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INSIDE:

Animal Health and Welfare Directorate and 3rd Korea-NZ Animal Health & Epidemiology Workshop

Improving biosecurity readiness: a new engine for FMD aerial spread plume modelling

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Editorial

Animal Health and Welfare Directorate and 3rd Korea-NZ Animal Health & Epidemiology Workshop

The Animal Health and Welfare Directorate is a relatively small but vital part of the animal health and welfare regulatory framework and provides science-based technical leadership in both areas.

The Directorate has three core functions: animal imports, exports and welfare. Animal imports sets the zoosanitary risk management standards for importation of biologicals, animals and animal products. Animal exports works with other government-competent authorities to facilitate export of live animals and germplasm. The Directorate is integral to MPI's biosecurity and animal health systems. Animal welfare is a dynamic and growing area, and evidence-based advice and standard-setting is crucial for informed and sensible debate. My team work tirelessly, whether servicing the two Ministerial advisory committees – the National Animal Welfare Advisory Committee and the National Animal Ethics Advisory Committee – or our outreach programme (our *Safeguarding* team acting as the conduit for new regulations and codes), in emergency management or providing high-quality technical advice into policy development and other parts of MPI. Both areas are active internationally and provide input into international standard-setting and at various levels in the OIE.

As Director, my role is to think broadly both for MPI and across the primary sector and provide system leadership in animal health and welfare for the good of New Zealand. The complexities, competing interests and ever-changing landscape of animal health and welfare present significant challenge, but also opportunity.

Domestically the ever-increasing biosecurity tension that exists for trade to be a two-way street presents a significant challenge, but one that must be managed in a developed country reliant on exporting primary produce. Animal welfare science must continue to be central to our exceptional welfare framework as increasing public and consumer demands are placed on how we farm our animals. Globally there are risks from emerging disease in wildlife as a result of human activity, the rise of zoonotics, the ongoing threat of transboundary disease, and increasing challenge of antimicrobial resistance. However, I believe we can continue to be forward-thinking, agile and brave, with ever-improving disease surveillance, industry-led assurance and innovative thinking that combines market incentives with regulation to drive better outcomes.

I see my Directorate as pivotal to providing focus and direction to help navigate the animal health and welfare landscape ahead. Being appointed as a Chief Technical Officer (CTO Veterinary) under the Biosecurity Act, and as Director of Animal Health and Welfare, gives me the opportunity and responsibility to provide science-based advice to senior leaders within government. It also creates the opportunity to represent and champion issues domestically and internationally. It's a responsibility that I'll relish and feel extremely privileged to have been given.

Shortly after my appointment, in November I was asked to lead the NZ delegation for the 3rd Animal Health and Veterinary Epidemiology Korea-NZ bilateral workshop, hosted by Korea in Seoul. Korea is a significant and valued export market and this collaboration between our animal-health agencies sits under the Korea-NZ free trade agreement signed in March 2015. This workshop was the third time that animal health experts from both countries have met to share ways of managing the biosecurity challenges that both countries face. The NZ delegation consisted principally of veterinarians and scientists from the Diagnostics and Surveillance Services (DSS) Directorate and built on the two previous workshops that have been reported previously (Watts 2016, 2017). The workshop was highly successful and the maturing relationship and collegiality was evident to me over the 3 days.

New Zealand facilitated and ran a hands-on tutorial showcasing our Standardised Analyses for Disease Investigation (SADI) platform – a practical epidemiological tool to help guide resource allocation and planning during disease outbreaks. Despite the language differences, the tutorial was a great success. It set the tone for candid discussions about the threat of African swine fever to pig production in Korea, and provided useful lessons from incursion investigations in both countries.

The benefits of these types of workshop cannot be underestimated, particularly when they build on previous workshops. They are hugely valuable to inform our domestic approaches and ground-truth policy, and to learn from a country that is under arguably greater threat of transboundary disease than we are. Development of key relationships and opportunities to share and collaborate bilaterally are rare, and I hope the ongoing benefit from the last 3 years of doing so continues in the years to come.

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Detection of infectious bursal disease virus serotype 2 (IBDV-2) in New Zealand poultry and waterfowl

Summary

Investigations were carried out on three unrelated free-range chicken broiler farms after IBD antibody ELISA reactors were detected via the poultry industry sero-surveillance programme. The investigation was part of ongoing efforts to inform and validate New Zealand's IBD surveillance programme. The investigation excluded the presence of pathogenic IBD serotype 1 viruses (IBDV-1) but identified a non-pathogenic IBDV serotype 2 (IBDV-2) virus, which was also identified in wild mallard ducks (*Anas platyrhynchos*). The investigation concluded that IBDV-2 was the most likely cause of the sporadic low-level seropositivity seen during the programme. These findings support New Zealand's claim that Gumboro disease (also known as infectious bursal disease, IBD) is absent from commercial poultry, and will pave the way for the development of serotype-2-specific serological and molecular assays. Such tests will enable the rapid exclusion of IBDV-1 in poultry flocks identified with serum reactors through the ongoing IBD sero-surveillance programme.

Introduction

Infectious bursal disease (IBD – avibirnavirus) or Gumboro disease is an acute, highly contagious viral infection of young chickens that can cause immunosuppression resulting in morbidity and mortality from secondary infection. The IBD virus is characterised by a bi-segmented, double-stranded ribonucleic acid (dsRNA) genome consisting of two segments (A and B). The virus has no envelope, a simple icosahedral capsid structure, and a diameter of 58–60 nm. This relatively simple structure renders the virus very environmentally resistant (Eterradossi & Saif 2013).

Two IBDV serotypes (1 and 2) have been identified, differentiated in vitro by the lack of significant cross-neutralising antibodies, and in vivo by the absence of cross-protection (van den Berg et al. 2000). Serotype 1 strains are pathogenic in chickens, with strains classified according to virulence into various pathotypes: sub-clinical (scIBDV), classic virulent (cvIBDV) and very virulent (vvIBDV). In other avian species serotype 1 is avirulent (Oladele et al. 2009; McFerran et al. 1983; Eterradossi & Saif 2013). Serotype 2 strains do not cause disease in any birds. Turkeys are considered the natural host of serotype 2 viruses, although these may also be isolated from other birds (Eterradossi & Saif 2013). Although IBDV serotypes 1 and 2 have been detected in waterfowl and other wild birds, neither has been confirmed as a cause of disease (Wilcox et al. 1983; Jeon et al. 2008; Kasanga et al. 2008).

In New Zealand, an IBDV serotype 1 virus was identified in clinically normal commercial poultry in 1993. Investigations concluded that the virus was most likely to have been introduced in a contaminated or mislabelled vaccine (Thompson 1994; Motha 1996; Ryan et al. 2000). The poultry industry subsequently took steps to eradicate infection by imposing movement controls and decontamination protocols on all farms that tested positive in an ongoing sero-surveillance programme. The last IBD-seropositive flock was detected in January 1999 (Ryan et al. 2000).

Since that time, the New Zealand poultry industry has operated a "Country Freedom Quality Plan" for IBD surveillance and accreditation of commercial flocks (Brooks 2003; Ryan et al. 2000; Mulqueen 2018). Serum reactors in the ELISA run through the commercial poultry laboratories are referred to the MPI Animal Health Laboratory (AHL) at Wallaceville for the virus-neutralisation test (VNT). MPI's Incursion Investigation Team, in collaboration with the AHL, has investigated a number of flocks in which serum reactors in the screening ELISA have produced low-titre (non-specific) reactors in the VNT. In all cases, investigations have excluded IBDV (Bingham et al. 2006; Bingham et al. 2010).

This report describes the key features of an investigation into historical samples taken in 2015–2016 from three free-range broiler farms with low-level seropositivity. The investigation was carried out as part of ongoing efforts to inform and validate the active surveillance programme and demonstrate that Gumboro disease is absent from New Zealand.

Broiler farms investigated

Investigations were carried out on three unrelated free-range chicken broiler farms (Farms 1-3, **Figure 1**) during 2015–2016, after the detection of IBD ELISA (IDEXX FlockChek IBDV, Maine, USA) reactors through the poultry industry surveillance programme. Low-titre serum reactors were confirmed in the serotype 1 IBDV VNT carried out at the AHL. The VNT used the New Zealand IBDV-1 virus isolated in 1993, and followed the test procedure detailed in the OIE Terrestrial Manual Chapter 2.3.12 (Motha 1996; OIE 2016).

Figure 1: Barn and outdoor run set-up at one of the free-range chicken broiler farms investigated

After confirmation of low-titre positive reactors in the VNT, the Incursion Investigation Team carried out a standard investigation into the clinical and pathological presentations, and laboratory findings evident on each farm, with the aim of determining the cause of the serum reactors and, importantly, establishing whether IBDV was present.

The farms all had healthy flocks (assessed throughout the ~6-week production cycle) with no clinical signs, increased mortality or pathology indicative of IBDV infection. Mortality figures were within industry standards, with all barns on all farms demonstrating excellent performance and low mortality figures. No differences in mortality were evident between barns with and without IBD serum reactors. Cumulative mortalities over the ~35-day production cycle for barns with and without serum reactors were 2.1–4.0 and 1.9–6.5 percent respectively.

No IBDV was suspected, given the lack of clinical signs and pathology, and follow-up work was aimed at understanding the cause of the serum reactors, to help inform the surveillance programme. A cross-sectional serological survey of all barns (~25 sera per barn) on every farm was carried out, and cloacal swabs and fresh and fixed tissues, including bursa, spleen and caecal tonsil, were collected on-farm and/or at processing.

Serum samples were tested by ELISA, with reactors detected inconsistently across the barns (**Figure 2**). The VNT, carried out on ELISA-positive sera (S/P > 0.2), returned titres predominantly ≤ 1:16 (though occasional higher). Histopathology of bursas from seropositive flocks was mostly within normal expected limits, with occasional bursas showing one or more changes including mild-to-moderate lymphocyte depletion, cryptosporidiosis or focal bacterial-associated heterophilic inflammation, and rare peri-follicular haemorrhagic change (thought to be consistent with electrical stunning prior to slaughter). There was no evidence of generalised necrosis and atrophy of bursal follicles as would be expected from pathogenic IBD infection. An RT-qPCR assay for IBD serotypes 1 and 2 (Hein & Trinidad 2006) produced positive results, indicating the potential involvement of an IBD-like virus.

Virus isolation was attempted (five serial passages) on positive tissue samples identified by IBD RT-qPCR using three cell lines and CAM inoculation of embryonated chicken eggs. Despite intensive attempts, no birnavirus was isolated as assessed by molecular assays run after each passage. This was not unexpected, as isolating IBD field viruses in vitro is often challenging (Soubies et al. 2018). A reovirus (endemic virus) was isolated in some samples and was confirmed by electron microscopy and PCR.

Molecular investigation of PCR-positive samples

To enable confirmation and further characterisation, samples that tested positive by the IBD RT-qPCR were examined using additional primer sets and whole genomic sequencing. Conventional PCRs included one targeting segment A VP2 gene (Eterradossi et al. 1998; Wu et al. 2007) and the other segment B VP1 gene (Le Nouën et al. 2006; Wu et al. 2007). Procedures followed the OIE Terrestrial Manual Chapter 2.3.12 (OIE, 2016). Only primers for the B segment produced PCR product (549 bp specific for IBDV sequences). This nucleotide sequence when blasted had up to 92 percent nucleotide identity to both serotype 1 and 2 IBD viruses (no discrimination) in GenBank. This result was consistent across all three farms.

Given that the primer sets targeting segment A failed to produce a signal, a number of primer sets were specifically designed, based on IBDV genome sequences of the serotype 1 and 2 viruses deposited in GenBank. Using this approach, bursas from the most recently affected farm (farm 3) were used to sequence the full genome of segment A (3,243 nucleotides). When blasted in GenBank this sequence had 88–89 percent nucleotide identity to IBDV serotype 2, and 84 percent identity to IBDV serotype 1 viruses. Partial sequencing of positive bursal tissue from the first two farms aligned with findings for Farm 3.

These results were considered to have excluded IBD serotype 1 viruses, and specifically the IBD-1 virus that entered New Zealand in the early 1990s. Findings appeared to indicate the presence of a poorly characterised avibirnavirus. The OIE IBD Reference Laboratory (Ploufragan-Plouzane, France) was engaged to assist with further characterisation of the virus (see below).

Wild waterfowl tested

As part of the investigation into potential sources of virus, cloacal swabs collected from wild mallard ducks (*Anas*

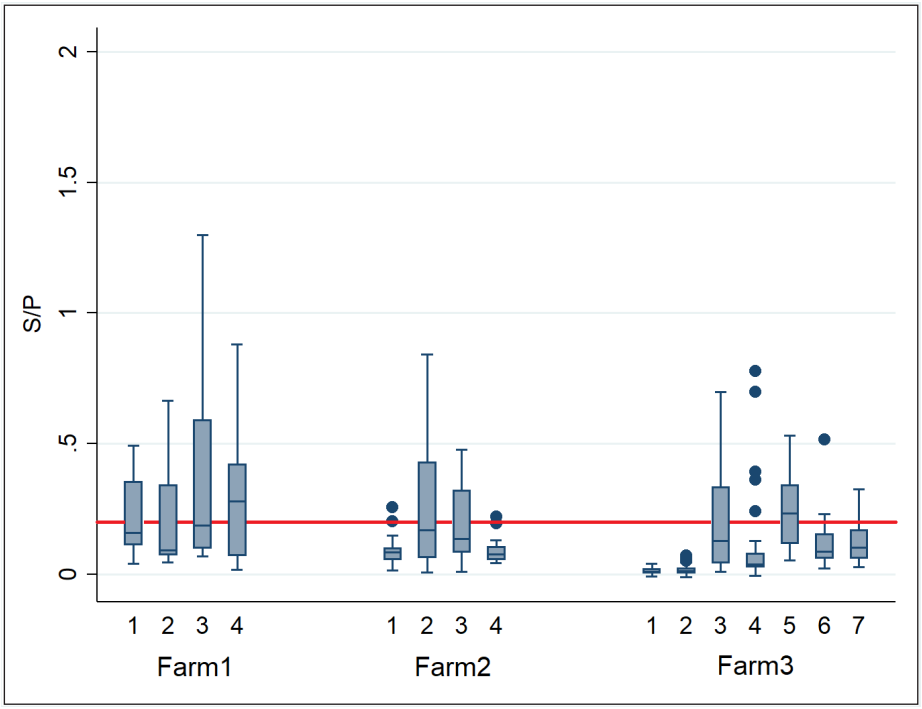

Figure 2: Box and whisker plots showing IBDV ELISA (IDEXX FlockChek IBDV) sample-to-positive (S/P) ratios from a cross-sectional sero-survey of individual barns on three farms. The red horizontal line indicates the manufacturer's cut-off at 0.2. The boxes span the interquartile range of values (25–75 percent), with the central line indicating the median value. The whiskers extend to 1.5x the interquartile range.

platyrhynchos) during MPI's Avian Influenza Surveillance Programme were tested using the PCR protocol and sequencing described above (Stanislawek et al. 2012). Samples were collected in the summer months (January–March) of 2016 from three locations in the North Island (Waikato, Bay of Plenty and Hawke's Bay. A low prevalence of PCR-positive cloacal swab samples was detected in all three areas: 3/320, 1/80 and 2/129 respectively. Positive samples were confirmed by sequencing undertaken at the AHL, where the analysis indicated a high similarity to the virus detected in the poultry broiler farms.

Confirmation of IBDV serotype 2

New Zealand poultry sequences from farms 1–3 were submitted to the OIE IBD Reference Laboratory (Ploufragan-Plouzane, France) and aligned with sequences from IBDV strains representative of the genetic diversity for IBDV (Abed et al. 2018). Phylogenetic analysis of segment A by neighbour joining revealed that the New Zealand sequence unambiguously clustered together with European (strain 23/82) and American (strains OH and Turkey/PA/00924/14) strains of serotype 2, although they also exhibited significant genetic differences consistent with the 88–89 percent identity reported above. Sequence examination of the VP2 gene revealed an insertion at position 249, which is considered typical for serotype 2 viruses. The submitted New Zealand sequences reveal new genetic data on serotype 2 viruses and are of interest to the scientific community.

Conclusion

Consistent with other reports, we conclude that a non-pathogenic serotype 2 IBDV is the most likely cause of the sporadic low-level serum cross-reactivity seen during New Zealand's IBD surveillance programme (Ashraf et al. 2006). Although it is not possible to definitively explain the origin of the virus in the broiler farms, phylogenetically similar serotype 2 IBD viruses were diagnosed in both the free-range broilers and wild New Zealand mallards. It is reasonable to expect the occasional introduction of IBD-2 from wild birds, especially under a free-range system.

The investigation described here demonstrates New Zealand's continued efforts to validate its claims that IBD serotype 1 virus is absent from New Zealand (Herrera 2019). Work underway will see further attempts to isolate the New Zealand serotype 2 virus, working in collaboration with the OIE Reference Laboratory. This work will pave the way to development of serotype-2-specific serological and molecular assays to help with the rapid exclusion of IBDV-1 (and confirmation of the involvement of IBDV-2), in future cases of serum reactors detected through the ongoing IBD sero-surveillance programme (Ryan et al. 2000, Mulqueen 2018).

