sick bees is

not necessarily the cause of disease, because it is a ubiquitous fungus. Fungal microsporidia are transferred among bees via the faecal-oral route, and within the hive the spores can be airborne (Sulborska et al. 2019). In regions where N. ceranae is present along with a reasonable density of bees competing for pollination (as is expected in commercial beekeeping), we can expect a reasonable prevalence of gut carriage in bees. Knowledge of the distribution and expected density of *N. ceranae* within New Zealand and within beehives is still work in progress for our apiculture surveillance system. Examination of the varroa treatment history of the beekeeping operation found that mortality was correlated with the use of a newly registered formic acid fumigant. Fumigation had resulted in extensive corrosion of metal objects such as queen excluders in the hive, indicating high levels of formic acid that can result in mortality. The use of formic acid, as distinct from contact insecticidal products, is recent in New Zealand, and where environmental temperatures and humidity are high there is greater risk of toxicity. The beekeeper was instructed to complete an adverse event form for MPI's agricultural compounds and veterinary medicines (ACVM) group. Exotic disease was excluded and the investigation closed.

An Auckland beekeeper called the exotic pest and disease hotline to report finding three dead hives out of 23 on four apiaries. Piles of dead bees were noticed by the manager of the orchard where the first one was placed, and subsequent checks indicated the other two were affected, all within 2-3 days in mid-October. An AsureQuality Apiary Officer was enlisted to help investigate. During springtime agricultural spraying is common, so toxicity was suspected as the primary cause. Exotic disease can also cause mass mortality of adult bees, with the major differentials being tracheal mite and Israeli acute paralysis virus (IAPV). Diseases present in New Zealand, such as Nosema, have also been associated with mortalities of adult bees. Samples of bees were collected and frozen and submitted to the PHEL for rule-out of exotic and endemic diseases and pests. A sample was also sent to a subcontracting laboratory for toxicological screening. Tracheal mite and IAPV were excluded by dissection

and molecular assays respectively. Multiresidue toxin panels were negative for all toxin analytes, including common pesticides and fipronil. Nosema testing showed the presence of Nosema (not speciated) at one orchard and Kashmir bee virus at another. Both of these agents are relatively common throughout New Zealand, and being inconsistently found among the orchards tested, were not thought to be the cause of mass mortality in this instance. No cause of the mortality was determined, but important exotic diseases and common pesticides were ruled out. The investigation was closed.

Small hive beetle excluded

An AsureQuality Apiary Technical Specialist called the exotic pest and disease hotline to report finding beetles and larvae in a mid-Canterbury apiary with two hives, one of which had failed. Infestation of the exotic small hive beetle Aethina tumida was considered a potential differential diagnosis. Examination of the bottom board of the failed hive revealed larvae (Figure 8) and beetles among digested wax, and these were submitted to PHEL (Christchurch). The beetle was identified as Saprinus detritus, the carrion pill beetle, (**Figure 9**), while the larvae were identified as Calliphora vicina (European blowfly), both present in New Zealand. These insects were attracted to the failed hive by the presence of decaying bees and hive products. An exotic disease incursion was ruled out and the investigation closed.



Figure 8: Larvae of the European blowfly (*Calliphora vicina*) among digested wax on the bottom board of a failed mid-Canterbury hive. Photo: Chantel Rich, AsureQuality



Figure 9: Carrion pill beetle, *Saprinus detritus* (Coeloptera: Histeridae) from a failed mid-Canterbury hive. Photo: Carol Muir & James Haw, PHEL Christchurch

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MARINE AND FRESHWATER

Quarterly report of investigations of suspected exotic marine and freshwater pests and diseases: October to December 2019

Diseased scampi, Chatham Rise

During routine scampi surveillance on the Chatham Rise, nine scampi (Metanephrops challengeri, Balss, 1914) out of 4,500 (0.2 percent) showed signs of "white scampi". This is a syndrome where freshly caught scampi appear pale in colour instead of the normal pink and red. Five of the scampi were fixed individually in 10 percent formalin, as well as five that appeared healthy. MPI was notified and the 10 scampi were submitted to the Animal Health Laboratory (AHL). Histology revealed that the healthy scampi had no abnormalities, but the five diseased animals had moderate to marked microsporidiosis in the skeletal and cardiac muscle. This was consistent with infection by Myospora sp., a microsporidian parasite endemic to New Zealand. As there were no biosecurity issues, the investigation was stood down.

Perkinsus in greenshell mussels

MPI was notified of chronic lowlevel mortality in greenshell mussels (Perna canaliculus) being held for research. The mussels had been held in three different environmental regimes, only one of which was experiencing mortalities. Histological testing at the AHL showed the presence of *Perkinsus* sp. at low levels in five of 10 animals and the species was identified as *P. olseni*. This species is endemic in New Zealand and has been previously detected in mussels in several areas of New Zealand. The results were conveyed to the notifier, added to the OIE quarterly report, and the investigation was stood down.

Paua mortality, Kaikoura

A new pāua (*Haliotis iris*) facility notified MPI of high mortality in adult broodstock collected locally. All animals were about the same size (125–135 mm). When collecting samples, they are sexed by pulling up the frill to check the colour of the gonad. While doing this, damage can occur and cause stress to the animals. Exotic marine and freshwater pest and aquatic disease investigations are managed and reported by Diagnostic and Surveillance Services Directorate, Wallaceville. The following is a summary of investigations of suspected exotic marine and freshwater diseases and pests during the period from October to December 2019.

The facility is maintained at a constant temperature of 14-15°C and the water had been run through the system with no animals for 7 days before the broodstock arrived. All water parameters were checked and were within normal range, and the stocking density was low. Some of the paua had been induced for spawning a few days after entering the facility, and both induced and un-induced animals were affected by mortalities. Sick animals were slow to respond to stimuli and did not right themselves. After all the sick animals were moved into a quarantine tank, a peak of mortalities occurred. Four days after the mortalities had begun to subside, a failure with the cooler in the facility caused the water temperature to rise by almost 2 degrees, to 16.5°C, and there were further mortalities.

Samples of animals from both mortality peaks were submitted to AHL for disease testing to rule out the endemic Perkinsus olseni, the exotic abalone virus gangleoneuritis virus (AVGV), and withering syndrome. Additionally, a general health screen was performed using histology, which microscopically examines sections of tissue for signs of disease. Ten representative animals were tested. AVGV, P. olseni and withering syndrome were ruled out by specific PCR, culture, and histology respectively. Histology revealed changes to the haemolymph, which are commonly associated with slow haemolymph flow, decreased muscle tone and possibly an increase in haemolymph proteins, which are all likely to be associated with stressors or underlying disease. In five of these pāua, bacteria were present in the gills, and in two of them bacteria were present in the haemolymph.

Pāua from the second mortality peak showed dominant growth of two

indigenous *Vibrio* spp. (most likely *V. splendidus* and *V. tapetis*) and histology revealed an active bacterial infection in the vascular channels, digestive gland and tubular kidney. These bacteria are abundant in the marine environment and several species, including *V. splendidus* and *V. tapetis*, can be pathogenic to aquatic animals. No other pathogens were detected by histology or bacteriology.

The cause of these mortalities was most likely multifactorial. The reason for the first peak of mortalities remains unknown but it is thought to have been due to a stress response induced during capture and confinement (including handling and induction of spawning). The second peak of mortalities occurred after a temperature increase in the facility. Even a change of just one Celsius degree can stress aquatic animals, reducing their immunity, providing an environment for the proliferation of bacteria and leading to disease. The cause of these mortalities was suggested by histology and bacteriology, which showed an active infection of Vibrio spp. and inflammation in the tissues around the foci of bacteria. Results were communicated to the facility, along with information on ways to reduce future stress and thus infection. As testing revealed no biosecurity concerns, the investigation was stood down.