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Improving biosecurity readiness: a new engine for FMD aerial spread plume modelling

This article aims to provide details and explain the capabilities of the recently upgraded system used by the Ministry for Primary Industries to model aerial spread of foot-and-mouth disease (FMD). This system is called PDEMS, and its key components are:

- a weather analysis and forecast model (called NZCSM),
- a dispersion model that accounts for turbulence (NAME-III), and
- an operational workflow control system.

We show results of a comparison between the older (2005) and upgraded versions, and provide background on FMD itself.

Aerial spread modelling

Over the last few years, NIWA has been working on upgrading the core components of its Plume Dispersion Emergency Modelling System (PDEMS). This service is designed to predict airborne spread of FMD (Gloster et al. 2010), and is a key tool in MPI's readiness and response system for potential FMD incursions. A modified version of PDEMS recently provided valuable data on potential incursion pathways for myrtle rust and the risk of its spread in New Zealand, to support the biosecurity response and its long-term management (Beresford et al. 2018).

PDEMS was initially set up as a key biosecurity infrastructure tool after the 2001 FMD outbreak in the UK. FMD is the most feared animal disease in New Zealand, as an outbreak could stop the export of most animals and animal products and cause a downturn in the economy. Infected cattle, deer, sheep, goats and pigs produce the virus and release it into the environment before and after the onset of clinical signs. The released viral particles then propagate infection through a number of direct and indirect pathways. Long-distance airborne transmission is a distinct feature of FMD, and spread by this route cannot be measured by tracing the movements of animals and fomites (objects that can transfer disease between places. In the case of FMD, these can include people, vehicles, machinery, non-susceptible animals, feed and products).

Airborne transmission can only occur when:

- enough virus is being released into the air by infected animals,
- climatic conditions are suitable to send and maintain viable virus particles at infective concentrations, and
- susceptible hosts are present at the destination.

Despite the relatively low chance of transmission by this route compared with other mechanisms of spread, failure to take it into consideration may delay the detection of new infections, leading to longer and more severe outbreaks.

How it works

Compared with other animal species, infected pigs produce and release large amounts of virus via the respiratory route, so airborne spread becomes a significant threat when properties with high numbers of pigs are infected. If an FMD infection was found in an intensive-rearing piggery, MPI would activate PDEMS to predict the threat of airborne transmission.

When PDEMS is activated during an emergency or for a test, MPI is able to estimate the concentration of virus released on specific days at specific locations, according to the stage of infection, numbers of infected pigs and at-risk pigs, and potentially strain type if known. This information is entered into PDEMS at the National Institute of Water and Atmospheric Research (NIWA), which models the movement of airborne viral concentration from the infected premises (IP) to the surrounding area or a particular location, using meteorological variables such as wind, temperature, solar radiation, humidity and estimates of local air turbulence. Modelling indicates where viral concentrations are above the threshold known to cause infection in key susceptible species. The data is sent back to MPI to be used as another source of information to prioritise field surveillance activities during a response. This takes into account the concentration of virus, the presence of susceptible animals in the plume-covered area, and susceptibility of animal species to airborne FMD viral concentrations (**Table 1**). Cattle are by far the most susceptible species, owing to their large lung volume (Alexandersen et al. 2003).

The core upgrade in the new PDEMS is the replacement of the now aged CALPUFF dispersion model with the NAME-III dispersion model. This model was developed by the UK Met Office and models the aerial dispersion and deposition of disease vectors such as viruses and insects as well as chemical pollutants, volcanic ash and radioactive particles from nuclear accidents. Meteorological data for NAME-III is derived from the other key component of PDEMS, the New Zealand Convective-Scale Model (NZCSM), a high-resolution numerical weather prediction model that operates with a 1.5-km resolution grid all over New Zealand and its surrounding waters. The use of NZCSM represents a significant improvement over the CALMET system used previously, as CALMET used data from NIWA's coarser 12-km resolution New Zealand Limited Area Model (NZLAM). PDEMS is run on NIWA's Wellington-based

TABLE 1: THRESHOLD OF AIRBORNE FMD VIRAL CONCENTRATION REQUIRED TO CAUSE INFECTION IN SUSCEPTIBLE SPECIES

FMD-SUSCEPTIBLE SPECIES	THRESHOLD OF AIRBORNE FMD VIRAL CONCENTRATION REQUIRED TO CAUSE INFECTION ($TCID_{50}/M^3/24$ HRS)
Cattle	0.06
Deer	0.6
Sheep	1.11
Pigs	7.70

High Performance Computing Facility (HPCF) (**Figure 1**), which has itself just undergone a massive update and includes a disaster-recovery HPCF in Auckland as operational backup. The observational components of PDEMS comprise two portable electronic weather stations and wind and temperature profiling sondes that can be deployed near an infected premises (IP) in the event of an incursion, to provide wind data for NAME-III.

Testing the system

In July 2018, a week-long exercise was conducted to test the new NAME-III-based version of PDEMS and see if it was fit to replace the older system (Turner et al. 2017). The aims of the exercise were to verify that the new PDEMS was comparable with the older version when using both the NZCSM-derived meteorological forecast data and observed winds at two IPs, and to refine configurations of the NAME-III component of the new version to achieve an optimal balance of speed and accuracy. The exercise was designed to be as realistic as possible, in that a target time of 4 hours was set for NIWA to undertake the modelling and deliver results to MPI after receiving daily viral emission profiles. In addition, the exercise tested the deployment of NIWA field teams and a portable electronic weather station (EWS) at a pig farm in Canterbury (one of the hypothetical source farms), which telemetered data back to NIWA where it was assimilated into one of the PDEMS simulations.

The evaluation took place over the week of 15–19 July 2018 at two real pig farms in Canterbury, designated IP1 and IP2, with 5,290 and 933 pigs respectively. Two specially configured EWSs were established 100 m apart at IP1 on 14 July and dismantled after midday on 20 July. Aerial profiles in the lowest 100 m of the boundary layer using wind sondes were made at several times on 15 and 16 July. These also measured drift in the surface layer at those times and also served as a point of comparison.

Various configurations of both old and new PDEMS versions were tested from 14 to 19 July with the aims of:

- achieving comparable results that would be easily usable by a GIS,
- optimal timing to produce results,
- a good comparison with "EWS mast-data-only" runs, where NAME-III was primarily forced with observations from the mast data at IP1; (these were done for 15–19 July) and
- evaluating data and how they were to be used for field surveillance.

Outputs from the viral plume concentration models were delivered to MPI via an ASCII raster layer and mapped in ArcGIS. Plumes were categorised by the thresholds (Alexandersen et al. 2003) required to infect four different susceptible species (**Table 1**.)

Results

Performance of the new model

The PDEMS model provided daily 24-hour average viral concentrations (DACs) across the study sites and the number of days when DACs were above thresholds that can infect cattle, deer, sheep and pigs. The test compared the shapes of the plumes and the area under the plumes where concentrations were above the threshold for infecting cattle for each day. Maps of these plumes from both PDEMS versions for each day of the test are shown in **Figures 2 and 3**.

Generally, the shapes of the plumes were broadly similar between the upgraded and older versions on most days, with most dispersion being in the south and southeast sectors, but there were some differences even among the "observation-driven" runs. This is because each configuration makes subtly different assumptions about meteorological parameters such as cloud cover that affect the amount of turbulence in the environment and thus the amount of mixing.

The upgraded NAME-III-based PDEMS plumes were around 40–70 percent the size of the older version. This was expected and is consistent with the results of an international inter-model comparison exercise of FMD-spread models. It was due to the conservative assumption of worst-case turbulent conditions in the older version. An example of such an assumption was that nocturnal cloud cover was always presumed to be zero, meaning clear nights and the development of strong inversions resulting in high night-time and early morning viral concentrations.

The NZCSM-based models make no assumptions and use realistic forecasts of cloud after initialising with satellite-observed cloud cover.

These maps also show the 3-km and 10-km buffer zones around the IPs to highlight how knowledge of the plumes may influence surveillance and organism management activities during an outbreak. It is current New Zealand policy that farms within the 3-km zone would be automatically placed on surveillance, owing to the risk of local spread. The 10-km zone corresponds to the area that would automatically fall under strict movement control, with hygiene stations at the perimeter when an IP is declared. A farm situated under the plume within the 3-km zone would likely be prioritised for greater surveillance than other farms within this zone. Properties with susceptible species under the plume outside of the 3-km zone would also need to be under surveillance.

To evaluate the performance of the input meteorological forecast data from NZCSM to the new PDEMS, a comparison of key variables against observed data from the deployed EWS at IP1 is shown in **Figure 4**. During the test period there was very little rainfall observed and this was also seen in the NZCSM forecast data (not shown). There was also generally good agreement with the wind speed and wind direction forecast data (interpolated to the IP1 location from the raw model grid data). Comparisons with the profiler drift observations made on 2 days during the experiment (**Figure 5**) were also encouraging, with all configurations producing plumes consistent with the profiler drift.

Modelling time

The time for the new PDEMS to complete all of its forecasts was under an hour – well within the requirement of 4 hours. The new PDEMS also has the advantage that it can be easily deployed to multiple processors on the supercomputer when results for different dates and/or source farms are needed. This ability to do multiple runs simultaneously could be very useful at the start of a response, as historic runs to track possible spread before detection might be required.

Discussion

Based on the test results, the new NAME-III-based PDEMS has been adopted and the old system has been retired. A key advantage to this is the future-proofing of the core dispersion modelling component of the system. NAME-III is a core modelling tool at the UK Met Office, with a dedicated research and development team, and has an active international user base. Further, NIWA is a core member of the Unified Model Partnership (https://www.metoffice.gov.uk/research/collaboration/um-partnership), which ensures it is involved in the future development of these tools and has access to the people and software needed to ensure PDEMS remains an up-to-date and useful service for MPI.

Beyond FMD, NIWA is continuing to add functionality to the PDEMS service, mainly by expanding it to cover a range of airborne pests and pathogens such as myrtle rust, gypsy moth and spider mite, and by developing the delivery of plume maps via a web map service.

Figure 1: Two of the three supercomputers that make up the HPCF are housed at NIWA's Greta Point campus, Wellington (photo: Dave Allen, NIWA)

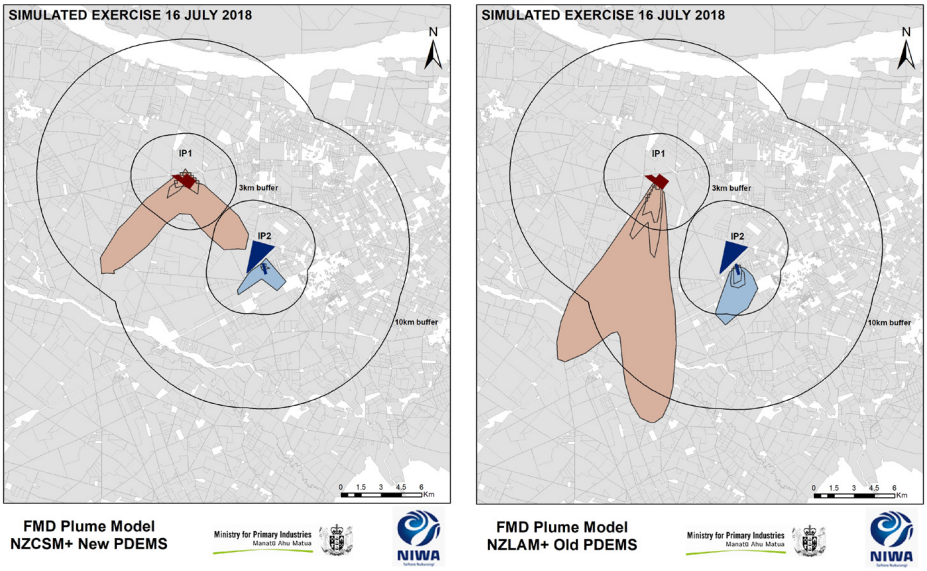

Figure 2: Maps showing the areas where 24-hour average viral plume concentrations exceeded 0.06 TCID50/m^3 (the threshold for infecting cattle) from the two hypothetical FMD viral sources near Burnham on 16 July 2018. These viral plumes were simulated by the upgraded PDEMS (left) and previous version (right). The areas where viral concentrations exceeded 0.06 TCDI50/m^3 were 35 km^2 and 84 km^2 respectively.

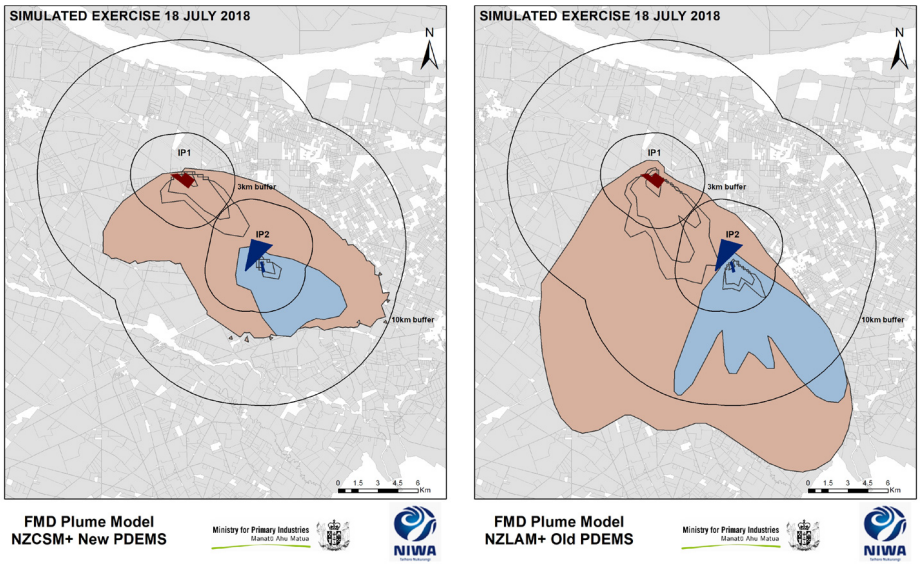

Figure 3: Maps showing the areas where 24-hour average viral plume concentrations exceeded 0.06 TCID50/m^3 (the threshold for infecting cattle) from the two hypothetical FMD viral sources near Burnham on 18 July 2018. These viral plumes were simulated by the upgraded PDEMS (left) and previous version (right). The areas where viral concentrations exceeded 0.06 TCDI50/m^3 were 183 km^2 and 390 km^2 respectively.

Acknowledgements

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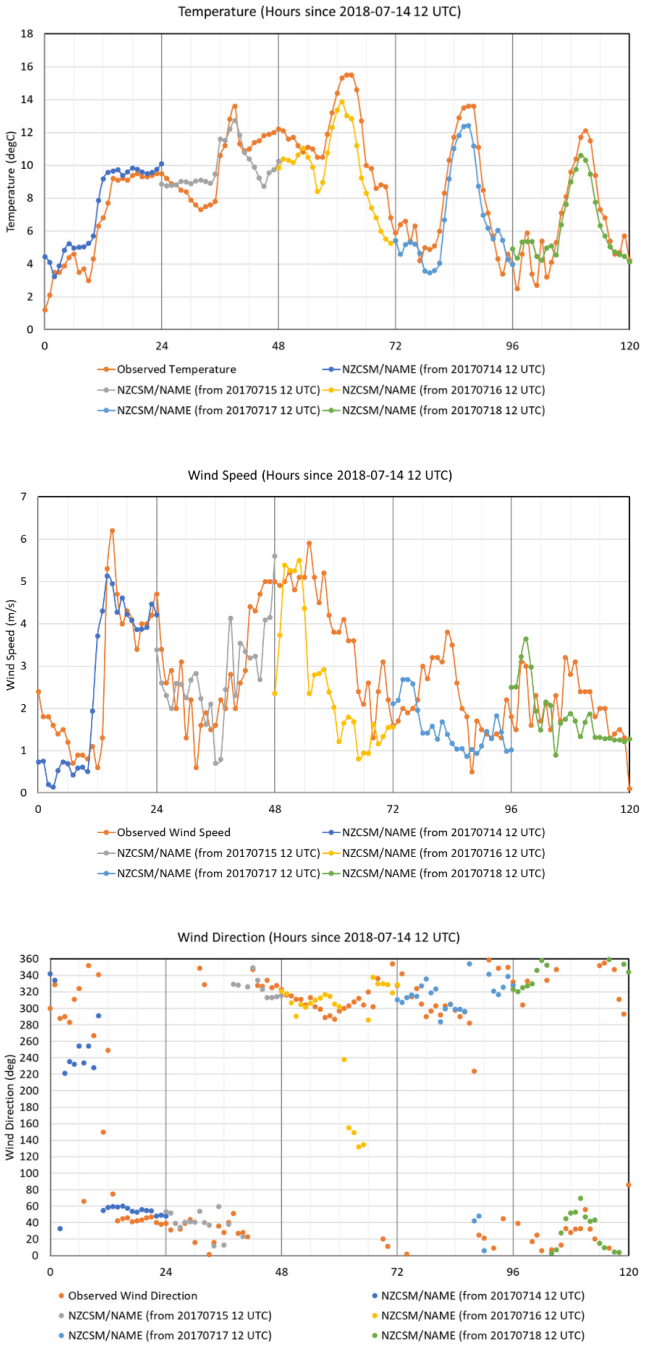

Figure 4: Time series of observed (orange line) and NZCSM forecast surface temperature (top panel), 10 m wind speed (middle), and wind direction (lower) for the duration of the exercise at IP1.

Figure 5: Drift (purple areas) of windsondes from 100 m above surface to ground above hypothetical source at 10:34 am and 11:37 am July 16, 2018 overlaid on Google Earth background. The windsondes were released from an altitude of about 100 m and drifted downwards and downwind to provide wind and temperature profiles in the lowest 100 m of the atmosphere.

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The New Zealand OIE foot-and-mouth disease control programme in Southeast Asia

Introduction

Over the last 3 years staff from the Ministry for Primary Industries (MPI) have assisted with foot-and-mouth disease (FMD) control in Southeast Asia, with a particular focus in Laos (PDR Laos) and Myanmar (**Figures 1 and 2**). Support has included veterinary and technical input, and high-level technical oversight of the programme itself. The NZ Ministry of Foreign Affairs and Trade (MFAT) has committed US$ 11,948,338 over the 6 years the programme will operate, representing one of New Zealand's largest investments in animal health and welfare outside of our borders. The aim is that technical experts from MPI and other NZ agencies will share their expertise and experiences, thus building on our strengths and providing the project with a distinctly NZ profile.

Figure 1: The original NZ team (project leader names for specific organisations in italics) conducting preliminary scoping around baseline activities to be carried out in Laos. From left: Drs Ian Dacre (former Project Coordinator OIE), Daan Vink (former MPI epidemiologist), *Cord Heuer* (Massey Epicentre), Tim Carpenter (former Massey Epicentre), and *Andrew McFadden* (MPI epidemiologist and NZ National Coordinator SEACFMD).

The NZ programme is part of the broader South East Asia and China foot-and-mouth disease (SEACFMD) campaign, run by the OIE (World Animal Health Organisation). MPI's contribution has been formalised through a Memorandum of Understanding with the OIE. The programme broadly follows methods laid out in the EUFMD and FAO framework (endorsed by the OIE) and the Progressive Control Pathway for FMD control, a framework that guides countries where FMD is endemic, through a series of incremental steps to better manage FMD risks (EUFMD/FAO/OIE 2018).

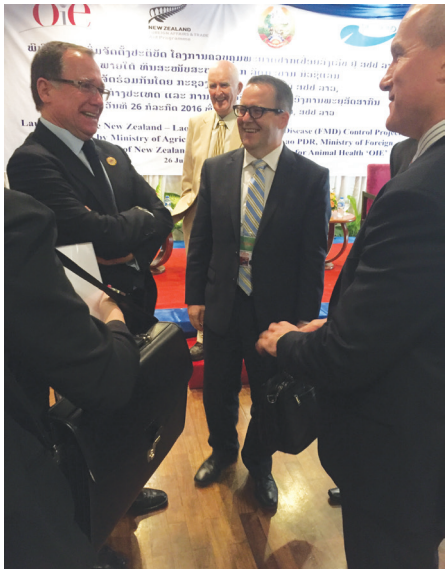

Figure 2: Opening ceremony on 26 June 2016 of the New Zealand funded OIE FMD control programme for Southeast Asia in Vientiane, Lao PDR. Minister Murray McCully (Left front; former Minister for Foreign Affairs and Trade) and Dr Ian Dacre (Right front, former Project Coordinator OIE).

A major development challenge for the livestock production sector in Southeast Asia is control of FMD, a production-limiting disease considered to have significant negative impacts on rural economies and incomes of rural households. FMD here, is also a potential source of introduction of the virus to NZ and other countries that are currently FMD-free. While some countries in the region are recognised as FMD-free (e.g. Indonesia, Singapore and the Philippines), in others FMD is present at varying levels of endemicity, creating the threat of pandemic spread when new virus strains emerge or when transboundary spread into neighbouring countries occurs.

Progress to date

The initial phase of the programme involved testing the theory that FMD could be controlled by targeting specific high-risk areas or "hot spots" of disease. The assessment was about trying to understand how we could use limited resources in the most efficient way. Although the NZ programme is substantial, only a small proportion of livestock in both Myanmar and Laos could, with the available resources, realistically be targeted for vaccination. Thus, a key output was developing efficient methods of FMD control from this pilot study that could be applied at a wider national or regional level to combat spread of disease in the future.

Spatial analytical methods were applied whereby the different spatial risk layers e.g. locations of where previous outbreaks had occurred, cattle density varying with location, location of transport routes where stock are moved etc. were overlaid on one another (added up using spatial techniques) to give an overall spatial risk map. In Myanmar the risk layers were assessed and their relative importance weighted by local knowledge (thus giving more influence of important factors to the overall risk map created). The resulting risk map created from the spatial risk layers (and weighted by local knowledge) was compared with a) current knowledge of where outbreaks had occurred (based on formal notifications of outbreaks to the OIE, village questionnaires on disease occurrence) and b) seroprevalence surveys that had been carried out as part of the programme. The results indicated that in Lao PDR it was not possible to accurately determine the level of risk in a way that could be used to decide where vaccination was most needed (Vink & Dacre 2017; Vink et al. 2018). However, in Myanmar the results indicated that this could be done to some extent.

The data collected as part of these risk assessments, for instance from questionnaires (**Figure 3**), seroprevalence data and post-vaccination monitoring, has informed our understanding of both epidemiology and the impact that FMD was previously having. Analysis has also allowed us to explore novel and efficient methods of surveillance. For instance, interviews with farmers and the village head may give an adequate picture of recent clinical outbreaks more cheaply than the traditional approach of using serological methods.

Figure 3: Survey questionnaires being developed in Myanmar. Local people meet to discuss and work through the questionnaire to ensure respondents will understand questions.

Results so far help us to understand the socio-economic impacts and measure the effectiveness of the vaccination programme in preventing transmission of the virus. At the end of the programme final surveys will be carried out to quantitatively assess the positive effects the programme has had on communities and disease control. The logistics of organising these activities are not simple and MPI staff have been integral to ensuring their success. Some of this organisational role has been through oversight and advocacy by the project steering committee in Myanmar and the project advisory group in Laos.

MPI staff have been involved in training as part of the programme, to improve in-country capability (**Figure 4**). This has generally been done by collaborating with other agencies, e.g. with the Epicentre, Massey University, to produce courses on surveillance, outbreak investigation and spatial analysis using geographical information systems. Training has included development of course material, formal presentations and facilitation of meetings (often in multiple countries).

Figure 4: Dr Kelly Buckle demonstrating sampling of animals to detect FMD in Myanmar as part of a recent outbreak investigation training course.

In addition, new practical applications of biosecurity protocols were developed in conjunction with the New Zealand animal health consultancy Epi-interactive, for use by low-risk FMD vaccination staff (**Figure 5**; Rawdon et al. 2017). The aim was to ensure that FMD virus was not transmitted inadvertently between villages by the vaccination teams. Capability has also been built up in the countries' laboratories, with training in quality assurance and molecular and serological testing by MPI staff, who also assisted directly with some of the baseline serological testing. In doing so our own capability has been developed, as there are no opportunities for our staff to test for FMD in NZ. In addition there are initiatives underway to understand the characteristics and performance of different FMD tests. This helps us fine -tune testing protocols for FMD.

Figure 5: Drs Ian Dacre (left) and Tom Rawdon (right) piloting the biosecurity protocols developed for FMD vaccination teams in Laos and Myanmar.

Periodically during the programme, adjustments have been needed to be made. To have a positive impact an aid programme needs to be sustainable; that is, activities need to continue beyond the life of the programme. When external funds are used for major expenditure such as vaccination, it is important that vaccine purchase can continue when donor funds are no longer available. Otherwise the programme grinds to a halt and the overall benefit of funding may be negligible. The NZ programme has sought to dovetail vaccination funding with increased local production in Myanmar, thus ensuring continuity of the programme once NZ funding has ended. In Lao PDR, where locally produced vaccine is not available, the focus in the remaining years will be on surveillance and response, with future vaccine provision being mainly focused on urgent supply to equip in-country disease-control staff during periods of cyclical outbreaks of FMD. Apart from ensuring sustainability, the NZ team has sought to explore novel methods to control FMD, and to challenge current thinking in search of improved efficiencies and cost savings in the Southeast Asian setting (Heuer et al. 2018).

Future work

MPI will continue to support MFAT as part of the steering committee and by providing technical input into the NZ FMD control programme until its completion in 2020. Another project that MPI will have considerable involvement in, is a pilot study to assess the feasibility of collecting samples from animals slaughtered (at an official slaughter plant) and detecting FMD virus. The objectives of this study will be to:

1. Assess the feasibility of detecting FMD virus from animals slaughtered at a slaughter plant.
2. Use data collected from strain typing and animal location to provide a clearer epidemiological picture of circulating strains.
3. Compare test performance from a pen-side (portable) PCR platform (currently being developed through a NZ-based PhD programme) to normal laboratory-based PCR methods.
4. Collect lesion data and tissue samples (lymph nodes, tonsils, palatine tissue) that may contribute to knowledge of FMDV carrier status in an endemic disease situation.

This work will contribute to research being carried out as part of PhD studies by two MPI staff (Kelly Buckle and Rudi Bueno).

Conclusions

The programme is an example of a synergistic relationship between several NZ government departments and academic organisations to assist with disease control in our regional community. In the course of the programme, MPI staff have gained just as much as they have given. There has been an opportunity to showcase skills to solve complex problems, and to learn how people in different countries and cultural settings solve real-life issues with OIE-listed diseases. Friendships at both a professional and personal level between NZ MPI staff and their overseas counterparts are another important benefit. Mutual respect and understanding different cultures carries

positive international relationships into the future. All of us involved look forward to continuing to develop these friendships during the second part of the programme.

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Quarterly report of diagnostic cases: October to December 2018

SVS Laboratories

Bovine

Two mature Friesian cows from different farms in Matamata-Piako presented for acute onset of blindness caused by hyphaema. Multiple haemorrhages were also seen in the mucous membranes and skin of one animal. Both animals had **thrombocytopenia** and platelet counts were estimated to be 20–40 x 10^9/L in one animal and < 10 x 10^9/L in the other (reference range 220–640 x 10^9). No treatment was given and the cows spontaneously recovered over the next week. Immune-mediated platelet destruction was considered the most likely cause, possibly following exposure to an agent which had adsorbed to platelet surfaces.

A 2-year old heifer from Hauraki presented with a fractured forelimb caused by **copper deficiency**. The post-mortem liver copper level was < 50 umol/kg (reference range 95–2,000). While not common, copper deficiency can be associated with fractures and other musculoskeletal abnormalities such as dropped hocks and flying scapulae.

A 4-month-old Jersey calf from Kaipara presented with bilateral dry sloughing of the ear tips. Biopsies were submitted and showed necro-ulcerative dermatitis with underlying vascular changes characterised by fibrinoid vascular degeneration, thrombosis, oedema and haemorrhage. Differential diagnoses included photosensitisation and ergotism. The submitting veterinarian reported that he had walked the paddock and found weed seed heads infected with ergot, and a clinical diagnosis of **ergotism** was made.

Six animals from a mob of seventy 15-month-old crossbreed heifers from Otorohanga presented clinically with neurological signs and were suspected to have thiamine deficiency or lead toxicity. Two of the animals subsequently died. Blood lead concentrations in three survivors exceeded 0.5 mg/L (toxic level > 0.35), confirming a diagnosis of **lead toxicity**.

A 3-year-old cow from Matamata-Piako presented with a firm 20-cm spherical mass on the lateral thorax. The overlying epidermis was recently ulcerated by trauma. Wedge biopsies submitted for histopathology showed sheets of polygonal cells with variable amounts of brown intracytoplasmic pigment, and a diagnosis of **melanocytoma** was made. Cattle infrequently develop melanocytomas and some of these are congenital neoplasms that are reported in young animals. In general these are benign tumours.

Four calves from the western Bay of Plenty initially presented with increased respiratory rates and harsh lung sounds. The calves died and on-farm post-mortem examinations showed consolidated areas of the lungs with nematodes. Tissue samples were submitted for histology and culture. Histologically, more than 90 percent of the pulmonary architecture was distorted by suppurative and eosinophilic inflammation consistent with **bronchointerstitial pneumonia**. ***Trueperella pyogenes*** was cultured from scattered abscesses. Bronchial lumina contained cross- and tangential sections of adult nematodes, and smaller bronchiolar lumina and adjacent alveoli contained tangential sections of nematode larvae, consistent with **lungworm** infection. Pneumonia is a common cause of morbidity in young calves and is often a multifactorial disease with viral and bacterial components. In this case, lungworm infection, which can be seen in young calves that have been put on grass early, was a likely co-morbidity factor that contributed to the severity of the pneumonia.

A 2-year old steer from Matamata-Piako presented for sudden onset of diffuse dermal lumps that were worse in the neck and groin areas (**Figure 1**). The lesions were not pruritic or suppurative. Cytology of impression smears were diagnostic for **cutaneous lymphoma** characterised by intermediate to large lymphocytes with 2–6 variably sized round to irregular nucleoli, and occasional atypical mitoses. Given the age of the animal, sporadic cutaneous lymphoma (non-BLV-associated) was most likely. These are considered indolent cutaneous lesions in 2–3-year-old cattle.

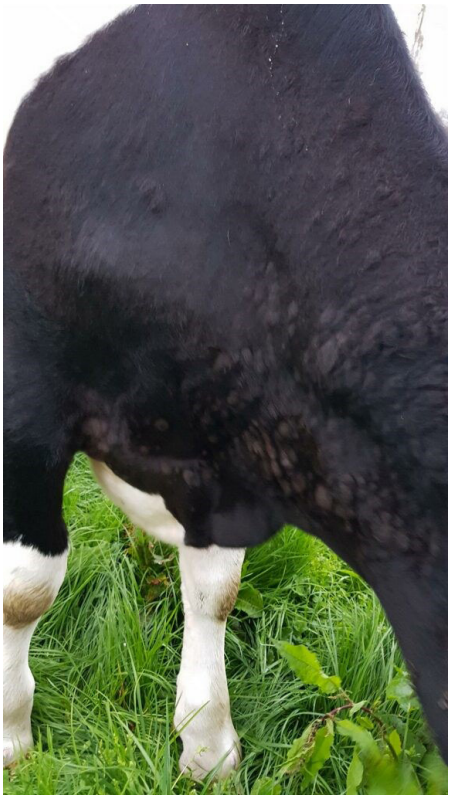

Figure 1. Two-year old steer with skin lumps (photo: Kylie Van Ras, MVP Vets)

Caprine

Five of six goats from Matamata-Piako were positive on ELISA serology for ***Mycobacterium avium ssp. avium*** and a diagnosis of **Johne's disease** was made. The primary clinical presentation of this disease in goats is weight loss, and clinical disease is often triggered by a preceding episode of stress such as parturition or new animals introduced into the herd. Unlike cattle, goats rarely have persistent watery diarrhoea.

A cohort of adult female Saanen goats from Whangarei presented with depression and neurological signs. The brains from two goats were submitted for histopathology. Abscessation, perivascular and meningeal mononuclear inflammation, and rarefaction were seen. In addition, intracytoplasmic gram-positive bacilli were present within

areas of inflammation in the brainstem, consistent with ***Listeria spp.*** infection. Most antibiotics used to treat *Listeria* spp. are readily pumped from the brain by transporters within the blood-brain barrier, so that antibiotics may not reach clinically effective concentrations in the brain, and result in failure to respond to treatment. Also, animals that are recumbent when treatment is initiated respond poorly and have a poor prognosis.

Exotic ruminant

A **cervical leiomyoma**, an **ovarian luteoma** and **cystic remnants of the mesonephric duct** were diagnosed on histopathology following post-mortem examination of an 18-year-old female sitatunga or marshbuck (*Tragelaphus spekii*) from Waikato. The uterus, ovaries, oviduct, urinary bladder and cystic lesion were submitted en bloc for histology. Leiomyomas are benign lesions of smooth-muscle cells and are common in the reproductive tract of many species. Luteomas are ovarian tumours derived from sex-cord stromal (or interstitial) cells. The most distinctive histomorphological feature of this luteoma was the abundant eosinophilic cytoplasm that contained numerous lipid-type steroid vacuoles. Many interstitial cell tumours are reported to be hormonally active. Mesonephric ducts are embryonic structures that are associated with sexual development and can give rise to cysts in all species, including sitatungas. Cystic remnants of these ducts are often an incidental finding at the time of necropsy.