Ascidians identified

MPI was contacted by a member of the public who had found several long red fleshy organisms washed up on a beach near Manganese Point, Whangarei Harbour. Photos were sent to the Marine Invasives Taxonomic Service (MITS) for identification as a sample could not be collected. A MITS taxonomist identified the organisms as the native colonial ascidian *Synoicum kuranui*, which is known to occur in Whangarei Harbour. *S. kuranui* often forms into a long sausage-shape in areas of high current.

Mass mortality of fish, Matarangi Beach

A member of the public reported a mass mortality of fish washed up on Matarangi Beach, Coromandel Peninsula. Photos were requested, which confirmed that the fish were jack mackerel (Trachurus sp.), all of a similar size. The AHL sent a sampling kit to the notifier to collect samples for rule-out of exotic disease, and Fisheries NZ was also notified in case this might be a dumping incident. The Waikato Regional Council was also notified. However, when the samples were received at the AHL they were of poor quality owing to transport delays, and no testing could be carried out. The results were inconclusive, but the possibility that the mortality event was caused by predators chasing prey on to the shore, or a dumping of unreported commercial fish, cannot be excluded.

Salmon mortalities at fish farm, South Island

A land-based salmon farm reported that more than 200 salmon had died over a 5-day period. The salmon farm had recently received a new transfer of live fish and considered it likely that poor transfer conditions, including a large amount of scale loss, was the cause of the mortalities. All transfers of new fish to this facility were put on hold while the dead fish were tested at the AHL to rule out exotic aquatic disease. Histology and bacteriology results were negative and transfer authorisation was renewed because the biosecurity risk appeared to be low.

Green liver in kahawai

MPI was notified after one of four kahawai (*Arripis trutta*) caught near Lake Ferry, Wairarapa, was found to have a mossy-green liver. The notifier said that apart from the colour, the liver was normal in appearance. Green liver syndrome has been reported in farmed fish as a sign of nutritional deficiency. However, the sample was too degraded for analysis and the case was closed as inconclusive.

Unusual jellyfish washed up on Gisborne beach

The MPI Communications team was contacted by a news reporter in Gisborne requesting information about jellyfish. A large number of jellyfish had washed up in the area over the previous weekend. Photos were sent to MITS for identification and an expert in Australia tentatively identified them as *Desmonema gaudichaudi* (Cnidaria: Scyphozoa), the speckled or spotted jellyfish. MITS was unable to confirm the identification without a specimen but *D. gaudichaudi* is indigenous to New Zealand and no biosecurity risk.

Lesions on mussels, Coromandel Peninsula

A member of the public found several greenshell mussels (Perna canaliculus) with unusual reddish orange growths on the gonads, and sent photos to a NIWA fisheries scientist for advice. NIWA was unable to rule out disease by examining the photos and contacted MPI. The mussels had been collected near Whitianga, and had been steamed and frozen before MPI was notified. As the cooking process had likely destroyed the causative agent, fresh samples were required to identify it. Several attempts were made to contact the notifier for more information but they did not respond.

Several days later another member of the public reported unusual orange/red "blisters" on the gonads of greenshell mussels collected at Whangamata. The photos showed signs that were very similar to those in the earlier notification, and samples were requested for laboratory testing to rule out exotic disease. The mussels had been collected at the mouth of a tidal estuary where the water is constantly moving, and the water temperature at the time was 18°C. Two affected mussels were tested and both had a marked trematode infestation of the gonad, most likely caused by Cercaria haswelli. This condition has previously been reported in P. canaliculus from both the North and South Islands, and may be common in some parts of New Zealand (M. Bradstock, pers. comm.). As testing revealed no biosecurity concerns, the investigation was stood down.

Suspect sea squirt, Coromandel

A member of the public called MPI to report a suspect *Clavelina oblonga* sighting near Coromandel town. This potentially invasive ascidian was first reported from Great Barrier Island, in May 2019. Five sites (including the initial site of detection) near Coromandel town were intensely investigated by divers but no *C. oblonga* were found. The relevant regional councils, MPI's Biosecurity Response team, and local mussel farmers were notified and the investigation was stood down.

The 2019 find was referred to the Response group and a delimiting survey was carried out which showed it was widespread in Smokehouse Bay, Great Barrier, at least 1.5 km out from the initial detection site. Owing to its limited distribution, it is important to know whether this organism has spread.

New to New Zealand bryozoan, Auckland

NIWA detected a new to New Zealand bryozoan species while carrying out an ecological survey in Viaduct Harbour, Auckland, and reported the find to MPI. The bryozoan appeared to be Nevianipora sp., a tropical genus not previously known to be present in New Zealand. A UK-based taxonomist has suggested it may be N. pulcherrima (Bryozoa: Diaperoeciidae) based on skeletal morphology. However, there is poor systematic resolution in this bryozoan family. A Marine Exotic Species Note (MESN) was prepared by NIWA with more detailed information. NIWA believes it is likely the species will become established as the temperature range in northern NZ is similar to that of its native range. However, the impact is likely to be low as none of the species in this family are listed as pests.

Unusual alga growing on mussel lines, Coromandel

A Coromandel mussel farmer reported an unusual alga growing on mussel lines. The algal growth was believed to have inhibited the settlement of mussel spat on lines in the upper 3 metres of the water column. Photos were sent to MITS and the alga was identified as *Colpomenia* sp. Four species of *Colpomenia* are present in NZ, including one non-indigenous species, so a sample was requested for rule-out. However, over the Christmas period the alga disappeared from the lines. The notifier will notify MPI again if it re-appears.

Large number of small jellyfish near Auckland

A member of the public notified MPI after she observed large numbers of small jellyfish around the Whangaparoa Peninsula over the course of a week. The notifier was concerned the jellyfish might be an exotic organism and dangerous to human health, and she sent photos to Biosecurity Surveillance and Incursion Investigation Aquatic & Environment Health for identification. Following a conversation with the notifier, the jellyfish were tentatively identified as crimson jellies (Turritopsis rubra), a common, fast-moving jellyfish species that proliferates in large numbers in coastal North Island areas. The species is not known to sting humans. The notifier was sent a digital copy of NIWA's "jiggling jellyfish" identification guide (Macpherson & Gordon 2019).

Mass fish mortality, Kawau Island

MPI received 11 notifications from members of the public after large numbers of dead fish were seen floating near Kawau Island in the Hauraki Gulf from 12 to 14 December 2019. The dead fish were identified from photos as blue mackerel (*Scomber australasicus*). Fisheries officers were notified and collected samples on 13 December but these were in an advanced state of decay, suggesting the fish had already been dead for several days.

Two members of the public sent samples to the AHL for analysis but both samples were in poor condition when they arrived and thus unsuitable for bacteriology and histology. However, AHL scientists were able to extract DNA from gill material and test it for pilchard herpesvirus, which periodically causes large mortalities of pilchards (*Sardinops sagax*). It is unclear from the literature whether blue mackerel are susceptible to this disease, but the samples tested negative for pilchard herpesvirus. Fisheries NZ supplied information suggesting the mortality might be related to commercial fishing. A purse-seine vessel fishing in the area six days prior to the first notifications had reported that gear failures over two successive days had resulted in the loss of about 4 tonnes of blue mackerel.

Exotic bridled goby, Great Barrier Island

MPI was notified that a bridled goby had been found in the Kaitoke wetland at Great Barrier Island. The fish was caught in a fyke net set overnight and retrieved the following day. A photo of the fish was sent to MITS and confirmed to be the Australian bridled goby, Arenigobius bifrenatus (Kner, 1865). This is a burrowing coastal and estuarine goby from temperate Australia. The first record of this species in New Zealand from in the Whangateau Harbour in 1998. It is thought to have arrived accidentally in shipping ballast water. As this is not a new to NZ species and is a range extension, the investigation was closed and DOC was notified of the detection.