Cervid

Three 1-year-old deer from Tasman presented with weight loss and ill-thrift. Serum was submitted to test for *Mycobacterium avium* ssp. *paratuberculosis* antibodies by the Paralisa test. All three animals had high levels of MAP antibodies, consistent with **Johne's disease**. Disease outbreaks typically affect young deer aged 8–15 months, resulting in reduced growth rates, muscle wasting, ill-thrift and diarrhoea.

About 10 percent of 92 one-year-old deer from Taupo were found to have increased rib fractures following transportation to the abattoir. Ribs were collected and submitted for histologic examination. In each affected deer, at least one costochondral junction was mildly enlarged and the physis, on cut section, was grossly irregular. The most striking histopathologic changes were a lack of mineralisation of the cortex, with increased osteoclastic resorption and an infraction line in at least one animal. A diagnosis of **osteomalacia** was made. These changes are most consistent with calcium deficiency, but in New Zealand this is not common among most grazing ruminants. In this case, the farm was reported to be supplementing phosphorus and the changes may reflect a calcium-to-phosphorus imbalance, with marginal to low normal dietary calcium in the face of excess dietary phosphorus. In other cases, vitamin D deficiency should also be considered. Liver copper analysis was performed to rule out bone disease caused by copper deficiency. Hepatic copper levels in 10 deer ranged from 50 to 190 umol/kg with a median of 115 (reference range 100–2,000, with 60–100 being marginal and < 60 being deficient). While copper concentrations were marginal in three deer and deficient in only one, the histological lesions were not consistent with copper deficiency. However, marginal copper concentrations in some animals could have compounded bone fragility as a result of decreased collagen cross-linking.

Equine

A 1-month old foal from Matamata-Piako had had diarrhoea since birth and developed bilateral tibiotarsal effusions and fever without significant lameness. On chemical analysis, serum amyloid A was 1,652 mg/L (reference range 0–8). A mild band neutrophilia was present and synovial fluid analysis revealed nucleated cell counts (NCC) of 56.3 x 10^9/L and 90.6 x 10^9/L (reference range < 1.0 x 10^9), with neutrophil percentages of 97 and 99 (reference < 10) and total solids (TS) of 49 and 52 g/L (reference range < 15) respectively. Bacteria were not seen on cytology and no organisms were cultured. An initial diagnosis of **suppurative arthritis** was made. Sixty hours after arthroscopic lavage and following regular intra-articular antibiotics, NCC and TS from one joint were essentially unchanged, whereas in the second joint the NCC had decreased to 15.8 x 10^9/L and neutrophils to 91 percent. TS by refractometer remained the same. After a second arthroscopic lavage and over the ensuing 6 days, NCC in both joints ranged from 12.9 to 22.9 x 10^9/L with 76 to 97 percent neutrophils and TS of 41 to 44 g/L. Chemical arthritis was suspected as a cause of chronically elevated NCC, neutrophils and TS. Three days after the final intra-articular antibiotic treatment NCC had fallen to 1.9 and 2.4 x 10^9/L, neutrophil count to 20 and 29 percent, and TS to 35 and 37 g/L. These results confirmed **chemical arthritis** and illustrate the effects of arthroscopy and intra-articular antibiotics on NCC and TS.

An adult gelding from Matamata-Piako had a sinus tumour, with mineral opacity on radiographs. The lesion was surgically excised and diagnosed as a **sinonasal osteosarcoma**. In general, sinonasal sarcomas vary in size but are often large and multilobulated, and may infiltrate into adjacent bony structures, resulting in facial deformities, loss of teeth, exophthalmus and nervous signs. Large neoplasms may also project into the meatus, narrow the lumen and interfere with airflow, resulting in stertorous breathing.

A 12-year-old gelding from Matamata-Piako presented with a mass on its neck. Histomorphology showed the tumour was composed of vascular spaces up to 700 x 600 microns and lined by mature endothelial cells. A diagnosis of **haemangioma** was made. A previous penetrating lesion or infection at this site may have caused a proliferative vascular hyperplasia or a vascular malformation. Regardless of the inciting cause, this type of lesion is slow-growing and complete surgical excision is usually curative.

A 15-year-old mare from Matamata-Piako presented with severe purulent endometritis. On cytology, numerous fungal hyphae, pseudohyphae and yeasts were present, but no bacteria and only rare neutrophils were noted (**Figure 2**). On culture, moderate growth of ***E. coli*** and a heavy growth of ***Candida albicans*** were found. Inter-uterine treatment commenced and 10 days later repeat cytology and culture revealed only a scant growth of staphylococci. **Mycotic endometritis** is uncommon and accounts for 1–5 percent of confirmed cases of equine uterine infections. Infection occurs after a significant disturbance in normal vaginal or uterine defences, or is a result of generalised immunosuppression. The transition from yeast to hyphae is considered a key factor

in the virulence of *C. albicans,* although is not necessary for infection.

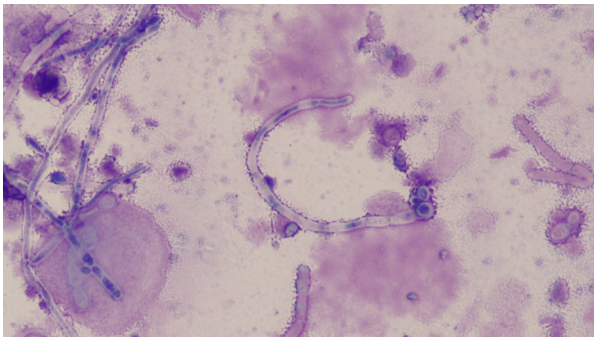

Figure 2: *Candida albicans* hyphae, pseudohyphae and yeast from an equine uterine swab

Uterine swabs were submitted from a 2-year-old Warmblood filly from Waipa for uterine cytology and culture. Moderate numbers of yeast cells were seen on cytology and confirmed as ***Trichosporon sp.*** on culture. A diagnosis of **fungal endometritis** was made. While *Candida* spp. (see previous item) and *Aspergillus* spp. are the most common fungal organisms isolated from equine endometritis, other isolates may include *Trichosporon* spp., *Cryptococcus neoformans* and *Fusarium* spp. Prolonged antibiotic therapy may be a predisposing factor in some cases of fungal endometritis. Transmission of organisms from stallions has not been documented.

A 2-year-old gelding in Waikato presented with round alopecic skin lesions. ***Trichophyton* sp.** was cultured and a diagnosis of **equine dermatophytosis** (ringworm) was made. Dermatophytes can be transmitted via contact with infected hair or from fungi in an environment contaminated by infected horses. Equipment such as brushes, combs, blankets and tack acts as a fomite and readily transmits fungi from one animal to another. Rodents and companion animals also can spread dermatophytosis to horses.

Poultry

Failure of endochondral ossification (**rickets**) was diagnosed in 3-week-old chickens from Waikato. The chickens presented with ongoing lameness and some were found dead. Grossly, birds had expansion of the proximal tibial physis. Histopathology confirmed failure of endochondral ossification characterised by zones of hypertrophy up to 5 mm wide, and limited osteoid deposition. The zone of proliferation had no significant lesions. In this case, histologic lesions support a **phosphorus deficiency**.

About twenty 3-week-old female broilers in a flock of 30,000 birds in Auckland presented with unilateral, apteric lesions with serocellular crusts on one wing. Lesions were submitted for histology. The diagnosis was **ulcerative and granulomatous dermatitis (Blue wing disease),** with cellulitis, myositis and synovitis. In some areas the lesions were characterised by coagulative necrosis, and associated blood vessels contained fibrin thrombi. Given the distribution and histomorphology of the lesions, **trauma** was suspected.

Two-month-old female breeder chickens in Auckland presented with enlarged tibiotarsal joints. On post-mortem examination, the joints had increased serous to gelatinous fluid. Histologically, the synovium was enlarged as a result of synoviocyte proliferation and monocytic inflammation. A diagnosis of lymphoplasmacytic and histiocytic **synovitis** was made. Changes in the synovium were suggestive of avian orthoreovirus (reovirus) or *Mycoplasma synoviae* infection. Reovirus infection is the most common viral cause of arthritis in chickens.

Avian

A **hepatic sarcoma** was diagnosed in an aged adult lovebird (*Agapornis* sp.) from Waikato. The submitting veterinarian noted hepatomegaly during post-mortem examination. On cut sections, the liver lobes were mottled tan/yellow and red. An unencapsulated spindle-cell tumour effaced and compressed the normal hepatic architecture. Sarcomas are the most commonly reported neoplasms in lovebirds and the lack of a primary lesion elsewhere in this bird was unexpected. In addition, white-to-yellow plaques were seen in the air sacs and on the surface of the right lung. Histology showed a granulomatous pneumonia and airsacculitis. ***Penicillium* spp.** were cultured, consistent with **mycotic granulomatous pneumonia and airsacculitis**. While not as common as *Aspergillis spp.* in birds, *Penicillium* is also pathogenic . Both lesions would have contributed to morbidity in this bird and either or both may have been associated with mortality.

Enucleation was performed in an adult kaka in Rotorua owing to loss of vision and chronic ulceration and oedema in the affected eye. Histology was performed and a diagnosis of **endophthalmitis** with ulcerative conjunctivitis, phacolytic uveitis, hyphema, a linearly bisected ossicle and retinal degeneration was made. The sharp and linear laceration through the bisected ossicle was suggestive of **trauma**, with a puncture wound suspected. Trauma would have resulted in the observed hyphema, inflammation and separation of the retina. A common sequela to retinal detachment is cataractous changes in the lens. In this case, the lens was almost fully emulsified.

Canine

A 3-year-old mixed-breed dog from the Bay of Plenty presented with depression and weakness after having gone missing for a week. Bloodwork at presentation showed mildly elevated muscle enzymes, borderline albumin despite evidence of dehydration, and mildly elevated serum potassium. Repeat bloodwork 3 days later revealed a five-fold increase in creatine kinase even though the dog had remained in the veterinary clinic on IV fluids over that time. It transpired that the dog had been fed feral pig meat and a tentative diagnosis of **pig-dog ("go-slow") myopathy** was made.

A 13-year-old spayed female dog in the western Bay of Plenty had a mammary mass and thoracic radiographs were reported as suspicious. Surgical biopsies were submitted for histology and a diagnosis of **inflammatory mammary carcinoma** was made. These carcinomas characteristically invade the superficial dermal lymphatic vessels, as was seen in this case. They are known to produce large amounts of Cox-2, which promotes metastasis via lymphatic and vascular invasion. Inflammatory mammary carcinomas, while rare, are considered the most aggressive malignant mammary carcinoma of dogs and humans. In general, at the time of diagnosis most neoplasms have already metastasised to distant sites including regional lymph nodes, lung (as suspected in this case based on "suspicious" radiographic changes reported by the clinician), liver, spleen and heart. Prognosis is poor to grave.

Three separate adult dogs and a litter of puppies in Tauranga all presented with diarrhoea. In all cases, *Giardia* sp. antigen was detected via faecal ELISA and a diagnosis of **giardiasis** was made. One adult dog was also positive for ***Campylobacter jejuni*** on bacterial culture, and another had **ascarid** eggs on faecal flotation. Giardia, *C. jejuni* and ascariasis are zoonotic, and in-contact persons, especially immunocompromised individuals (including very young and aged persons) were advised to take appropriate precautions.

Feline

Two adult cats in Tauranga and one adult cat in Hamilton presented with diarrhoea. All three tested positive for ***Giardia* sp.** antigen by ELISA and one was also positive for ***Campylobacter jejuni*** following bacterial culture of faeces. As in the canine cases listed above, in-contact persons were advised of the zoonotic potential of these organisms.

Lagomorph

Acariasis (consistent with ***Cheyletiella* sp.**) was diagnosed in an adult male Giant Flemish rabbit in Marlborough after it presented with hair loss. The rabbit was being treated weekly with a topical imidacloprid and moxidectin product (a treatment for *Psoroptes cuniculi* ear mites). A hair pluck was submitted for cytology and showed numerous biting mites that varied in size and developmental stage, with prominent, curved mouthparts and paired legs. *Cheyletiella* spp. are non-burrowing mites that feed on keratinised cells, and infection is colloquially known as walking dander. This is a zoonotic organism and human cases are associated with an infested animal in the household.

Gribbles Veterinary Pathology

Bovine

Three out of 200 bulls died on a Northland farm after showing signs of acute epistaxis, dyspnoea, coughing and anorexia. Necropsy of a 15-month-old Angus cross bull revealed multifocal to coalescing raised yellow nodules throughout the liver, with adhesions. There were also pleuritic adhesions and pulmonary consolidation, raising concern about possible tuberculosis. Histopathological examination of the lung and liver revealed severe subacute necrosuppurative pneumonia and hepatitis (abscesses) with intralesional filamentous bacteria that were Gram negative. They were also acid-fast negative with Ziehl-Neelsen stain, which ruled out *Mycobacterium* spp. No bacteria were isolated on aerobic culture of a liver abscess. However, previous antibiotic treatment may have affected the viability of any bacteria present, preventing isolation. The possibility that the bacteria seen on histopathology were anaerobic could not be ruled out, as anaerobic culture was not performed. The clinical and pathological findings were considered to be consistent with **caudal vena cava syndrome.** This is often associated with initial ruminal acidosis causing bacterial rumenitis with subsequent septic emboli in the caudal vena cava, eventually resulting in liver and lung abscesses. Pulmonary thromboembolism may cause acute haemorrhage and death.

A cow of unspecified age from Northland showed signs considered by the owner to be consistent with polioencephalomalacia. The farm had had four similar cases in the previous week and the brain of the cow was submitted for histopathological examination. There was a moderate subacute encephalitis, predominantly within the brainstem and midbrain and extending along cranial nerves. This was considered to be consistent with a bacterial encephalitis caused by ***Listeria monocytogenes***. This occurs in adult ruminants, usually in the winter and spring. The bacteria are in the environment (especially in silage and soil) and in the faeces of ruminants. It is thought that an 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, or occur in outbreaks associated with feeding of silage.

Two 6-week-old Friesian calves from a group of 38 in Northland died suddenly, one of them after having a seizure. Histopathological examination of a range of tissues from both calves revealed no definitive cause of death, although symmetrical foci of vacuolation were noted in thalamic nuclei near the third ventricle in the brain of one calf. Another calf died subsequently, and histopathological examination of a range of tissues again showed no definitively significant lesions. A diagnosis of lead toxicity was considered, since peracute lead toxicity may not cause any histological lesions. A section of kidney was stained with Ziehl-Neelsen stain and this revealed acid-fast intranuclear inclusions. A sample of fixed liver from that calf was found to contain 16.3 mg/kg of lead (> 5 mg/kg generally considered toxic), confirming the diagnosis of **lead toxicity**. Further investigation revealed the presence of lead-based paint on wooden rails in the calf-rearing shed.

A 10-week-old Friesian cross calf from Hauraki had a hard rostral mandibular mass that appeared to contain fragments of teeth. Histopathology of samples from the mass revealed disorganised and inflamed fibrous, bony and dental tissues, including structures resembling immature teeth. A diagnosis of **compound odontoma** was made. This is thought to be a developmental abnormality or hamartoma, rather than a true neoplasm.

Twenty of 650 calves from North Otago presented with neurological clinical signs and 12 died. Histological examination of the tissues from two of the dead calves revealed changes consistent with **polioencephalomalacia.** This is quite frequently seen in calves, the most common cause being thiamine deficiency, which is thought to be induced by dietary changes including the ingestion of thiaminase-containing plants that cause a functional deficiency of thiamine. Sulphur toxicity may also be implicated, as well as lead and salt toxicity. History, response to treatment (intravenous administration of vitamin B1) and access to known toxins help differentiate between the different causes.

Six animals in a group of 160 three-month-old dairy calves on a mid-Canterbury farm showed neurological signs including blindness and one had died. One of the remaining affected calves was sacrificed and the brain submitted for histological examination. There was patchy bright fluorescence of parts of the cerebral cortex when the brain was examined under ultraviolet light. Histopathological examination revealed lesions typical of **polioencephalomalacia**. This disease is common in calves from November to February each year.

Two of 50 dairy calves on a mid-Canterbury farm were found dead and a third animal that was recumbent and

in respiratory distress died soon after. At necropsy the calves were found to have fibrinous peritonitis and pleuritis. ***Pasteurella multocida*** was recovered in pure culture from the liver of one calf. This episode was typical of **septicaemic pasteurellosis**, which in weaned calves in New Zealand is associated with a specific type B *P. multocida*.

A calf-rearing unit in the Nelson area with 400 calves experienced an acute outbreak of **pneumonia** with 12 sick calves, three of which died. Tissues were received from one dead calf and histological examination revealed a fibrinous pneumonia consistent with either *Histophilus somni* or *Mannheimia haemolytica* infection. A heavy growth of ***Mannheimia haemolytica*** was cultured from the lung. This bacterium is a common isolate from sheep pneumonias but is not so common in pneumonia of cattle in New Zealand.

Several cases of severe anaemia caused by **theileriosis** were diagnosed in individual adult dairy cows from a number of properties in Waikato during this quarter. Clinical theileriosis in New Zealand is most commonly associated with the **Ikeda strain** of ***Theileria orientalis***.

Calf diarrhoea was a common reason for testing in Waikato in this period. In the early spring rotavirus and cryptosporidium were the most common findings. Coccidial infection was more common in animals more than 1 month old. Several outbreaks of gastrointestinal disease in older calves during late spring and early summer (November and December) were reported, and dual infection with **coccidia** and ***Yersinia pseudotuberculosis*** was diagnosed after testing.

A 20 x 15 x 10 cm mass excised from the flank of a 4-month-old male Jersey calf from Taranaki was dark in colour and histological examination confirmed a **melanoma**. Melanocytic tumours make up only about 5 percent of all bovine tumours and most of them occur in the skin and subcutaneous tissue. They tend to occur in young cattle and it is thought that they may have a congenital aetiology. The literature suggests they are expected to exhibit benign behaviour, and complete surgical excision is associated with a favourable outcome.

A Jersey cow presented with a sudden onset of a very large fluctuant fluid-filled lesion on the right side of the neck. There was about 4 litres of serous fluid and necrotic tissue within the mass. Cytological examination of the fluid revealed moderate numbers of neutrophils with very large numbers of bacteria present, mainly rods, confirming a suppurative septic inflammatory lesion **(abscess).** Culture of the fluid yielded a heavy growth of ***Trueperella pyogenes***.

About 10 of 160 weaner calves from the King Country were found dead. These dead calves as well as several other live ones had variably severe **diarrhoea**. Histological examination of tissues from one of the calves revealed a multifocal suppurative enteritis with intralesional colonies of bacteria. There was also severe villus blunting and atrophy. These lesions were considered typical of **yersiniosis.** Culture of the intestinal contents of this calf and also the faeces of three of the other affected calves yielded ***Yersinia pseudotuberculosis***. There were also moderate numbers of coccidial organisms present in all three faecal samples, suggesting that the aetiology may have been multifactorial in this case.

A 6-year-old Friesian cross cow from a farm in Taranaki had reduced milk yield and was inappetant. On rectal examination faeces were normal in consistency but more scant than expected. The cow's temperature was 38.2°C and no abnormal gastrointestinal "pings" were heard. She had tachycardia and a systolic heart murmur. No jugular distension was noted and the jugular pulse was considered normal but the mucus membranes appeared pale. Serum biochemistry revealed a severe hypomagnesemia of 0.09 mmol/L (reference range 0.59–1.08) accompanied by a moderate non-regenerative anaemia with a haematocrit of 0.19 (reference range 0.24–0.46), low haemoglobin (68 g/L; reference range 80–140), low RBC count (3 x 10^{12}/L; reference range 5–7.7 x 10^{12}) and increased numbers of nucleated RBCs (106 per 100 white cells, including occasional rubricytes). A diagnosis of **anaemia caused by hypomagnesemia** (also known as **Taranaki anaemia**) was made.

About 10 of 60 mixed-age South Devon cows from Wairarapa experienced late-term abortions over a 3–4-week period. Histology on three fetuses and associated placentas did not yield significant histological findings. Microbiological culture of the fetal stomach contents was negative. A complete blood count performed on two of the affected cows about 2–3 weeks after they had aborted suggested they were in late recovery from a previous anaemic episode. Organisms consistent with *Theileria* spp. were identified on the blood smear and PCR confirmed the presence of ***Theileria orientalis*** **Ikeda strain**. It was suspected that **theileriosis** may have been the cause of the late-term abortions in these cows, as other common causes were ruled out.

About a hundred rising-2-year-old beef cattle on a North Otago farm broke through a fence onto a kale crop. Over the ensuing days, nine animals died and a further 15 developed neurological signs and recumbency. There were no significant gross findings on necropsy of two of the animals. A full set of formalin-fixed tissue samples was collected. The brains of both animals had areas of rarefaction and neuronal necrosis within the cortical grey matter. Vascular endothelial cells were swollen and vesicular and meningeal and perivascular spaces were infiltrated by gitter cells. These findings were considered typical of **polioencephalomalacia**. Possible mechanisms included disruption of rumen microflora with consequent thiamine deficiency, or excess dietary sulphate.

An outbreak of **sporadic bovine encephalomyelitis** occurred on a North Otago dairy farm. Affected calves were 4–6 weeks old and had been on grass for 2–3 weeks. About 10 calves became weak and pallid, and were straining to defaecate. At least five died. As the outbreak progressed, some calves developed hindlimb ataxia and opisthotonus. Faecal samples from five calves were negative for coccidial oocysts. Serum biochemistry testing on two calves was unremarkable. Necropsy revealed fibrinous exudate within the pericardial and peritoneal cavities. Histopathological examination revealed severe subacute lymphohistiocytic and suppurative meningoencephalitis, polyserositis and vasculitis. The diagnosis was confirmed by the detection of ***Chlamydia pecorum*** by PCR on EDTA blood and pericardial fluid.

Five 2-year-old Friesian heifers on a South Taranaki dairy farm had reduced milk production and became sick and lethargic over a period of several days.

The affected heifers had diarrhoea and inappetance. One heifer died and was necropsied. The caecum and large intestine contained bloody fluid material. Histological evaluation of the distal small intestine and large intestine revealed necrotising and eosinophilic enterocolitis with loss of crypt epithelium and nests of coccidial oocysts. Culture of the liver and faeces was negative for *Salmonella* spp. These findings were compatible with a diagnosis of **coccidiosis.**

Three 2-year-old heifers from a herd of 520 Friesian cows in Manawatu developed proliferative wart-like lesions located mostly on the head and neck. In one case the udder was also affected. The lesions involved both skin and subcutaneous tissue and had a granular texture. A sample was collected from one of the larger masses (15 x 7 x 10 cm), fixed in 10 percent formalin and submitted for histopathological examination. There was pyogranulomatous cellulitis and granulation tissue punctuated by bacterial colonies embedded in characteristic, brightly eosinophilic radiating material (club colonies). These findings confirmed a diagnosis of **actinobacillosis,** which was suspected to have been caused by trauma associated with cattle feeders.

Two unusual malignant tumours were diagnosed in cattle on Taranaki farms during this period. In one case, biopsies were submitted from a large mass in the left flank of an adult Friesian cow. Histological examination revealed highly pleomorphic mesenchymal cells arranged in irregular vascular structures, compatible with a **haemangiosarcoma.** The other tumour presented as marked swelling of the base of the horn in a 13-year-old beef steer. Examination of an incisional biopsy revealed pleomorphic spindle cells arranged among deposits of osteoid and chondroid matrix. The tumour was diagnosed as an **osteosarcoma.** These are rarely reported in cattle and tend to involve the bones of the head.

About 10 percent of a herd of 300 Friesian cross dairy cows on a South Taranaki farm developed proliferative lesions between the toes. Hind and forefeet were affected. Some of the lesions were raw and ulcerated; others crusty and papillated. Five samples were biopsied for histological evaluation and silver staining. All had similar lesions that varied in severity and chronicity, and were characterised by marked epidermal hyperplasia and hyperkeratosis, erosion and ballooning degeneration. Three biopsies had dense swathes of silver-positive **spirochaetes** infiltrating the stratum spinosum and papillary dermis. The histological findings confirmed the clinical suspicion of **bovine digital dermatitis**. Further discussion with the farmer suggested the possibility that the infection had spread through use of a footbath with an inadequate concentration of formalin.

There were several outbreaks of **pinkeye (keratoconjunctivitis**) in cattle in mid and North Canterbury in this quarter. In some cases the herds had been vaccinated against this condition within the last few weeks using an inactivated *Moraxella bovis* oil-emulsion adjuvant vaccine, but new cases were still developing. Most of the outbreaks involved adults but on one property about 10 percent of calves were affected. In one outbreak nearly half of the 900 milking cows on the property were affected over a period of several weeks. When swabs from the eyes of the affected animals in these outbreaks were cultured, the majority were found to be infected with ***Moraxella bovoculi***. *Moraxella bovis* was not isolated. In the case involving calves, IBR virus (bovine herpesvirus-1) was also detected by PCR testing. *M. bovoculi* can be isolated from normal cattle and also from cattle with keratoconjunctivitis, but its exact role in the pathogenesis of disease is uncertain. However, the fact that it was consistently found in these outbreaks is of interest, though unaffected cattle in the peer group were not tested for comparison.

Routine monitoring of copper and selenium concentrations in liver biopsies occasionally revealed some unexpected results this quarter. In one case six animals from a group of mixed-age crossbred cattle in mid Canterbury were tested and had an average liver selenium concentration of 59,397 nmol/kg , with a range from 16,769 to 126,032 (adequate range 850–15,000). Concentrations greater than 32,000 are generally considered to be indicative of **selenium toxicity**. No history was available so the reasons for the excessive concentrations were not determined, but excessive supplementation was most likely.

In late November, a sudden drop in milk production accompanied by loss of condition and watery diarrhoea in mixed-age milking cows on a property in the Waimate district prompted laboratory testing. Faecal samples were received from five animals. ***Salmonella* Bovismorbificans** was isolated from all five, with heavy bacterial growth in four cases, confirming the attending veterinarian's suspicion of **salmonellosis**.

Eighty of 350 milking dairy cows on an Otago farm developed a severe vaginitis. A pooled sample of vaginal material from a number of affected cows was tested, along with a swab from a single affected cow. Both samples were positive for ***Ureaplasma diversum*** by PCR but no comparative testing of unaffected animals was undertaken and the significance of this finding is not clear.

After a group of 700 2–3-month-old Friesian heifer calves were vaccinated with a clostridial vaccine, about 400 developed **injection site lesions** that looked like abscesses. Culture of material from these lesions did not yield any bacteria, and cytological examination revealed a pyogranulomatous inflammatory reaction containing degenerate neutrophils and macrophages but no bacteria could be identified. The vaccine had been injected intramuscularly rather than by the recommended subcutaneous route. Such reactions are often more severe in animals of this age than in older animals, and they usually resolve without treatment over a period of months.

Three 6-week-old unweaned beef calves from a large group on a sheep-and-beef farm in Central Otago were found dead. Necropsy of one of the dead calves revealed it to be in very good body condition. There were pale streaks over the epicardium and several litres of straw-coloured fluid in the pleural cavity. Histopathological examination of the heart revealed changes consistent with **congenital white muscle disease**. The concentration of selenium in the liver of this calf was so low that it was undetectable by our method. There had been management changes on this farm during late winter and it was suspected that the pregnant cows had not received selenium supplementation in late pregnancy that was usual on this property.

Ovine

A lamb from Kumeu had a history of progressively severe lameness over a period of 2 weeks. Radiographs revealed a mottled appearance of the bone and the lamb was euthanased. Within the proximal tibia and the mid cannon bone (metatarsus) there was a mild to moderate subacute **osteomyelitis** with bone remodelling. This was considered likely due to a bacterial infection secondary to bacteraemia originating from an omphalitis.

Two 8-month-old Wiltshire hoggets from the Auckland region died. One showed antemortem signs of acute lethargy, collapse, icterus, pyrexia, tachycardia and tachypnoea. The sheep had been fed a mixture of sheep pellets and tree branches, including trimmings from *Pittosporum* spp. and puriri (*Vitex lucens*). At necropsy, the urinary bladder wall appeared inflamed, the liver was yellow and the kidneys were grey to black. The urine was dark red to black. Antemortem blood samples showed a mild anaemia, with a haematocrit of 0.2 (reference range 0.22–0.4). There was gross and microscopic evidence of haemolysis, including lysed red blood cells seen in the blood smear and an inflammatory leukogram with a neutrophil count of 23.3 x 10^9/L (reference range 0.4–5 x 10^9), monocytes 1.3 x 10^9/L (reference range 0–0.6 x 10^9), eosinophils 2 x 10^9/L (reference range 0–1 x 10^9) and basophils 0.3 x 10^9/L (reference range < 0.1 x 10^9). Serum biochemistry showed mild azotaemia with increased urea (13.3 mmol/L; reference range 3.9–11.3), creatinine (150 µmol/L; reference range 80–145) and bilirubin (185 µmol/L; reference range 0–8). These changes were considered suspicious of haemolytic anaemia with associated haemoglobinuric nephrosis. A sample of fresh liver contained 5,340 µmol/kg of copper (adequate range 95–3,000). Histopathology of samples of liver, kidney and bladder showed a chronic hepatopathy with bridging fibrosis, "copper cells" (pigment-laden macrophages) and hepatocyte karyomegaly as well as haemoglobinuric nephrosis in the kidney and multifocal thrombosis in the bladder wall. A diagnosis of **chronic copper toxicity** was made. It was speculated that this might have been caused by a combination of dietary copper intake, lack of other elements that could reduce copper uptake, and possibly an additional dietary hepatotoxin. Puriri and pittosporum trees are not generally considered toxic but it has been reported that *P. tenuifolium* fed to rats resulted in a weight gain when dried leaves formed 30 percent of the ration, but a loss of weight when this was increased to 60 percent (Connor 1977).

A 3-day-old East Friesian lamb from Otorohanga suddenly died. Histopathological examination revealed lesions in the lungs and liver that were considered typical of **septicaemia.** Omphalophlebitis was considered to be the likely route of infection, but fresh tissues were not cultured so the specific aetiological agent could not be identified.

An outbreak of **leptospirosis** was diagnosed on a Central Hawke's Bay sheep farm. Initially, eight 1-month-old lambs were found dead about 10 days after docking. Reported necropsy findings included yellow (jaundiced) carcasses and dark-coloured kidneys. This correlated with the histological changes, which included hepatocellular necrosis, interstitial nephritis and renal tubular haemaglobin pigment. A MAT test of heart blood taken from a recently dead lamb showed titres of > 1:1,600 to ***Leptospira*** **Pomona** and ***Leptospira*** **Hardjo**.

In early October eight of 40 lambs aged 2–4-weeks on a Waitaki farm died suddenly after vaccination, anthelmintic treatment and selenium supplementation. The investigating veterinarian suspected **acute selenium toxicity**. This diagnosis was confirmed when the liver selenium concentration in one of the lambs was found to be 139,883 nmol/kg (toxic level > 32,000 nmol/kg).

Camelid

Skin biopsies were examined from an adult alpaca in the Auckland area that had had pruritic skin disease for a few months. There was a mild chronic-active hyperkeratotic perivascular dermatitis with rare superficial mites, consistent with ***Chorioptes bovis.***

An 11-year-old alpaca from Northland had a hard mass on the left rump that was slowly increasing in size. Histopathological examination revealed a subcutaneous mass composed of thick and anastomosing bone trabeculae, consistent with an **osteoma**. These are uncommon tumours, mainly found on craniofacial bones. They exhibit slow, progressive growth over months and may persist indefinitely without becoming malignant.

A 2-year-old female alpaca in Waikato had severe chronic skin disease consistent with **hyperkeratosis**. Biopsies of the affected areas confirmed a primary hyperkeratosis with subcorneal intraepidermal and follicular pustules and pyogranulomatous furunculosis considered likely to be from a secondary bacterial infection. Causes of hyperkeratosis without epidermal hyperplasia include zinc-responsive dermatitis, idiopathic hyperkeratosis, ichthyosis and mange. As this animal had had the lesions from an early age and there had been no change during the period of zinc supplementation for facial eczema, a diagnosis of **ichthyosis** was considered most likely.

Cervine

Twelve red deer stags from the Paeroa area had velvet removed and two were found dead the next morning. On histological examination the most significant findings were in the lung and liver. There was marked pulmonary oedema and haemorrhage with eosinophilic infiltrates. Such findings have been described in stags that experience **xylazine-related deaths**. There did not appear to be pre-existing disease in the lung, liver and other examined tissues.

A low copper concentration (51 umol/kg) was found in the liver of a stag from the Paeroa district, which died from xylazine-related complications after velvet removal. Of a further four cohort animals, three also had low serum copper levels, with two of these below the minimum detection level of the test and one marginal. **Copper deficiency** is diagnosed when liver copper concentration is < 60 umol/kg or serum copper is < 5 umol/L. Deer with copper below these levels are at risk of clinical disease or impaired growth rates.

Equine

A **bacterial urinary tract infection** caused by ***Enterococcus*** **spp**. was diagnosed in a 16-year-old mare from the Bay of Plenty. Sediment in a urine sample showed a marked pyuria and bacteriuria, and culture yielded a heavy growth of *Enterococcus* spp. Urinary tract infections are unusual in

horses. In this case there was a chronic neurological and muscular dysfunction of the bladder as a complication of a surgical procedure performed a few years before. *Enterococcus* spp. are commonly found in the lower urinary tract, and compromising factors involving the urinary tract can lead to opportunistic infections.