Suspected wasting disease of seagrass, Auckland

A member of an estuary environmental group notified MPI about large patches of dead seagrass seen while photographing seagrass (Zostera muelleri) beds at St Heliers Beach, Auckland. Her personal research indicated the cause might be wasting disease caused by the marine slime mould Labyrinthula zosterae. This organism is established in NZ and has caused significant declines in seagrass beds in the past, for example at nearby Okahu Bay in the 1960s. Labyrinthula zosterae has previously been detected in healthy seagrass beds, but blooms may occur when the plants are stressed and conditions are favourable (e.g. low light, high temperatures and high salinity). Seagrass beds are also sensitive to changes in environmental conditions and are known to change over time. As the cause appears likely to be either environmental conditions or an organism already present in NZ, the decline is outside of the scope of MPI's remit.

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PLANTS AND ENVIRONMENT Plant health surveillance and incursion investigation report: October to December 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 (IIs) 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.

Incursion Investigators received 369 plant and environment notifications during the 3-month period from October to December 2019 (**Figure 1**), continuing the trend of increased post-border notifications compared to the same quarter in 2018 (309). Investigators immediately stood down 90 cases because biological risk was ruled out, and 22 more cases were redirected to other agencies responsible for managing the pest concerned. The resulting 257 cases that required further investigation to rule out a biological risk, represented a 20 percent increase compared with the same quarter in 2018 (207). Significant cases involving pests of concern commonly notified this quarter included a variety of seed with dubious import pathways and the brown marmorated stink bug.

Seeds not compliant with Import Health Standard

MPI has a multi-layered biosecurity system to deal with risk. This includes import risk analysis, international agreements, permits, diagnostics, Import Health Standards (IHSs), pathway risk analysis, detector dogs and passenger and mail inspection. Despite the robustness of the system, not all risk goods will be detected; for example e-commerce trade in seeds is a difficult pathway to manage.

The Biosecurity Surveillance & Incursion Investigation Plant Health (BSIIPH) team investigated 28 seed cases this quarter and investigators concluded only five were negative for the presence of a biological risk. To protect our primary industries from exotic pests and diseases and minimise costly eradication events, the importation of seed into New Zealand is regulated by the applicable IHS and non-compliance may result in prosecution.

Seed and nursery stock can be imported into the country only for species approved by MPI, listed on the Plants Biosecurity Index (PBI) and compliant with the IHS 155.02.05: Seeds for Sowing. Packages must have a clear customs declaration describing the contents and identifying the species, which must not be listed in Schedule 1 or 2 of the Trade in Endangered Species Act 1989 unless accompanied by the appropriate permits from the Department of Conservation (DOC). In addition, packages must display the scientific name and be free of any weed seeds or other contaminants such as soil. Non-compliant seeds often arrive via the e-commerce pathway from China, the US, UK, Greece, Egypt and Australia. MPI is often notified via social media networks of potentially incorrectly imported vegetable seeds and ornamentals being traded in New Zealand. All cases are investigated and, in most instances, the seeds and

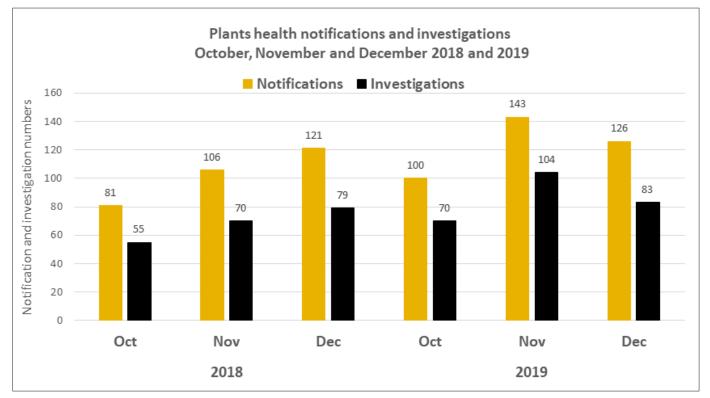


Figure 1: Plant health notifications, investigations and other outcomes, October–December 2018 and 2019

any resulting plants are destroyed. Some example are provided below.

Seeds from an overseas website discussed on Facebook

A member of the public alerted MPI to a Facebook posting where suspected illegally imported seeds were being grown in pots. Using MPI's Intelligence Team, the II obtained contact details of the person of interest (POI) and determined that 44 packets of "edible seeds" had been ordered from the Chinese website "Wish". Only one seed line sprouted, which was supposed to be sugarcane but the plant did not look like it so the POI sought advice from Facebook plant hobbyists. In response, Facebook members warned the importer of the biosecurity risk posed by imported seeds from non-reputable sources.

Apparently, the packet description was in Chinese script and the POI was unsure what it said or whether botanical names were provided. One packet was simply labelled "persimmon". The POI had previously received a letter from MPI relating to other non-compliant seed imports that had been seized at the Auckland International Mail Centre (AIMC). Her explanation for planting these seeds was that "since they were delivered they must have undergone biosecurity clearance at the border". All the potted plants were destroyed with the assistance of Horizons Regional Council (HRC), Taumarunui. It is frustrating that even though members of the public are advised by MPI how to correctly import seeds, they continue to purchase from overseas websites, thereby risking New Zealand's biosecurity.

Cucamelon seeds sold on Trademe

A member of the public who had previously been investigated for importing cucamelon seeds alerted MPI to a Facebook discussion about "recently purchased" cucamelon (Melothria scabra, Cucurbitales: Cucurbitaceae) seeds being sold on Trademe. Cucamelon, also known as dragon's eggs or Mexican sour gherkin, is not listed on the PBI. It is not known to be present in New Zealand and therefore is a suspect new organism under the Hazardous Substances and New Organisms (HSNO) Act 1996. Since the notifier's originally imported cucamelon seeds had been destroyed, there was concern as to the

origin of the new Trademe auction. Facebook members were contacted and a subsequent phone interview with the new seller revealed the seeds had been purchased earlier in the year and not recently as implied. With the assistance of Trademe administrators, the investigation determined the seeds had been purchased in the earlier auction but were not known about during the earlier investigation so had not been seized. Two potted plants and the remaining seeds were incinerated by the owner.

In comparison to the previous seed investigation, the notifier in this case had taken on board the seriousness of biological risk associated with importing and growing seeds and co-operated with this investigation. While online trading of risk goods creates much work for plant health investigators, the forum can also work in MPI's favour by educating a wide audience.

Brown marmorated stink bug investigations

New Zealand is on red alert for an invasion of the brown marmorated stink bug or BMSB (Halyomorpha halys), considered one of this country's greatest biosecurity risks. It feeds on a wide variety of plants, including pipfruit, stonefruit, grapes and kiwifruit. If established here, it could potentially feed on all our fruit and vegetable crops. Since 2018, farmers across Europe have reported record crop devastation, in some instances so high that the cost of production is not viable. The bugs are very good at spreading on agricultural machinery, vehicles and inanimate goods and this "hitch-hiker" behaviour poses the greatest risk of the bug reaching New Zealand. For example, in 2018 there were 17 male and 9 female live stinkbugs accidentally imported with a pair of shoes from West Virginia, USA. Fortunately the purchaser was aware of BMSB and immediately called the MPI exotic pest and disease hotline (0800 80 99 66).

During this quarter 60 suspected BMSB cases were reported and an investigation was initiated for each one, resulting in 20 positive BMSB identifications where urgent measures were undertaken by the II to mitigate the risk of establishment. A further 37 cases investigated were negative for BMSB (see **Table 1**), which illustrates the species diversity, region and host range of notifications

received that were negative for BMSB but still required investigation. BMSB notifications are treated with urgency and some positive cases are described below.

Suspect BMSB, Christchurch Airport

A solitary live suspect BMSB was reported to MPI by Qantas staff at the oversized luggage belt in the Christchurch International Airport terminal. At the time of detection the insect was sitting on the strap of a passenger's backpack. The duty Chief Quarantine Officer (CQO) was notified and arranged submission of the bug for identification to PHEL, where it was confirmed to be a female BMSB, non-reproductive and unmated. The traveller was an American citizen en route to Asia via Australia after travelling through New Zealand. The investigation revealed that after spending one night in motel accommodation on arrival in Christchurch, he spent the following 17 days travelling in a campervan around the South Island. For most of this time he was accompanied by his son, who arrived in Queenstown from South Australia. They travelled to Gore Bay, Arthur's Pass, Mt Cook, Wanaka, Queenstown, Curio Bay, Portobello, Temuka and Akaroa, staying in camping sites and sleeping in the campervan and a tent the son had brought with him from Australia.

Investigators contacted the father and son and inspected their belongings for BMSB. People associated with the facilities they had used during their trip were contacted and BMSB pamphlets were provided for staff and for public awareness. The campervan was inspected and nooks and crannies sprayed with an insecticide as a precautionary measure. In addition, the vehicle was sealed and a can of insecticide applied to the interior. On inspection no BMSB were found. The camping equipment was inspected and frozen for a week at MPI and no BMSB were found. It was possible that the BMSB might have arrived at the airport in other luggage and just happened to find its way on to this passenger's backpack.

Live BMSB in suitcase ex Italy

MPI was notified of a suspect BMSB found among the luggage of a family returning to New Zealand after 3 months in Emilia Romagna, northern Italy, where they had observed large numbers

Table 1: Suspect live BMSB notifications investigated and determined to be negative (36 native and one exotic species)

negative (36 native and one exotic speci Species	Region	Host
Native to New Zealand	Keylon	11051
Coreid bug, Acantholybas brunneus	Auckland	Aluminium furniture
Brown soldier bug, <i>Cermatulus nasalis</i>	Auckland	Commercial nursery
	Auckland	Tie-down straps
	Awakeri	Kiwifruit orchard
	Hamilton	Residential kitchen
	Paengaroa	Kiwifruit orchard
	Papamoa Beach	Garden
	Port of Tauranga	Chair
	-	Kiwifruit packhouse
	Tauranga	Kiwifruit orchard
	Tauranga	
	Tauranga	Garden centre
	Tauriko	Kiwifruit orchard
	Te Puke	Curtain
	Waimauku	Persimmon orchard
	Wellington (Lower Hutt)	Vehicle
	Wellington	Vehicle
	Whangarei	Farm
	Whakamarama	Avocado orchard
	Auckland	Air conditioning units
	Auckland	Silver beet
	Auckland	Vehicle
	Auckland	Mail
Brown shield bug, Dictyotus caenosus	Christchurch	Pallet at Transitional Facility
	Invercargill	New farm machinery
	Pukekohe	House doorstep
	Temuka	Holiday park
	Te Puke	Kiwifruit orchard
	Te Puke	Lawn
Native bronze beetle, Eucolaspis sp.	Auckland	Aeroplane cabin
Pittosporum shield bug, <i>Monteithiella humeralis</i>	Christchurch	Hotel
	Nelson	Residential house
Green vegetable bug, Nezara viridula	Lower Hutt	Roof racks
Schellenberg's soldier bug, <i>Oechalia</i> schellenbergii	Marlborough	Plant
Forest shield bug, Oncacontias vittatus	Tauranga	Ceramic plant pot
Family Reduviidae	New Plymouth	Vehicle
Exotic to New Zealand		
Western conifer seed bug, <i>Leptoglossus</i> occidentalis	Christchurch	Office at Transitional Facility

of stink bugs on window shutters at their accommodation. A family member and entomologist had alerted the group to the possibility of live insects hitchhiking in their personal belongings and advised taking extra care when packing. On arrival into New Zealand, the family specifically asked border clearance staff to inspect their luggage because they were aware of the heightened risk. Quarantine Officers (QOs) at Auckland International Airport found one stink bug, which was identified by a PHEL entomologist as *Acrosternum* sp. (Hemiptera: Pentatomidae), not known to be present in New Zealand.

As the QO found no further insects, the luggage was released to the family. On returning to their homes in the Bay of Plenty and Waikato, in closed rooms family members unpacked their luggage and found two and three suspect BMSB respectively, which they placed in the freezer. The insects were submitted to PHEL who identified a male and female BMSB (in the Bay of Plenty case); a male and female BMSB and one Acrosternum heegeri (in the Waikato case). This investigation highlights the elusive nature of this pest. When BMSB have been found in clothing, all items have been inspected and sometimes washed in hot water. If the II believes any risk remains, the risk goods are fumigated.

Positive – Biosecurity response initiated

The complexity and biosecurity risk associated with some notifications results in the investigation being transferred to MPI's Response Group who, with the assistance of the IIs and PHEL, conduct responses to eliminate, reduce or contain the threats and potential impacts of biosecurity incidents.

Dodder seed contamination in clover seeds from Egypt

AsureQuality (AQ) notified MPI that Cuscuta sp. seeds were found as a contaminant in a 5-tonne shipment of berseem clover (Trifolium alexandrinum) from Egypt. The shipment arrived in late December 2018 and was cleared by MPI in early January. The importer requested germination and purity tests from AQ for the new sowing season. The seed contamination level detected by the purity test was one Cuscuta seed in a 60-gram sample. Cuscuta spp., commonly known as dodders, are parasitic plants and regarded as a weed worldwide, with adverse effects on native flora and on crop production.

Of the seed shipment, 1.9 tonnes had already been distributed domestically for sowing for pasture or as a cover crop. A hold was put on the seed line. The *Cuscuta* seeds were submitted to PHEL Botany and identified as dodder (*Cuscuta pedicellata*, Solanales: Convolvulaceae). This dodder is native to the eastern Mediterranean, central Asia and northeastern tropical Africa, and parasitises legume crops. It is not known to be present in New Zealand. Border Clearance Services (BCS) was engaged to collect samples from the importer's warehouse at five times the International Seed Testing Accreditation (ISTA) standard, consistent with Border Operating Procedures. AQ tested for the presence of further contaminants and found a total of 44 contaminant seeds, representing 6 species. The contaminants included dodder (Cuscuta *pedicellate*) x 2, white clover (*Trifolium* repens) x 2 red sorrel (Rumex acetosella) x 1, chicory (Cichorium intybus) x 36, wheat (Triticum aestivum) x 1 and alfalfa (Medicago sativa) x 2. The latter two species require additional measures to meet entry requirements under the IHS, and Egypt is not recognised as an approved country for the importation of T. aestivum. Germination tests were conducted on the C. pedicellata seeds to determine if they were viable, and returned positive results. The white clover, red sorrel and chicory are listed as basic on the PBI and do not require additional measures under IHS 155.02.05.

Discussions between BSIIPH, BCS, Plant Imports and Risk Assessment teams determined that destruction of the affected seed line was the only viable option to manage the risk. However, given that much had already been sold on the domestic market, it was not feasible to manage that risk under investigation and the investigation was transferred to the Response Group to manage the residual risk.

Investigation positive; urgent measures prevent establishment

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.

Ant investigations

BSIIPH investigated nine notifications of possible exotic ants. Only two investigations established exotic species.