Avian

A captive male saddleback (*Philesturnus rufusater*) of unspecified age from the Auckland area had intermittent upper respiratory signs for one month. Necropsy revealed a granulomatous lesion in the region of the syrinx. Histopathological examination showed a severe subacute ulcerative necrotising heterophilic tracheitis with intralesional fungal hyphae consistent with *Aspergillus* spp. confirming a **fungal granuloma** and in the mesentery adjacent to the intestines there was a chronic active granuloma with intralesional mixed bacteria. As the bacterial granuloma was older than the fungal infection, the fungal infection was considered to be the cause of death but may have been secondary to immunosuppression from the underlying bacterial infection. The mixed bacteria in the granuloma within the mesentery adjacent to the intestines were considered likely to be enteric bacteria from a previous perforation of the intestinal tract, or possibly from a yolk sac infection.

A zebra finch (*Taeniopygia guttata*) from an Auckland zoological collection was found unable to fly, and then died. Histopathological examination of a range of tissues showed multifocal granulomatous inflammation in the intestines, lungs, liver, skin, bone marrow, skeletal muscle, trachea and spleen. Ziehl-Neelsen staining revealed acid-fast bacilli within macrophages, consistent with **mycobacteriosis**, probably caused by ***Mycobacterium avium***.

A 3-year-old ring-necked lorikeet (*Barnardius zonarius*) from Canterbury was found dead. Histology revealed a suspected **viral hepatopathy** characterised by the presence of large amphophilic intranuclear inclusion bodies within degenerating hepatocytes. PCR on the liver was negative for Pacheco's disease and polyomavirus, but **adenovirus** was not tested for and remains a possible differential in this case. All these viruses can produce similar hepatic lesions with the presence of intranuclear inclusion bodies.

Canine

A 1-year-old male French bulldog from Putaruru developed an **acute diarrhoea** and a faecal sample was received for testing. An ELISA test was positive for **giardia**, **ascarid** eggs were seen on a faecal egg count and ***Campylobacter upsaliensis*** was isolated on bacterial culture. It was considered likely that all these contributed to the diarrhoea, with the ascarid parasitism possibly the most significant of these findings.

A 7-year-old spayed Border Collie farm dog from Taranaki developed clinical signs of jaundice and depression. An MAT antibody titre of 1:1,600 for ***Leptospira* Hardjo** was considered to support a diagnosis of **leptospirosis** along with these clinical signs.

A 3-year-old female Greyhound from Whanganui had persistent diarrhoea, occasional vomiting and lost weight over a period of 3 weeks. ELISA testing of a faecal sample was negative for giardia and cryptosporidium, and a faecal egg count was negative. However, bacterial culture revealed a significant infection with ***Salmonella* Bovismorbificans.**

A male Schnauzer of unknown age from Whanganui dug up and ate a possum that had been killed with a first-generation anticoagulant poison. The activated partial thromboplastin time was increased at 22 seconds (reference range 10–15) and the prothrombin time was increased at 56 seconds (reference range 7–11). This suggested **secondary poisoning by anticoagulants**.

Feline

A 12-year-old female Bengal tiger (*Panthera tigris tigris*) from a Taranaki zoo developed a mass about 8 cm in diameter within the subcutaneous tissue of the neck. Initial fine-needle aspirates revealed a population of large round cells with abundant green-black cytoplasmic pigment on cytological examination. The mass was excised and submitted for histology, which confirmed a densely cellular neoplasm composed of nests, packets and streams of neuroendocrine cells containing variable quantities of cytoplasmic melanin. There were features of malignancy and large central areas of necrosis, confirming a diagnosis of **malignant melanoma**.

A 5-year-old neutered male Domestic Shorthaired cat from Wellington had a 24-hour history of mucoid, foul-smelling diarrhoea but remained bright, responsive and normothermic. A faecal sample was positive for both **giardia** and **cryptosporidium** on ELISA testing and ***Toxocara cati*** eggs (100 per gram) were found in the faeces. It was considered likely that this cat was suffering from a **multifactorial diarrhoea**. The cat was wormed and treated with a course of metronidazole and azithromycin. The diarrhoea stopped and the owner reported that the cat now had much more energy than previously.

Two 2-month-old Burmese kittens (a male and a female) with no abnormal clinical signs were tested because a littermate had died, with necropsy findings suggesting lipaemia and a cardiac defect. There was a marked increase in triglycerides, at 20.54 mmol/L in the female and 14.59 in the male (reference range 0.11–1.42). Haematology tests on the female revealed anaemia (haemoglobin 63 g/L; reference range 80–140, and haematocrit of 0.15; reference range 0.24–0.45). PCR for *Mycoplasma* spp. was negative. The kitten was tested again 16 days later and triglycerides were within normal range, at 0.89 mmol/L. This was therefore considered to be a case of **transient hyperlipidaemia and anaemia syndrome**. The cause is not known (Blackstock 2009).

A faecal sample was tested from a 1-year-old cat from Southland that had had diarrhoea for 5 days. ***Salmonella* Typhimurium phage type 160** was isolated, confirming a diagnosis of **salmonellosis.**

Reptilian

A five-year-old bearded dragon (*Pogona* sp.) from Auckland was lethargic and off-colour. Examination of a blood smear revealed a white blood cell count of 2,368 x 10^9/L, with the majority of the white cells (1,941.8 x 10^9/L) being intermediate to large mononuclear cells with a high nuclear to cytoplasm ratio. This was consistent with **acute leukaemia** of possible monocytic, lymphoid or granulocytic origin. There are several previous reports of leukaemia in bearded dragons in scientific literature.

A 4-year-old female bearded dragon housed in a cage with three others was

found dead. Necropsy revealed a very large gall bladder but no gross lesions. Culture of the contents of the gall bladder yielded a heavy growth of ***Salmonella* Wangata**, a bacterium usually found in poultry. A presumptive diagnosis of **salmonellosis** was made.

Guinea pig

An adult female guinea pig from Auckland was unwell for 3 days then died while having a seizure. It had recently weaned a litter of pups. Histopathological examination revealed, within the lungs, kidney, liver, intestines, brain, heart, stomach and pancreas, numerous epithelial and mononuclear cells with intranuclear or intracytoplasmic eosinophilic viral inclusions. This was considered consistent with guinea pig **cytomegalovirus infection** (cavid herpesvirus-1). There was also a peracute bacterial enteritis. This herpesvirus infection is common in guinea pigs, but is often subclinical or latent. Acute systemic infections can occur in immunocompromised animals, and this can occur in pregnancy. The virus can cross the placenta and cause in-utero infections.

Skin biopsies were examined from an adult guinea pig from Northland with a severe skin condition. There was a severe chronic-active hyperplastic and hyperkeratotic dermatitis with numerous mites, consistent with **acariasis**. The mites were considered most likely to be ***Trixacarus caviae*** (guinea pig sarcoptic mange mite), which can be zoonotic. Other possibilities include *Chirodiscoides caviae* (guinea pig fur mite), which is not generally considered zoonotic.

New Zealand Veterinary Pathology

Bovine

Two calves 2–3 weeks old from a group of about 30 in Waikato presented with a 2-3-days' history of scouring with no pyrexia. Faecal samples from the two affected animals were received and ***Salmonella* Bovismorbificans** was cultured from both. Cryptosporidium oocysts were not detected, both calves were negative for coronavirus and one of the pair was positive for **rotavirus** on ELISA. It is possible that the rotavirus was contributing to clinical signs in one animal.

Several Friesian/Jersey cross calves about 2 months of age in Waikato presented with sudden-onset inappetance, dehydration (hollow eyes), sometimes a mild scour and no pyrexia. Four of 50 were affected, with two deaths. Faeces and blood samples were received from one of the ill calves, along with multiple formalin-fixed samples from one of the dead calves. Faecal analysis revealed light to moderate numbers of **coccidia** oocysts, and faecal culture was negative for *Yersinia* sp. and *Salmonella* sp. Histology of the ileum and colon revealed moderate to abundant coccidial oocysts, macrogamonts, and occasional large schizonts in the mucosa, accompanied by marked necrosis and eosinophilic to lymphoplasmacytic enteritis. A diagnosis of marked **coccidiosis** was made, with associated necrotising enteritis that may also have led to septicaemia in the dead animal.

A single 6-month-old crossbred calf from a group of 30 in Waikato was much smaller than the rest of the mob, with severe emaciation and evidence of chronic scouring. On post-mortem examination the abomasal wall was thickened, with fluid gut contents. Faecal egg count demonstrated severe **endoparasitism**, with 7,500 strongyle eggs per gram. Faecal culture did not yield significant bacterial pathogens, and histopathology of liver, lung, kidney and heart did not reveal any further significant findings.

A mob of 20 beef bulls in the Far North had loss of condition affecting five animals. A pooled liver-fluke antibody ELISA test yielded a high positive result (%S/P > 150%), which is correlated with a high prevalence of **liver fluke** infection (interpreted as > 50 percent of the animals in the pool infected, although this can be skewed, especially in small pools if some individuals have an unusually high burden).

A mob of 40 Friesian calves aged 3–5 months in Waikato had scours, sometimes bloody, affecting six to 10 animals, with one death. Post-mortem examination of the dead calf revealed marked dehydration, with bloody fluid in the large intestine. Faeces were very scant, but a direct smear preparation demonstrated coccidia oocysts. Although tissues for histology were moderately autolysed, vast numbers of coccidial organisms were still visible within epithelial cells. A diagnosis of severe necrotising enteritis consistent with **coccidiosis** was made. Faecal culture did not reveal significant bacterial pathogens.

A single Friesian cow 7–9 years of age from Whangarei had progressive non-responsive nervous disease. She had been off milk and unusually aggressive, with a drooping right ear and circling to the left. The animal was euthanased and samples were submitted for TSE surveillance. Histology of the brain demonstrated a severe chronic encephalitis, consistent with **listeriosis**.

Two rising-2-year-old Friesian bulls from a mob of 300 in the Far North presented with scouring despite recent drenching. No pyrexia was present. Serum copper levels were decreased in both animals, at 6.5 and 5.0 umol/L (reference range 8.0–22.0). One animal was also positive on Johne's ELISA. **Johne's disease** was diagnosed in one animal. However, the sensitivity for this test in clinically affected animals is 85–90 percent so the result for the other animal may have been a false negative. **Copper deficiency** may also result in scouring in adult cattle, and may have also be contributing in these cases.

Three 2-year-old Holstein-Friesian cows from Matamata-Piako presented with a history of skin lesions, with pruritic alopecic and crusty patches of the face and neck, some with purulent exudate. The condition appeared to be spreading among members of the herd. Smears from the lesions were evaluated cytologically, revealing mixed inflammation with structures consistent with dermatophyte arthrospores in two out of three animals. A presumptive diagnosis of **dermatophytosis** was made, based on these findings, although fungal culture failed to confirm this.

An outbreak of conjunctivitis occurred in rising-1-year-old steers on a farm in Waikato. Conjunctival swabs from three affected animals all yielded a moderate growth of ***Moraxella bovis***. This infection is the cause of **pink eye/infectious bovine keratitis**, which occurs most commonly in late spring and summer. Young animals are most susceptible.

A farm in South Waikato had poor growth grates in eight 4-month-old Friesian/Jersey cross calves from a group of 38 animals. Of the affected group, three had increased lung sounds and two had some degree of scour. Animals had previously testing negative to

bovine viral diarrhoea, were regularly drenched with dual oral drench, and received supplementary vitamin B12 and minerals. Faecal analysis showed low numbers of coccidial oocysts in all animals, but a moderate growth of ***Yersinia pseudotuberculosis*** was isolated from faecal culture of all eight animals. **Yersiniosis** was considered most likely to be the primary disease process, although coccidiosis may have been a contributing factor in some animals. Other infections may also have been present, given the increased lung sounds.

A single adult Friesian cross cow from Horowhenua presented with fleshy swelling of the left side of the face. Wedge biopsies submitted for histopathology revealed extensive pyogranulomatous to eosinophilic inflammation, with club colonies surrounded by Splendore-Hoeppli material. A presumptive diagnosis of **actinobacillosis** was made based on histological findings.

Three calves from a group of 40 in Kapiti presented with increased respiratory rate and effort, lethargy and ataxia. One calf was seizuring and was euthanased. Histopathology of the brain revealed areas of laminar cortical necrosis consistent with **polioencephalomalacia**. This lesion can be due to thiamine/vitamin B1 deficiency, which is most commonly seen in well-fed young animals but can also be caused by thiamine deficiency secondary to rumen acidosis. Other causes of polioencephalomalacia include salt poisoning and lead poisoning, and lesions have also been associated with high sulphur intake.

Ovine

A 1-year-old Beltex ram from a group of four in Hamilton died suddenly overnight, and a herdmate had died 1 week earlier following 2 days of illness during which it had failed to respond to empirical treatment with NSAIDs and antibiotics. At postmortem the liver was found to be discoloured orange to brown, with blue to black kidneys and crimson urine. Histology of the liver revealed acute necrotising hepatitis and cholestasis as well as acute haemoglobinuric nephrosis. The liver copper level was 4,300 umol/kg (reference range 95–2,000). A diagnosis of **copper toxicity** was made, with histological evidence that this had led to a haemolytic crisis and subsequent renal injury. The hepatic lesions may have been complicated by a combination of copper toxicity and acute hypoxic injury.

Caprine

A 4-year-old milking goat from Manawatu showed rapid loss of condition and scouring. It was euthanased and post-mortem examination revealed marked mesenteric lymphadenomegaly, sometimes with areas of necrosis. The terminal small intestine was also thickened and appeared corrugated with dilated serosal lymphatic vessels. Histology confirmed granulomatous enteritis and lymphadenitis, as well as multifocal granulomatous hepatitis consistent with **paratuberculosis/Johne's disease**.

Equine

A 7-year-old Warmblood cross mare in Central Hawke's Bay presented with retained fetal membranes after foaling. Biochemistry revealed slightly low serum calcium (2.71 mmol/L, reference range 2.8 –3.3) with normal serum albumin and low serum creatinine (85 umol/L, reference range 97–144), indicating loss of body condition. This degree of **hypocalcaemia** is not associated with hypocalcaemic tetany, but is thought to be a factor in decreased uterine tone or contractility (similar to ileus).

After a 4-year-old Standardbred gelding in Waikato had been grazing on an old shooting range it presented with neurological and respiratory signs. The blood lead level tested on an EDTA sample was 0.48mg/L (toxic level > 0.33), confirming a diagnosis of **lead toxicity.** The peripheral neuropathy associated with lead toxicosis in horses is thought to be caused by peripheral-nerve segmental demyelination, contributing to the clinical signs noted. Respiratory signs can occur owing to laryngeal/pharyngeal paralysis and dysphagia leading to aspiration pneumonia. In cases of chronic toxicity, where blood lead can be below the level considered toxic, diagnosis can be made by initially treating with calcium disodium EDTA, which chelates the lead from bone and increases the levels in plasma and urine.

A 10-week-old Thoroughbred filly foal in Matamata-Piako presented with increased respiratory sounds, which were found on ultrasound scan to be associated with cranial lung lobe consolidation and abscessation. Clinical examination revealed mucopurulent tracheal discharge, which on culture yielded a heavy growth of ***Rhodococcus equi.*** A PCR test for *R. equi* VapA gene (virulence factor) is also available, although this test should not be regarded as a substitute for bacterial cultures, which are required to detect concomitant bacterial infections and for antimicrobial sensitivities.

A 4-week-old Thoroughbred foal in Waikato presented with a markedly enlarged umbilicus, which was unresponsive to antibiotic treatment. Following surgical resection, the umbilical lesion was found to be filled with purulent material that on culture yielded a heavy growth of ***Trueperella pyogenes***.

A yearling Thoroughbred filly and a Thoroughbred foal on an Auckland property presented with submandibular abscesses. Bacterial cultures of abscess swabs from both horses yielded light growths of ***Streptococcus equi*** **ssp.** ***equi***, confirming a diagnosis of **strangles.**

Camelid

A single 9-year-old female alpaca from Napier presented with poor body condition (score 1/5), weakness, pale mucous membranes and increased respiratory rate and noise on auscultation. There were four other alpacas in the group, which were grazed with goats and drenched annually. There was no evidence of scouring, but reduced feed intake had been noted. Clinical signs improved transiently with empirical treatment (antibiotics, anti-inflammatories, vitamin B12), but 5 days later the animal deteriorated. Haematology revealed severe **regenerative anaemia** (haemoglobin 25 g/L, reference range 113–190), numerous nucleated RBCs and moderate polychromasia. Moderate hypoalbuminaemia (25 g/L, reference range 35–44) suggested protein loss (e.g. blood loss, urinary or GIT loss) with concurrent pseudohypocalcaemia caused by loss of some of the albumin-bound fraction of total calcium (1.81 mmol/L, reference range 1.89–2.59). Serum biochemistry demonstrated severe **hypophosphataemia** of 0.18 mmol/L (reference range 1.9–3.4). This could be seen with decreased intake or absorption, and may be seen over winter in thick-coated alpacas in some areas, owing to hypovitaminosis D. Creatinine was also

low (62 umol/L, reference range 72–238), an indication of poor body condition or muscle mass, which was consistent with the clinical history. Faecal analysis showed **strongyle** and ***Trichuris*** eggs (25 and 50 per gram respectively). Although these are quite low numbers that may not be clinically significant in many species, severe clinical disease has been documented with little or no faecal shedding of egg or oocysts (Cebra & Stang, 2008). Clinical signs were explained by the severe anaemia, which may have been multifactorial in this case. The severe hypophosphataemia may have resulted in extravascular haemolysis, but given the management history, endoparasitism may also have been a factor.

Porcine

A single castrated adult male kunekune pig from Wellington was underweight, with a lesion present in the nasal septum associated with a nose ring. One other pig present on the property showed no signs of illness. The underweight pig was euthanased and the nasal lesion was submitted for histopathology. The formalin-fixed specimen received included a 30-mm-diameter ulcerated area with a protruding multinodular mass measuring 30 x 25 mm. Histopathology revealed **squamous cell carcinoma** with secondary inflammation. Squamous cell carcinoma in uncommonly reported in pigs and usually consists of a primary oral neoplasm that has potential for metastasis to regional lymph nodes and beyond (Kleinschmidt, Puff & Baumgärtner, 2006; Swenson et al., 2009).

Rodent

A single guinea pig from Nelson presented with a well-defined area of alopecia and scaling on the rump, with no fluorescence under Wood's lamp. Skin scraping was negative for mites, and a KOH preparation of a hair-pluck did not reveal fungal hyphae or arthrospores. A fungal culture was performed to confirm, and it yielded a growth of ***Trichophyton* sp**. Most clinical cases of dermatophytosis in guinea pigs are caused by *T. mentagrophytes*, although *Microsporum canis* may also cause lesions. *Trichophyton* spp. do not fluoresce under Wood's lamp, which explains the negative result in this case. Dermatophytosis is potentially zoonotic, and spores may persist in the local environment.

Avian

A 23-year-old captive greater spotted kiwi had a 5-day history of inappetance with raspy respiration. Areas of pulmonary haemorrhage and consolidation were identified on post-mortem examination, and fungal culture of lung tissue yielded ***Aspergillus* sp.**, as well as a scant mixed bacterial growth (most likely due to contamination, especially in a post-mortem sample). *Aspergillus* spores can be present in litter or nesting material used by kiwi in captivity – usually only in low numbers but on occasion there may be high environmental loads, increasing the risk of aspergillosis in otherwise healthy animals (Glare, Gartrell, Brookes & Perrott, 2014). It is not known in this case whether the risk may have increased by an underlying disease process or immune suppression.

A conjunctival lump was removed from a 3-month-old kiwi chick in Rotorua. Histopathology of the lesion revealed the tissue to be lined by squamous epithelium with central fibrous tissue containing multiple feather follicles with associated bands of smooth muscle. These findings are consistent with **conjunctival dermoid**, a form of choristoma that has been rarely reported in birds (Busch, 1985). To the author's knowledge, this lesion has not been previously reported in kiwi.

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Quarterly report of investigations of suspected exotic diseases

Exotic disease investigations are managed and reported by the Ministry for Primary Industries (MPI) Diagnostic and Surveillance Services Directorate, Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from October to December 2018.

Exotic vesicular diseases ruled out

A veterinarian in Blenheim called the MPI exotic pest and disease hotline to report oral mucosal erosions, coronary band separation, corneal oedema and bloody urine in a single animal from a mob of 1-year-old cattle. The mob had been in contact with sheep within the previous few weeks. Oral erosions and coronary band lesions can be caused by exotic vesicular diseases such as foot-and-mouth disease, but also by endemic diseases such as malignant catarrhal fever (MCF) caused by ovine herpesvirus-2 (OHV-2). The animal was moribund and subsequently euthanased. A full set of tissues were taken for histology and additionally blood was tested by ELISA for bovine viral diarrhoea virus (BVDV) and by PCR for OVH-2. Histologically, perivascular inflammation was present within the kidney, mucosa and haired skin, consistent with clinical MCF. BVDV ELISA was negative. PCR for OVH-2 was positive. The clinical and epidemiological picture, histological results, and molecular results supported a diagnosis of MCF, and enabled rule-out of exotic disease. The investigation was closed.

A non-farming member of the public called the MPI exotic pest and disease hotline to report having heard secondhand of an outbreak of foot-and-mouth disease on a local orphan lamb-rearing farm. Reportedly one staff member had also contracted the disease. The duty Incursion Investigator identified the veterinary practice servicing the farm. Discussion with two veterinarians who had attended the farm in the previous days and weeks confirmed the Incursion Investigator's suspicion that the notifier was describing an outbreak of orf. Foot-and-mouth disease was ruled out and the investigation closed.

An MPI meat plant veterinarian in Wairoa called the MPI exotic pest and disease hotline to report a burst vesicle-like ulcer on the nose of a single cow from a mob of 29 beef animals. Exotic causes of vesicular disease in cattle include foot-and-mouth disease virus, among others. Endemic causes of ulcers in cattle are many, including infectious and toxic diseases, and trauma. The farmer confirmed that the 29 cattle had been housed together, with no foreign visitors or imports to the farm within the past 3 weeks, and he had seen no disease in any other animal on the farm. The farmer had not noticed the lesion prior to load-out. The notifying veterinarian provided photographs of the affected animal, and closely examined it and the remaining animals, which were all healthy with no lameness or drooling. The affected animal had only the one nasal lesion and no oral or foot lesions. Exotic vesicular disease was excluded based on history and clinical signs in both the affected animal and herdmates, together with epidemiology. The animals were re-checked the following morning and were still all healthy, with no signs of exotic vesicular disease. The mob was slaughtered as per usual practice, and skin from the lesion was collected into formalin for microscopic examination. Blood samples were also collected for exclusion of endemic disease if needed. Histology supported a traumatic cause of the lesion, and no evidence of infectious disease agents was evident. Exotic disease was excluded as a cause of the lesion; trauma was confirmed as the likely cause, and the investigation was closed.

Lumpy skin disease ruled out

A veterinarian called the MPI exotic disease hotline to report a farm where about six out of 400 3-year-old cows were affected with nodular lesions in their hides. An exotic disease investigation was initiated to exclude lumpy skin disease virus (LSDV) as a possible causative agent.

On affected animals multiple lesions were present, which were generally circular in shape, 1–5 cm in diameter) and distributed around the body, but particularly on the neck, shoulder and flank. Another animal had been affected with similar lesions at the same time the previous year.

Several biopsies (fixed and fresh) were collected from three of the affected animals. PCR tests were negative for LSDV and bovine herpesvirus type 2 (the cause of pseudo-lumpy skin disease). Histological sections indicated that demodecosis could have been responsible for some or all of the lesions seen. The most significant feature from all sections was a variable, mixed, often eosinophilic inflammation in the dermis and a nodular granulomatous inflammation with furunculosis. On several sections from one affected cow, demodectic parasites were observed in the dermal layers. Exotic LSDV was excluded and investigators concluded that demodecosis was the likely cause of the lesions observed.

Malignant catarrhal fever confirmed

A veterinarian called the MPI exotic pest and disease hotline to report an outbreak of malignant catarrhal fever (MCF) in a group of 162 heifers. MCF usually presents as sporadic mortality, but outbreaks resulting in a number of affected animals have been recorded (Pardon et al. 2009; Moore et al. 2010). In this outbreak 7 percent (12/162) of animals died over a period of 16 days (9–25 September 2018). A characteristic of the outbreak was that cattle presented with clinical signs after a change in the weather, e.g. following a cold snap. There was no indication that a concurrent condition formed part of the sequelae. No significant changes were seen in CBC or blood biochemistry carried

out on several of the affected animals, and gross pathology and histology of tissues collected were consistent with MCF as a sole diagnosis. Blood from several affected cattle was negative for bovine viral diarrhoea (BVD) by PCR. Conventional PCR for herpesvirus was carried out on the buffy coat of blood in EDTA collected from affected animals. DNA sequencing from the PCR product produced from the reaction confirmed ovine herpesvirus 2.

Anthrax ruled out

A veterinarian contacted MPI about a mixed-age ewe that had died suddenly in transit to a slaughter plant. The ewe was one of 350 in the shipment, with none of the other animals displaying any signs of disease. A blood smear was collected from the dead ewe. No organisms consistent with *Bacillus anthracis* were seen after staining with polychrome methylene blue.

Haemorrhagic septicaemia ruled out

A veterinarian notified MPI via the exotic disease hotline of a farm where cattle were affected with pneumonia that could fit the clinical picture of disease caused by *Mycoplasma bovis* or exotic strains of *Pasteurella multocida* (haemorrhagic septicaemia). Five from a group of 14 three-year-old beef cattle had signs of pneumonia. They had rectal temperatures of > 40°C and appeared to have lost condition. Samples (serum, whole blood and nasal swabs) were collected from the affected cattle but tested negative by PCR for both *M. bovis* and *P. multocida*. Hence, exotic causes of pneumonia were excluded. The animals were later determined to have been affected by lungworm and improved after anthelmintic treatment.

Ischaemic teat necrosis excluded

A dairy veterinarian called the MPI exotic pest and disease hotline to discuss teat lesions in a dairy heifer. The veterinarian was considering whether ischaemic teat necrosis (ITN), an emerging condition in the UK but not yet reported in New Zealand, should be considered as a differential.

One heifer of 70 in a 350-head spring-calving herd developed lesions on two rear teats 7–10 days after calving. Bruise-like lesions commenced at the teat ends before turning black and falling off. There was no smell associated with the lesions and the heifer was not seen to chew or lick at her teats. The heifer did have unusual udder and rear teat conformation. There were no other teat conditions in the herd. Based on the clinical signs and the progression of the lesions, the duty Incursion Investigator and farm veterinarian agreed that the presentation was inconsistent with ITN and the lesions were attributable to cup damage caused at milking by poor sealing which was due to teat and udder conformation. Exotic disease was ruled out and the investigation closed.

ITN was first reported in 2004 (Blowey 2004) and there has been an anecdotal increase during 2014–2015 in mainland UK, where it is seen primarily in heifers during the first 2–3 months of lactation (Clegg et al. 2016). It can affect just a single animal in a herd (Hayley Crosby-Durrani, pers. comm.). Red-to-black lesions develop on the medial aspect of the base of the teat and then may extend down the teat (University of Liverpool, n.d.). There can be intense irritation, with cows licking or chewing at the teats and more severely affected animals removing entire teats. Some report a fetid smell associated with the lesions (Clegg et al. 2016). The pathogenesis and epidemiology of ITN is poorly understood. Digital dermatitis treponemes may be involved (Clegg et al. 2016).

An epidemiological study is currently being conducted in the UK with a view to determining incidence and risk factors. Samples from affected and unaffected animals are also being subjected to metagenomic analysis to attempt to ascertain the potential for an aetiological agent Hayley Crosby-Durrani, pers. comm.

Exotic causes of sheep abortion excluded

A veterinarian from North Canterbury called the MPI exotic pest and disease hotline to report an outbreak of abortions in a large mob of Romney sheep, concurrent with a non-specific lameness in some ewes. Ewes began aborting 2–3 weeks prior to expected lambing, and continued throughout lambing. There was no disease in the ewes. Outbreaks of lameness can be associated with foot-and-mouth disease (FMD) but this was ruled out by negative FMD virus PCR tests on serum from 60 ewes before proceeding with other testing. Outbreaks of abortion can be caused by several exotic agents, including *Chlamydia abortus* (the agent of ovine enzootic abortion disease) and *Coxiella burnetti* (the agent of Q fever), and by endemic infectious agents such as border disease virus (a pestivirus) and the agent of hairy shaker disease, *Campylobacter fetus*. Other possibilities include *Listeria monocytogenes* infection, *Helicobacter* spp., and *Toxoplasma gondii*. Abortion in sheep may also be related to *Leptospira* serovars, particularly *L. interrogans* serovar Pomona.

Serum samples from 60 ewes were tested by ELISA for *Chlamydia* spp. antibodies and for Q fever antibodies, with 12/60 (20 percent) and 36/60 (60 percent) positive respectively. Placental and fetal tissues did not show lesions consistent with Q fever, and tissues from three fetuses were negative for *Coxiella burnetti* by PCR, and it was concluded that the low reactor rate was most likely representative of cross-reaction. The positive reaction to *Chlamydia* did not differentiate between *C. abortus* (exotic) and *C. pecorum* (endemic; the cause of sporadic bovine encephalomyelitis and an occasional cause of sheep abortion). Serology of 25 ewes was also negative for *L.* Pomona. Stomach contents of five fetuses were negative on culture for *Salmonella, Listeria* and *Campylobacter*, and two fetuses were negative by PCR for *Toxoplasma* and *Helicobacter*. Towards the end of the outbreak, a few spindly lambs were identified, and these tested positive by serum PCR for hairy shaker disease, but other fetuses earlier in the outbreak were negative. PCR tests on heart, blood, lung, and placenta of three fetuses were positive for a *C. pecorum*-specific PCR, as were liver, lung, stomach contents and placenta from four fetuses. Immunohistochemistry for *Chlamydia* spp. was positive on the fixed placentas from two aborted lambs, showing *Chlamydia*-like inclusion bodies with positive staining, often associated with areas of inflammation and necrosis. *C. pecorum* was confirmed as the probable agent of this abortion storm, and exotic agents were excluded. The investigation was closed.

EVA ruled out

An MPI Animal Health Laboratory scientist called the exotic pest and disease

hotline to report that one of 18 horses in pre-export quarantine had tested positive for equine viral arteritis (EVA), with a virus neutralisation test (VNT) titre of 1:12. A titre of less than 1:4 is considered negative. New Zealand is free of EVA. Re-testing of the horse at 10-day intervals was unrewarding as serum cytotoxicity impaired test interpretation. In the meantime a review of the horse's history pointed to the test result being a false positive. The horse had been imported from Australia 15 months prior, with a negative EVA VNT. It had not been vaccinated for EVA and had tested negative along with 29 in-contact horses 3 months prior to the positive test. Seventeen horses that had been in contact in the months leading up to the positive test were negative when this horse tested positive. The horse had not displayed any clinical signs consistent with EVA. Another serum sample submitted 5 weeks after the initial positive test yielded an EVA VNT titre of 1:3, confirming that the previous test was most likely a false positive. After natural infection, EVA titres would be expected to remain elevated for years. Exotic disease was ruled out and the investigation closed.

Theileria equi excluded

A racehorse bound for Hong Kong tested positive for *Theileria equi* on pre-export serum antibody testing. A repeat blood was collected and tested for antigen by PCR, and antibody by IFAT. Both tests were negative. The investigation was closed.

An AHL scientist contacted the duty Incursion Investigator to report a *T. equi*-positive indirect fluorescent antibody Test (IFAT) in pre-export testing of a 3-year-old Thoroughbred. The horse had been born in Australia and imported into New Zealand a year earlier. *T. equi*, one of the agents of equine piroplasmosis, is exotic to New Zealand and Australia. The IFAT had been subcontracted to an overseas commercial laboratory. Using competitive enzyme-linked immunosorbent assay (cELISA) testing, the same blood sample had tested negative at the AHL. However, to meet importing country requirements a negative IFAT test was required. Given the inherent difficulties of interpreting the IFAT test, the original blood sample and a repeat blood sample from the horse were submitted to an OIE reference laboratory for piroplasmosis but negative results for *T. equi* were returned on both cELISA and IFAT tests from both samples. Exotic disease was ruled out and the investigation closed.

Streptococcus parauberis confirmed

A commercial veterinary pathologist contacted MPI to report finding an apparently new-to-New Zealand species, *Streptococcus parauberis*, in a culture from an equine hoof abscess by MALDI-TOF. *S. parauberis* had apparently also recently been detected in mastitic bovine milk. This agent is not commonly reported in the veterinary literature, but has been occasionally reported as a pathogen of various species of fish. It is not reportedly emerging, and this detection is considered to be a report of an agent that has been present in New Zealand for some time. The isolate was verified by the AHL and subsequently banked for future reference. The investigation was closed.

Brucella canis excluded

A veterinarian in the Bay of Plenty called the exotic pest and disease hotline to report orchitis in a canine patient. The major exotic cause of orchitis to exclude is the exotic bacterial agent *Brucella canis*. Severe clinical signs required removal of the testicle, which was submitted for histology and revealed the presence of bacterial colonies within the spermatic cord of the teste. This was not suggestive of the patterns expected with infection by *B. canis*. Serology was negative for antibodies to *B. canis* on a *B. canis* card test, further supporting exclusion of this agent as a cause of the orchitis. The investigation was closed.

A veterinary pathologist called the exotic pest and disease hotline to report possible *B. canis* infection in a Wellington-based laboratory Retriever dog. A swollen, painful testicle had been submitted for histological examination. The dog was diagnosed with suppurative and necrotising epididymitis, which can be have a variety of endemic bacterial and non-infectious causes, but also be caused by *B. canis*. A serum sample from the dog tested negative to antibodies for *B. canis*, and the investigation was closed.

Canine heartworm excluded

A veterinarian notified MPI via the exotic disease hotline of a 13-year-old dog with suspected canine heartworm as a potential differential diagnosis for heart disease. The dog had been imported from China 1 year previously. Blood submitted to the AHL (Wallaceville) tested negative for canine heartworm antigen and microfilariae by ELISA and Knotts concentration test respectively.