A member of the public bought five paintings at a private sale in Hawai'i. One of them was submitted to the Auckland Art Gallery for conservation work. A painting conservator discovered live insects causing extensive damage in the frame so the painting was subjected to an anoxia treatment for 3 weeks. This is a treatment commonly used for artworks and artefacts to avoid damage to valuable items, and requires that a very low level of oxygen is achieved and maintained for at least 21 days, with temperature and humidity controlled. On finding that the larvae were still alive at the conclusion of the treatment, the gallery contacted MPI. The notifier was advised to remove the painted canvas from the frame, seal it in plastic and place it in a freezer overnight before submitting it to PHEL for identification. Live West Indian drywood termite Cryptotermes brevis (Blattodea: Kalotermitidae) and two ant species, little fire ant (*Wasmannia auropunctata*) and a dead yellow crazy ant (Anoplolepis gracilipes) (Hymenoptera: Formicidae), were identified from the sample. The life state of the little fire ant could not be determined. A PHEL entomologist, along with Fly Busters Antiants (FBA) Consultants, conducted an inspection of the gallery to determine whether ants and termites had escaped from the frame. Monitoring stations were deployed for ant surveillance but no ants were detected. However, considering the cryptic nature of the termites and ineffectiveness of the anoxia treatment. two IIs conducted a site inspection of the property of the owner of the paintings. Other paintings and the wooden packing crates were inspected. Though the risk of an infestation appeared to be low, the packing crate was sent for fumigation and destruction as a precautionary measure. Substantial damage was evident in the frame, with obvious frass, but it was not clear whether this was due to recent activity or an old infestation. All frames were delivered to the Auckland Museum and subjected to an MPIapproved treatment. Over a period of days, oxygen was reduced to an effective level by introducing on-site-generated nitrogen gas, then the frames remained in the anoxic atmosphere for 21 days. All variables were constantly monitored, logged and adjusted over the treatment period. In lieu of a treatment certificate, Auckland Museum provided the II with the data output for the duration of the treatment as validation. The gallery was informed that the biological risk had been mitigated and the frames could be returned to their owner.

During the compliance inspection of a vehicle recently imported from

Singapore, live ants and eggs were found underneath rubber seals in the boot. Once disturbed, the ants retreated further into the vehicle and the inspection was halted so as not to further disturb them. An approved MPI ant treatment contractor was commissioned to spray the perimeter of the vehicle and all risk areas such as the underside and wheels to prevent the ants from dispersing. PHEL Entomology identified the ants as the ghost ant (Tapinoma melanocephalum, Hymenoptera: Formicidae), not known to be present in New Zealand. The vehicle was further treated by fogging with insecticide to kill all the ants. Following treatment, an unusual dead wasp was found in the vehicle and identified by PHEL Entomology as Aphidius sp. (Hymenoptera: Braconidae: Aphidiinae). The Aphidiinae is a subfamily of tiny parasitoid wasps that use aphids as their hosts. Several species have been introduced and released worldwide for aphid pest biological control, including in New Zealand. The MPI Treatments & Inanimate Pathways Team was informed.

Wood borers

There were eight investigations of suspect exotic borer beetles. Two of them were positive for an exotic species Heterobostrychus aequalis, (Coleoptera: Bostricidae) in barber chairs and a garden umbrella imported from China. The risk was mitigated by freezing the risk goods. The importers and the Chinese manufacturer were informed about the New Zealand IHS for wooden ware from all countries, https://www. biosecurity.govt.nz/dmsdocument/1221woodware-from-all-countriesimport-health-standard. The other six investigations established no biosecurity issues: beetles were found to have been dead for a long time or the species was determined to be already established in New Zealand and not a high-impact pest.

Suspect new bacteria in agricultural chemicals

MPI was notified of suspected new to New Zealand bacteria isolated from an agricultural product manufactured in Germany and imported for biological control of *Pseudomonas syringae* pv. *actinididae* or psa (Pseudomonadales: Pseudomonadaceae). The product contained *Aureobasidium pullulans* (Dothideomycetes: Dothioraceae), a yeast used as a biological agent against Psa. In 2019, 5,536 kg of the product

was imported in 14 batches consisting of three separate consignments. Samples from each batch were submitted to Plant & Food Research (PFR) for qualitycontrol testing, including checking the levels of A. pullulans and possible contamination. The tests revealed that most batches were contaminated: only two tested negative. Samples from four contaminated batches were sent to Manaaki Whenua Landcare Research (MWLR) for identification. MWLR found two bacterial species not previously reported from New Zealand, in three of the batches. One species was Enterobacter hormaechei (Enterobacteriaceae), a bacterium associated with the human gut and considered an opportunistic pathogen in immunocompromised people; it is not a plant pathogen. The second bacterium could not be identified as it was a species new to science. After receiving the identification results from MWLR, the importer initiated recall of all product as directed by the II. Investigation determined that 74 kg of the product had been released to two retailers based in Pukekohe and Cambridge and subsequently sold to kiwifruit growers. All the products were removed from sale and contained, except for 1 kg that had been sprayed onto an orchard. However, that batch had tested negative for contamination. The biosecurity issue and human health risk had been contained. A Notice of Direction under s122 of the Biosecurity Act 1993 was issued requesting that all products imported in 2019 be sent for destruction by deep burial. Interwaste was contracted to complete the task. The German manufacturer was informed about the contamination of the product and its failure to meet New Zealand import requirements.

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), treatment was applied. There may be some residual risk associated with items that could not be retrieved.

Pine pollen stopped at border

An importer submitted a complaint at the Auckland International Mail Centre (AIMC) after he learned that a parcel had been detained on arrival in New Zealand in September 2019. He was seeking clarification as to why the parcel was stopped when the same product, purchased online 5 months earlier from the same supplier, had been cleared without problem. The importer advised AIMC that he had received five products in May: nettle root extract, pine pollen tincture, Ashwagandha spagyric tincture, nettle root tincture and pine pollen megadose. The II established that the pine pollen product had been infused with 80 proof vodka for 30 days, and was therefore not a biosecurity issue. The September consignment also contained pine pollen megadose, as well as ground roots and stems of Cistanche tubulosa (Lamiales: Orobanchaceae), a parasitic desert plant traditionally used for medicines and foods in China. In order to clarify the border requirements concerning these products, the MPI Plant Import Team contacted the importer to explain the relevant sections of IHS: Stored Plant Products for Human Consumption, where, according to section 5.12(1), pollen of Pinus spp. is prohibited. This product was destroyed. However, the ground C. tubulosa roots and stems fell under section 5.4 (dried herbs, spices, roots and beverages etc.) and this material was cleared to enter New Zealand.

Uncleared woodware from Indonesia

The Incursion Investigation Team was notified by BCS of a non-compliance issue where uncleared goods (woodware and wood furniture) had been released from a Transitional Facility (TF) prior to inspection and clearance by an MPI QO. The goods had been manufactured in Indonesia and imported by a wholesaler into Australia, where they were stored prior to shipment to New Zealand. The importer was asked to place a hold on the goods pending inspection by border staff, as per the Border Operating Procedures. The goods were found to be free of any contaminants and the consignment was cleared. However, one item had been sold before the II initiated contact with the importer. No contact details for the customer were held by the importer. Given that BCS had cleared the remaining items, the risk of a contaminant being present on that good was considered low and no further action was taken. The Australian Department of Agriculture's strict biosecurity requirements were taken into account: the goods would have undergone inspection at the Australian border prior to being released to the wholesaler.

Fungal cultures for mushroom cultivation from the US

An importer received a direct delivery by express courier from the US of a consignment of fungal cultures (Pleurotus ostreatus, Agaricales: Pleurotaceae) growing on petri dishes. These were starter cultures for mushroom propagation. Since the packaging was unopened on arrival, the importer realised the cultures had not been inspected by MPI at the border, nor validated by PHEL as the fungal organism stated on the manifest, and this was an IHS requirement: Import health standard for microorganisms from all countries. PHEL had received a number of supposedly P. ostreatus samples from border staff which, on analysis, were identified as hybrids or new species historically misidentified as P. ostreatus. To import a new species into New Zealand, importers are required to seek approval from the Environmental Protection Authority (EPA) and obtain an import permit from MPI Plant Imports. The II alerted the MPI Target Evaluation Team who contacted the importer and sent one of their team to collect the consignment, which was quarantined until the cultures were formally identified by PHEL. The consignment was redirected to Border Clearance Services (BCS) for assessment of the importation documents by the Target Evaluation Team and to initiate the border assessment pre-clearance process. PHEL mycologists determined that some of the cultures were a hybrid of at least two fungi that belong to the *P. ostreatus* species complex, but the identity of the parents could not be resolved; the other cultures were not *P. ostreatus*. The importer was advised that as the cultures were not pure P. ostreatus the consignment was not eligible for entry, and was required to either re-ship or destroy the consignment. Not all import requirements are specified in the above IHS, and more information can be found on the MPI website, import

permit and here: steps to importing fungi for growing.

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 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.

New to New Zealand yeast and bacteria

The current popularity of kombucha (a fermented tea beverage) and its commercial production led to taxonomic clarification of the fermentation agents present in the symbiont culture of bacteria and yeasts (SCOBY) used in kombucha production. Initial diagnostic testing of SCOBY was undertaken by MWLR under contract to a commercial kombucha producer, and identified microbial species not previously reported as present in New Zealand, despite kombucha's historical use here. Cultures of three microbial species were submitted to PHEL's Mycology and Bacteriology Team for validation of the species, and to Plant Health Incursion Investigation for review of their biosecurity significance to New Zealand. The three bacteria were identified as Acetobacter musti, Acetobacter papaya (Rhodospirillales: Acetobacteraceae), and the yeast Picha manshurica (Saccharomycetales: Pichiaceae). All three are known to be associated with natural fermentation processes in nature. They are not plant pathogens and there was no evidence of any biological risk associated with these species. Reference cultures were lodged with the International Collection of Microorganisms from Plants (ICMP), and MWLR has applied to EPA for clarification of their new organism status under the HSNO Act. No further MPI actions were planned.

New to New Zealand fungus

The fungus *Mariannaea camptospora* (Hypocreales: Nectriaceae) has not previously been detected in New Zealand, but was recently identified during an investigation into unusual disease symptoms observed on radiata pine trees, Pinus radiata (Pinales: Pinaceae), at Kaukapakapa, Auckland. Although little information is available on M. camptospora, it is considered likely a saprophytic fungus. Certainly it was not considered the cause of the symptoms observed on the P. radiata trees, as it was isolated from only one of multiple samples collected. Known microbial pathogens were also isolated and are considered more likely candidates for disease causation. Mariannaea spp. are mostly known to colonise dead plant material including wood, bark and pine-needle litter. They also occur in soils and have been isolated from diseased plant roots. Of the 15 currently accepted Mariannaea spp., only *M. elegans* is reported as present in New Zealand, where it has been associated with kohekohe, Dysoxylum spectabile (Sapindales: Meliaceae), P. radiata and matai, Podocarpus spicatus (Pinales: Podocarpaceae) as a saprophyte. Internationally, M. camptospora has been isolated from dead oak wood, Quercus sp. (Fagales: Fagaceae), forest soils and Podocarpus sp. No reports were found suggesting M. camptospora is a plant pathogen, and its distribution in New Zealand is limited to this single record. The site was in a rural area, with no obvious pathway of entry into New Zealand. Established populations of this fungus are assumed to exist at this location. It is considered very unlikely that the distribution of this fungus within New Zealand is limited to this single location, so populations are highly likely to be elsewhere. No further action is planned in response to this detection.

Golden dodder in wetlands, Waikato

The Department of Conservation (DOC) reported a localised low-cover infestation of golden dodder, *Cuscuta campestris* (Solanales: Convolvulaceae) at Whangamarino wetland and Lake Whangape in Te Kauwhata, Waikato. *Cuscuta campestris* is an annual parasitic plant, native to North America, which can simultaneously parasitise several host plants and create dense mats over the top of host vegetation. Seed production is prolific: a single plant can produce up to 16,000 seeds, which can persist in the soil for up to 10 years. Golden dodder has a wide host range and is a serious pest of crops from the family Leguminosae, such as alfalfa (Medicago sativa) and clover (Trifolium sp.) and, to a lesser extent, sugar beet, Beta sp. (Caryophyllales: Amaranthaceae), onions (Allium sp., Asparagales: Alliaceae) and tomatoes (Solanum lycopersicum, Solanales: Solanaceae). Investigation into the regulatory status of this organism found that C. campestris does not appear on the National Pest Plant Accord (NPPA), but is listed as an unwanted organism under its synonym C. arvensis. However, the plant has basic entry requirements under the IHS 155.02.05 Seeds for Sowing. It has been naturalised since 1944, with a rare and localised distribution. On discovery of its naturalised status and wide distribution, a preliminary risk assessment was conducted by the II and PHEL. The climatic range for this species is tropical to subtropical. The optimum temperature range for seed germination is around 30°C, which may explain why this species has not become as aggressively invasive here as it has overseas. The risk of spread from the Waikato sites was uncertain, but likely to be low as the first record of the Lake Whangape population dates back to 1993. It is unknown how the infestation originated on this site and whether its presence was due to spread from one site or to multiple incursions. Given the wide distribution and naturalised status of *C. campestris* in New Zealand, it was considered not feasible to control. contain or eradicate this species and DOC was advised accordingly.

New to New Zealand beetle, Whanganui

In 2016 a single live beetle found in a Whanganui house was identified as *Phacodes personatus* (Coleoptera: Cerambycidae), an Australian longhorn beetle not previously recorded in New Zealand and known to attack dead and dying trees. At the time, Incursion Investigators considered the detection of a single individual insufficient evidence to demonstrate the species had established and that an on-going population was present. However, two further independent detections of solitary P. personatus beetles now provide strong evidence this species is established in New Zealand: on Eucalyptus sp. trees at Turakina Beach settlement (January 2018), and near Marton (January 2019). The furthest distance separating any two of these three locations is 32 km.

New Zealand has a number of subcortical-feeding cerambycids originating from Australia, mostly associated with *Eucalyptus* spp. The timing of adult beetle detections was consistent with similar subcorticalfeeding longhorn beetle species that attack Eucalyptus spp., have a single generation per year, and a peak adult flight time of January to March. Literature suggests the distribution of *P. personatus* is limited to New South Wales, Victoria and Tasmania, where it is associated with silver wattle, Acacia dealbata (Fabales: Leguminosae), green wattle (A. decurrens), black sheoak, Allocasuarina littoralis (Fagales: Casuarinaceae) and dally pine, Psoralea pinnata (Fabales: Leguminosae). A single report cited radiata pine (Pinus radiata) as a host in Tasmania, although enquiries to Tasmanian forest entomologists by Scion were unable to confirm this. The overall risk to New Zealand from P. personatus is considered low, and discussions with the forestry industry and Scion entomologists support this view. It has been added to databases of species detected in New Zealand and no further action is planned.

New to science virus in native plant

SPS Biosecurity reported unusual vein patterns on leaves of a tutu, Coriaria arborea (Cucurbitales: Coriariaceae) plant collected on the Kepler Track in Fiordland National Park during High Risk Site Surveillance (HRSS). The symptoms were limited to a single area and similar to those seen with herbicide damage. PHEL Virology extracted two undescribed viruses belonging to the genus *Badnavirus* and the family Alphaflexiviridae. Ongoing diagnostics by PHEL may help to characterise these viruses, determine whether they are hostintegrated or infectious, and understand the role of these two viruses. It was assumed that the symptoms observed were related to the alphaflexivirus but Koch's postulates have not been tested (i.e. the virus has not been re-isolated from the diseased host, grown in pure culture and the specific disease reproduced when the pure culture is inoculated into a healthy susceptible host.) It is possible that these are native species that have been undetected. DOC was notified of this investigation since it was associated with a plant in a national park.

New to New Zealand viruses in hebe, Whanganui

A sample from hebe (Veronica sp., Lamiales: Plantaginaceae) showing viral-like symptoms was collected from Queens Park, Whanganui, during HRSS. An undescribed Emaravirus and a partial genome of a Badnavirus were detected. Inheritance of endogenous badnaviruses has previously been reported in several New Zealand native plants, including hebe. In this instance, the PHEL virologist believed the Emaravirus was the causal agent. Hebe samples from other areas in Queens Park, and additional samples from Cooks Gardens, about 400 m away, were collected to determine the spread of these viruses. The undescribed Emaravirus was detected in all the hebe plants sampled in Queens Park, including the endemic Veronica bollonsii. None were detected from Cooks Gardens. PHEL Virology is continuing diagnostics to try and characterise the undescribed Emaravirus and to determine whether the Badnavirus was host-integrated or infectious. The origin of these new viruses is unknown, but given their association with a native plant they could be native or endemic to New Zealand.

New to New Zealand fungus on feijoa

The owner of a commercial feijoa orchard (Acca sellowiana, Myrtales: Myrtaceae) saw patterns and spots on the foliage of some feijoa plants in the orchard and was concerned the symptoms were caused by a disease. Diagnostic tests conducted by PHEL mycologists determined the fungus to be Anthostomella ravennica (Xylariales: Xylariaceae), a saprobic species described in 2016 and previously known only from European marram grass, Ammophila arenaria (Poales: Gramineae) in Italy. This fungus does not appear to pose a biosecurity risk; it is a saprobe (possibly an endophyte in living leaves) that has likely been in New Zealand for some time and is not associated with the spots seen. Many other species from this genus are present in New Zealand.

Investigation for high impact pests; negative

These investigations resulted from reports of suspected high-impact pests or diseases and were proven to be not present in New Zealand, or investigations established that they were already in the country.

Fruit-fly investigations

During this quarter, the Plant Health Team investigated five notifications of suspected adult fruit flies (Diptera: Tephritidae). All were negative and were stood down, following advice from PHEL Entomology after studying photos provided by the notifiers. However, one unknown larva found alive in a mandarin from Australia turned out to be a member of the family Tephritidae. The single live larvae was reported as being found inside a mandarin purchased from an Auckland supermarket. The mandarin had no Price-Look-Up Code (PLU) label but the notifier believed it had originated from Australia. Two larvae found in the fruit by the PHEL entomologist were identified morphologically as Tephritidae and qPCR techniques confirmed the species was island fly (Dirioxa pornia). Tracing indicated that the mandarin was likely to have been imported from Australia, where D. pornia is considered a minor nuisance pest to horticulture. A meeting was held between all concerned MPI departments and the consensus was that this was an incidental import and no further action was required. It was considered that the climate was unlikely to be suitable for establishment in New Zealand. The Minister of Agriculture was briefed on the situation.

Unknown mould on metal safe from Vietnam

A customer of a national hardware chain who had purchased a metal safe manufactured in Vietnam, reported mould on the surface of the safe. On checking retail stock in-store, another safe from the same consignment was also found to have surface mould. The retailer found that the silica gel sachets used to protect the product from moisture damage were swollen, indicating that moisture had seeped into the safe and provided ideal conditions for mould to grow. As a precautionary measure, the store was instructed to clean the safes with a hypochlorite-based product to kill mould. However, as the customer was still concerned about a possible biosecurity issue, further information regarding the importation of the consignment was requested. The safes were identified as arriving

into New Zealand in 2017. They had been held at a distribution centre in Auckland where two additional safes from the consignment were found to have the same mould. The distribution centre inspection team noted the pallets appeared to have been wrapped under humid conditions and moisture had seeped into the safes. The surface mould was therefore deemed not to pose a biological risk.

Suspect velvet leaf, Coopers Beach

A property owner reported observation of an unusual plant in an adjacent property which, when investigated online, looked similar to velvetleaf, *Abutilon theophrasti* (Malvales: Malvaceae), a pest plant regulated in New Zealand. However, photos provided by the notifier were examined by a PHEL botanist and determined not to be velvetleaf, but most probably a young princess or empress tree (*Paulownia tomentosa*, Lamiales: Paulowniaceae), a species established in New Zealand.

Suspect sirococcus blight on cedar, Ashburton

Disease symptoms on three Himalayan cedar trees (Cedrus deodara, Pinales: Pinaceae) on a residential property in Ashburton were thought to be similar to those caused by sirococcus shoot blight, Sirococcus conigenus (Diaporthales: Gnomoniaceae), a serious disease of *Cedrus* spp. overseas, but absent from New Zealand and an unwanted organism. The II contracted a forest biosecurity specialist to visit the site and collect foliage, branch and soil samples from and below the affected trees for PHEL mycologists to examine. Site observations suggested that although the symptoms were not considered indicative of any currently known biotic disorders on New Zealand Cedrus sp., root-related problems or environmental factors (including herbicides) were possible causes. Diagnostic tests ruled out the presence of S. consigenus, but isolated a number of tree pathogens that may have caused or contributed to the observed symptoms. These included *Pestalotiopsis* sp. (Xylariales: Sporocadaceae), Ilyonectria sp. (Hypocreales: Nectriaceae), Microsphaeropsis sp. (Pleosporales: Microsphaeropsidaceae) and Pythium sp. (Peronosporales: Pythiaceae). Regardless, the presence

of *S. consigenus* or any other exotic pathogen was ruled out.

Suspect spotted winged drosophila

A home owner reported live insects on ripe strawberries grown in her Hamilton garden, and suspected they were the exotic spotted wing drosophila larvae (Drosophila suzukii) (Diptera: Drosophilidae). PHEL Entomology ruled out Drosophilidae larvae from a photo provided by the notifier. No strawberries were available for sampling so the II advised the notifier to wait for her strawberries to ripen, then inspect them again for larvae. A specimen was then submitted to PHEL Entomology, and yielded the spotted snake millipede, Blaniulus guttulatus (Julida: Blaniulidae), one of the most common millipedes in New Zealand gardens. They may feed on live plant tissue (roots and tubers) and on fallen fruit, and in areas of high population density can be a pest.

Negative - Other

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

Stored products

There were 15 investigations of organisms associated with imported stored products, such as rice, dried figs, chilli, cereal and cane sugar. All investigations found common pests of stored products that are already established in New Zealand.

Fresh produce

Another 14 notifications were associated with fresh produce such as strawberries, mandarins, bananas, mangoes and grapes. Two yielded organisms exotic to New Zealand: mango seed weevil, Sternochetus mangiferae (Coleoptera: Curculionidae), found alive in mango from Australia; and a snail (dead) of the family Achatinidae, on bananas (country of origin not provided). Mangoes from Australia had been investigated for a report of a lepidopteran pupa consistent with the family Tortricidae. Mangoes from Australia undergo irradiation, rendering the weevil sterile, and since the mango stickers indicated Australian origin the weevil did not pose a biological risk.

The rest of the investigations revealed common insect pest species already established in New Zealand and not a biosecurity issue. They were mainly moths (e.g. noctuids *Helicoverpa armigera* and *Chrysodeixis eriosoma*), the tortricid *Cydia pomonella*, flies (families Psychodidae and Drosophilidae) and black rot, most likely the fungus *Alternaria* sp. (Pleosporales: Pleosporaceae).

Travellers

Eight investigations were carried out on insect pests or fresh fruit and nuts imported by travellers visiting or returning to New Zealand from the Philippines, Bali, Australia, Thailand, Nepal and Fiji. Insects were found when unpacking luggage and thought to have been "incidentally imported" into the country. In one case the manuka beetle, *Pyronota festiva* (Coleoptera: Scarabeidae) was discovered. This species is endemic to New Zealand and obviously not an incidental import.

Parcels, mail, packaging

There were 28 investigations that included risk goods imported from China, the US, Korea, Australia, Brazil, Ireland, Indonesia, Taiwan, South Africa and Canada. Two cases were reported from TFs at Tauranga and Auckland, where insects were found on wooden pallets and containers that had been devanned several days prior to detection, making it difficult to associate the insect with an imported good. In other cases there was no biosecurity issue because the species were present in New Zealand or were not viable. Dead organisms do not pose a biosecurity issue and often these investigations can be quickly closed. Often in these cases poor photos or no specimens are provided and the investigation outcome remains negative.

Spider investigations

This quarter the BSIIPH team investigated 20 cases of possible exotic spiders, all negative for any biological risk (**Table 2**).

One case was referred to the MPI Compliance Team. It involved a Hokitika POI reported as having goods undeclared at the border: two baby tarantulas (Araneae: Theraphosidae) from Sydney, brought in January 2019, and a coral fragment collected from Rarotonga in November 2019. A search warrant was issued and MPI Compliance Officers with an II inspected both the POI's residential house and working place. No live spiders were found. No coral was recovered but the POI provided a Trade in Endangered Species Act notice verifying seizure by border staff. The POI asserted that the notification of exotic spiders was "unjustified" and that the accusation had no substance and was most likely the result of a personal dispute with an ex-partner (the original notifier). When interviewed, the ex-partner remained adamant that the spiders had been smuggled into New Zealand, in January 2019. However, since no live spiders were found, no biosecurity issue was established and the investigation was closed.

found on a "weedy" Cyperus sp. (Poales: Cyperaceae) at the side of a track in Gittos Domain, Blockhouse Bay, Auckland. PHEL Entomology advised that the specimen was morphologically identical to two specimens deposited in the New Zealand Arthropod Collection (NZAC) and labelled as Agandecca sp. Specimens had been collected in 1980 and 1985 from the Northland and Wellington regions respectively. However, after consultation with Australian experts on this taxonomic group, it was concluded that the New Zealand specimens did not fit the description of Agandecca. They most probably are an

pecies	Region	Host
lower spider, Diaea sp.	Geraldine	Banana
Slater spider, Dysdera crocata	Auckland	Crate at TF
	Bunnythorpe	Banana
Ohunh asidar Esizahan anatuka	Auckland	Truck
	Auckland	Mailboxes
Orbweb spider, Eriophora pustulosa	Christchurch	Outdoor lights
	Parawera	Washing powder
Huntsman spider, Isopeda villosa	Auckland	Automotive parts
White-tailed spider, Lampona cylindrata	Palmerston North	Flat-packed outdoor furniture
Daddy longlegs spider, <i>Pholcus</i> phalangioides	Tauranga	Container at TF
Tunnel web spider, <i>Porrhothele</i> antipodiana	Lower Hutt	Residential garage
	Wellington	Box with baby bouncer
False katipo spider, Steatoda capensis	Christchurch	Garage door
House cobweb spider, Steatoda grossa	Christchurch	Surgical cabinets
No specimens provided	Auckland	Suitcase of a traveller
	Hamilton	Illegally imported tarantula
	Hokitika	Illegally imported tarantula
	Kaiapoi	Package
	Masterton	Whole-leaf tea
	Wellington	Imported barbecue

Inconclusive

These investigations have been stood down because results (or absence of results) cannot determine the presence or absence of a biological risk, and a decision has been made that further investigative activity is not warranted.

Suspect new to New Zealand plant hopper

A suspect new to New Zealand plant hopper (Hemiptera: Achilidae) was

undescribed species that has been present in New Zealand since at least the 1980s, with a wide distribution from Northland to Wellington. The family Achilidae has a worldwide distribution, though it is a poorly known group. Compared with other members of the Fulgoroidea that feed on plants, the Achilidae (particularly the nymphs) are typically fungal-feeders, found in leaf litter, under logs or under the bark of host plants. There are no reported pest species and there have not been any reports of any Achilidae in New Zealand causing harm to plants. Owing to rare collections of this species in New Zealand and little knowledge, it is unknown whether it is endemic, native or adventive to New Zealand, or what its specific host associations are. The investigation concluded that this species is unlikely to pose a biological risk and the investigation was closed.

Pine tree death, Kerikeri

The Incursion Investigation Team received a referral from Northland Regional Council biosecurity staff regarding a single dead Pinus pinea (Pinales: Pinaceae) tree at the Aroha Island Ecological Centre, near Kerikeri. At the time of notification the nature reserve was closed to the public owing to the hazard posed by the dead tree. As there was no obvious cause of the previously apparently healthy tree's sudden death, the centre's manager sought advice on how to rule out a biosecurity issue, determine whether the tree's death resulted from microbial pathogens, and whether these pathogens might pose a risk to other trees in the reserve. The II advised the council to fell the tree, remove cut wood from the site and use it as firewood, and to mulch the remaining branches. Fungal staining was evident on the surface of the felled trunk. Samples of the stained wood were provided to PHEL Mycology for analysis but no pathogenic fungi were found. The cause of the tree's death remains unknown but since no pathogens were identified it may have been abiotic factors. No further action was considered necessary.

Oak tree dieback, Cambridge

An arborist at the Waipa District Council notified a PHEL mycologist about oak tree decline in the Cambridge oak arboretum. Two trees had been removed earlier in 2019 and samples submitted to Scion but nothing conclusive was found. With similar signs presenting themselves in a third tree, the opportunity to collect samples was offered to PHEL in the lead-up to the tree's removal. Oak tree decline has been globally recognised, with the conclusion that no single pathogen readily accounts for the decline and a more likely explanation is a complex of primary and secondary pathogens capable of causing severe defoliation, dieback and thinning of tree canopies. Information about the

arboretum management practices, watering provisions and source of tree stock were gathered. However, during the week after notification, the oak tree sprouted new growth and showed signs of improvement. Plans to cut down the tree and submit samples for analysis have been placed on hold pending surveillance on the resilience of the tree.

Carolyn Bleach Manager – Biosecurity Surveillance & Incursion Investigation Plant Health Team Diagnostic and Surveillance Services Directorate Ministry for Primary Industries Carolyn.Bleach@mpi.govt.nz

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

0800 80 99 66

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

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

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

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Gribbles Veterinary Pathology

- AUCKLAND Courier: 37–41 Carbine Road, Mount Wellington, Auckland 1060 Postal: PO Box 12049, Penrose, Auckland 1642 Tel: 09 574 4701 Fax: 09 574 5304
- HAMILTON Courier: 57 Sunshine Ave, Hamilton 3240 Postal: PO Box 195, Hamilton 3240 Tel: 07 850 0777 Fax: 07 850 0770
- PALMERSTON NORTH Courier: 840 Tremaine Avenue, Palmerston North 4440 Postal: PO Box 536, Palmerston North 4440 Tel: 06 356 7100 Fax: 06 357 1904
- CHRISTCHURCH
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