Canine distemper investigation inconclusive

A veterinary pathologist called the exotic pest and disease hotline to report finding lesions in a dog that were consistent with canine distemper virus infection. The 8-month-old Huntaway presented to his veterinarian with parvovirus-like melaena and distemper-like muscle fasciculations, especially of the front limbs. Autopsy showed frank haemorrhage throughout stomach and gut, and extra-dural haemorrhage in the brain. Microscopically there were regions of inflammation within the lungs and the gastrointestinal system, including intranuclear and cytoplasmic inclusions resembling distemper virus inclusions. PCR of formalin-fixed paraffin-embedded tissues was negative for canine distemper virus, but this assay is not optimised for this sample type, and it was considered it might be a false negative result. Unstained slides were sent overseas for immunohistochemistry (IHC), an assay which stains antibody-specific proteins within fixed tissue. IHC showed clear, strong staining for CDV within lesions of the gastrointestinal system and lungs, confirming the likely causative role of a distemper virus in this dog. The owner did not know whether the dog was vaccinated, as no records had been kept and it had been treated with the other farm dogs. Canine distemper virus is notifiable under the Biosecurity Act 1993, and the virus is considered not to be present in its wild type in New Zealand. The causative agent of the current case is not known but there was speculation that the dog might have had access to seals, which can carry phocine distemper virus; or to mustelids, which have their own distemper virus. A separate case of distemper virus, from January 2018 in Auckland, was likewise confirmed by IHC but had also been negative on PCR. More work remains to be done on these sporadic, suspect cases of distemper in dogs, to clarify the causative agent and whether they are related to vaccination.

Canine distemper excluded

A practicing veterinarian called the MPI exotic pest and disease hotline to report a 5-year-old supposedly unvaccinated entire male Collie/Huntaway dog that had presented with a 3-day history of anorexia, vomiting, diarrhoea, nasal discharge and neurological signs – all consistent with canine distemper virus (CDV) infection. However, the dog was afebrile, haematological exam was unremarkable, it had not been imported and it had had no contact with imported dogs. A nasal swab, EDTA blood, and serum samples were submitted to the Animal AHL (Wallaceville). PCR tests conducted on the EDTA and nasal swabs were CDV-negative. At the same time the one in-contact dog began to display similar signs and was euthanased by the owner. The presented dog recovered after 4 days of symptomatic treatment. Therefore a toxic ingestion event was raised as the most likely cause of the clinical signs in both dogs.

Meanwhile, serology subcontracted to an overseas lab returned a significant titre (384) for CDV in the serum neutralisation test (SNT). A 4-week repeat serum sample was collected and sent to the same overseas lab. Subsequently both the initial serum and the repeat sample were run on the same plate and returned significant but stable SNT titres of 256 and 128 respectively. For antibody titres to be considered significantly different, a fourfold difference must be demonstrated in paired serum samples tested at the same time. The stable titre suggested that that the recent clinical episode in the dog could not be attributed to CDV. Only historical infection or vaccination could explain the stable titres. The MPI investigating veterinarian spoke to the dog owner, who then recalled that in fact the dog had been vaccinated. Exotic disease was ruled out and the investigation closed.

Leptospira interrogans serovar Canicola excluded

An MPI veterinarian contacted the Incursion Investigation team to report a dog possibly infected with *Leptospira interrogans* serovar Canicola, which had died in quarantine after a short illness including vomiting, reduced appetite, and pale mucous membranes. Clinical pathology indicated renal failure was the likely cause of the illness. *L.* Canicola is an exotic cause of renal disease in dogs, and serum from this dog was positive (2:400) to the microscopic agglutination test. This result was followed up by PCR testing for antigen in the urine, which was negative. This indicates that no active shedding was occurring, and makes it unlikely that the dog succumbed to *L.* Canicola infection. The owners refused autopsy of the dog, precluding investigation into the cause of the sudden renal failure. The investigation was closed.

Outbreak of feline vomiting investigated

A Wellington-based veterinarian called the MPI exotic pest and disease hotline to report having been notified about at least 14 cats with self-limiting vomiting lasting a few days to a week, including occasional mild diarrhoea, no other clinical illness and no haematology changes. One month prior to this notification, MPI was notified about vomiting in cats that had occurred immediately after the Canterbury cat show (late August 2018), where up to 55 cats were reportedly affected. As the Wellington case presented with a similar clinical syndrome it was assumed that the two outbreaks were related. Causes of vomiting in cats are not well defined but can potentially include any agent causing inflammation of the gastrointestinal tract and/or metabolic diseases affecting nausea centres. This condition was assumed to be infectious owing to the large number of animals affected and the apparently rapid spread. Worldwide, infectious causes of vomiting in cats include endoparasitism, bacterial infection (e.g. *Helicobacter*-associated disease) and viral infection (e.g. feline panleukopenia). A syndrome matching this one, with acute onset of self-limiting vomiting, is not described in the literature. According to the Wellington veterinarian, there were no significant abnormalities in four cats for which haematology and blood chemistry had been done. Most of the affected cats were reportedly up to date on vaccination. The veterinarian was asked to collect samples for rule-out of common infectious agents but only one faecal sample was collected, probably because sampling took place after the main epidemic. This sample, from an affected cat with blood-tinged vomitus, tested negative by commercial panel for *Clostridia perfringens*, feline coronavirus, feline panleukopaenia, *Giardia* spp., *Salmonella* spp., *Trichomonas* spp., *Campylobacter coli* and *C. jejuni*, but was positive for *Cryptosporidium* spp. Two other affected cats reportedly also tested positive for *Cryptosporidum* spp. on faecal testing. *Cryptosporidium* has not been reportedly associated with vomiting in cats, although it can be associated with diarrhoea in some (Santin 2013). This outbreak of apparently infectious self-limiting vomiting in domestic cats apparently propagated widely at the cat show, and spread thereafter through populations of non-show cats in several regions. However, no cats were reported to have died or experienced any other significant clinical signs. No aetiological agent was identified, although laboratory testing was limited by numbers of samples and the speed and resolution of the outbreak. If further cases occur, they should be reported to MPI in order to facilitate diagnosis. The investigation was closed.

Infectious bursal disease excluded

As part of routine infectious bursal disease (IBD) surveillance carried out by the Poultry Industry of New Zealand, serum reactors in the screening ELISA are tested by VNT at the AHL (Wallaceville). As part of routine monitoring, a free-range broiler barn was found to have 10 serum reactors (from a sample of ten birds collected at processing) in the IDEXX IBD ELISA. Samples were referred to the AHL, where a number of low-positive titres in the VNT (2 x 1:3; 1 x 1:4; 1 x 1:6; 1 x 1:8; 2 x 1:12; 2 x 1:16; 1 x 1:64) were identified. Mortality monitoring and daily bursal evaluation of any mortalities was also carried out by the farm's veterinarian, with no abnormalities detected. At processing, serum samples (25), and fresh (80) and fixed (10) bursae were collected from all barns. Serological screening using the IDEXX IBD ELISA was followed up with VNT testing, with all titres low (predominantly < 1:16). Histopathology of bursas from seropositive flocks showed that follicles were of expected cellularity and organisation, with no evidence of collapse or inflammation as would be expected from pathogenic IBD infection. Testing of all the fresh bursae (pools of 10) from

VNT-positive barns was carried out using real-time PCR for IBD serotypes 1 and 2 (Hein & Trinidad 2006). Follow-up of PCR-suspicious pools was carried out using conventional reference PCRs as described in the OIE Terrestrial Manual (Eterradossi et al. 1998; Le Nouën et al. 2006; Wu et al. 2007) and thereafter using full genomic sequencing of segment A. The full nucleotide sequence when blasted in GenBank had 88–89 percent nucleotide identity to IBDV serotype 2, and 84 percent identity to IBDV serotype 1 viruses. These fndings were considered to have excluded IBD serotype 1 viruses, and to indicate the presence of a poorly characterised avibirnavirus. The OIE IBD Reference Laboratory (Ploufragan-Plouzane, France) was engaged to assist with further characterisation of the virus, and identified that the sequence clustered together with both European and American strains of IBDV serotype 2. The investigation excluded the presence of IBD serotype 1 viruses and identified the presence of an IBDV serotype 2 virus (non-pathogenic). See also the further report on p. 4)

In another case of suspect IBD identified during routine surveillance, a free-range broiler barn was identified with two serum reactors (from a sample of 10 birds collected at processing) in the IDEXX IBD ELISA. Samples were referred to the AHL, where low-positive titres (1:6, 1:12) were identified by VNT. MPI's Incursion Investigation team followed up with a cross-sectional survey of the next batch of birds placed in all barns on the farm (25 sera per barn). Mortality monitoring and bursal evaluation of daily mortalities was carried out by the farm's veterinarian, with no abnormalities detected.

Serological testing of sera from all barns, carried out at AHL using the IDEXX IBD ELISA, identified no reactors, negating the need for further follow-up serological or molecular assessments. Exotic disease was excluded and the investigation was stood down.

Avian mortalities investigated

A Waikato veterinarian called MPI via the exotic pest and disease hotline to discuss two deaths in a backyard chicken flock consisting of fifteen 5-year old Red Shavers. The chickens were noticed to be unwell one morning after having been fine the previous evening. One presented in respiratory distress and was described as making a rattling noise when breathing. Another was hunched over with its head lowered and eyes closed, and had a darkened comb. Both died within 4 hours. Unfortunately the chickens were disposed of and unsuitable for necropsy. Aged modern brown layers are prone to developing a variety of tumours that can present as these did, but two deaths among 15 birds on one day is unusual, so exotic diseases such as avian influenza and Newcastle disease had to be ruled out. The absence of clinical signs among the 13 in-contact birds suggested against exotic disease, but nevertheless cloacal and oropharyngeal swabs from the in-contact birds were requested by the duty Incursion Investigator. These swabs were submitted to the AHL in avian transport medium, and avian Influenza and Newcastle disease were ruled out by PCR testing. The investigation was closed.

Lake Sinai virus variant 2 confirmed

An MPI scientist called the exotic pest and disease hotline to report finding that up to 45 percent of samples tested positive for Lake Sinai virus variant 2 during honey bee hive background disease surveillance. There has been an increase in detections of new or previously unreported bee viruses in New Zealand, as a result of increased research and more sensitive laboratory tests. This is not the first detection of Lake Sinai Virus variant 2 in New Zealand bees, but it is the first time this virus has been found in large numbers of hives. This finding is most likely to indicate a background level of the virus, which has been tenuously but not convincingly associated with disease overseas. The investigation was closed.

Small hive beetle ruled out

An apiary expert phoned MPI to report that a beekeeper had found a beetle resembling small hive beetle (*Aethina tumida*) on the bottom of a beehive in the Marlborough Sounds. A scraping from the bottom of the hive contained many beetles of multiple species but PHEL entomologists determined they were all endemic. The investigation was closed.

EFB ruled out

A beekeeper in Auckland reported a suspect occurrence of half-moon disease in one of his hives. The clinical presentation of half-moon disease is very similar to that of the exotic disease European foulbrood (EFB). The Incursion Investigator arranged for an AsureQuality Apicultural Officer (AO) to discuss the finding with the notifier and assess the likelihood of EFB, which could then be tested for if it was considered a possibility. The AO determined that the hive had lost its queen and now had a laying worker bee in its place. None of the signs observed were consistent with EFB so the investigation was closed.

Exotic ticks investigated

A hospital laboratory technician called the MPI exotic pest and disease hotline to report having received a specimen of a tick from a local doctor. The tick had been removed from the chest of a holidaymaker who had recently returned from rural Queensland. The un-engorged tick was submitted to PHEL (Tamaki) and identified morphologically as belonging to the genus *Ixodes*. Unfortunately it could only be identified to genus level, as it was damaged (mouthparts missing) and the use of formalin to preserve the specimen meant that molecular assays failed. No further ticks were found. Establishment was prevented and the investigation was stood down.

A member of the public in Auckland called the exotic pest and disease hotline to report finding a tick on her dog after a walk at a local reserve. She was concerned that the tick might have transmitted a disease to her dog. The tick was sent to PHEL, where it was identified as the endemic longhorn cattle tick, *Haemaphysalis longicornis*. This tick is not known to be a viable vector for canine disease in New Zealand. This country is free of Lyme disease (caused by *Borrelia burgdorferii*) and many other tick-vectored rickettsial diseases, and the notifier was reassured that her dog was very unlikely to have acquired any disease from this tick. The investigation was closed.

A Marlborough medical centre called the exotic pest and disease hotline to report finding a tick on a New Zealand resident who had returned 2 weeks previously from an 11-day holiday in Queensland. At the request of the duty Incursion Investigator the tick was submitted to the PHEL (Christchurch), where it was identified as an adult female Australian

paralysis tick, *Ixodes holocyclus*. This is the tick most commonly encountered on humans returning from Australia, where its normal range spans almost the entire east coast (Heath & Hardwick 2011). It can cause life-threatening paralysis in domestic animals through envenomation, and in humans it can cause localised swellings, anaphylactic reactions and tick paralysis. It can also transmit *Rickettsia australis*, the agent of Queensland tick typhus, and *Rickettsia honei*, the agent of Flinders Island spotted fever. Recent research has also identified it as a carrier of two novel "*Candidatus Neoehrlichia*" spp. and a novel *Ehrlichia* species (Gofton et al. 2015). The medical practice was advised to raise any human health concerns with a Medical Officer of Health. Rarely is more than one tick found on a returning traveller, and even if there was more than one present, or even tick eggs, the strict bioclimatic requirements of *I. holocyclus* mean that survival or establishment are highly unlikely (Heath & Hardwick, 2011). Therefore with the destruction of this tick the incursion event was terminated and the investigation was closed.

Swine exotic diseases excluded

An Auckland-based veterinarian called the MPI exotic pest and disease hotline to report that a 4-month-old Kunekune piglet was showing clinical signs consistent with several exotic pig diseases. The piglet had been obtained along with a healthy littermate 4 days previously from a breeder. Clinical signs included a fever and cyanotic (blue) ears. Exotic rule-outs for ear cyanosis in pigs include porcine respiratory and reproductive syndrome virus, classical swine fever virus, and African swine fever virus. Endemic causes of ear cyanosis include septicaemia from a number of bacterial causes. Serum and whole blood were submitted to the AHL for antibody and antigen tests respectively, for all three exotic differential diagnoses. All tests were negative and the investigation was closed. The piglet recovered with supportive therapy and was doing well at last re-check.

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Quarterly report of investigations of suspected exotic marine and freshwater diseases: October to December 2018

Exotic marine and freshwater pest and aquatic disease investigations are managed and reported by MPI Diagnostics & Surveillance Directorate, Wallaceville. The following is a summary of investigations of suspected exotic marine and freshwater diseases and pests during the period from October to December 2018.

Didemnum vexillum and *Sabella spallanzani* range extension, Matakana

A regional council biosecurity officer contacted MPI to report what was suspected to be *Didemnum vexillum* and *Sabella spallanzani* on a boat that had been lifted out of the water at Matakana River, Mahurangi. The officer was asked to collect samples, which were submitted to the NIWA Marine Invasives Taxonomic Service (MITS) for identification. MITS confirmed that these species were *D. vexillum* and *S. spallanzani*. This represents a minor range extension for both species. The council was notified and the investigation stood down.

Yersinia ruckeri in salmon, Christchurch

A veterinary pathologist from a private laboratory in Christchurch called the MPI pest and disease hotline to report a positive test for *Yersinia ruckeri* (the aetiological agent of enteric redmouth disease) in salmon samples they had received from a facility. This is an important disease of salmonids and can cause significant economic losses, with different strains of *Y. ruckeri* known to have differing pathogenic effects. The serotype 01b (Biotype 1) of *Y. ruckeri* is considered endemic in New Zealand and has previously been isolated from salmon hatcheries on the east coast of the South Island. It is generally considered a production disease and an indicator of underlying environmental or husbandry issues, which can be prevented by improving water quality and reducing stress on the fish. Endemic *Y. ruckeri* is generally confined to salmon cultured in fresh water, and is not considered a threat to marine systems. Exotic strains, including the Hagerman strain, are Unwanted Organisms under the Biosecurity Act 1993, so an investigation was initiated to rule out the exotic strain. The samples were sent to the Australian Animal Health Laboratory for serotyping, which confirmed the endemic strain of *Y. ruckeri* (01b). As this is an endemic disease, the notifier was contacted and the investigation closed.

Blisters on geoducks

Researchers reported blisters on adult geoducks and warts on juvenile geoducks. The notifier suspected that the blistering might have been caused by mechanical damage. Samples were collected by a warranted MPI official, to legally remove the organisms from a controlled area. Samples of affected and unaffected adults and juveniles were submitted to the MPI Animal Health Laboratory (AHL) for diagnostic testing. In all samples, histology showed mixed populations of fungi, bacteria and other organisms on the surface of the periostracum, which is not unexpected.

A PCR test for apicomplexans (APX) was also carried out on two samples. APX is a large and diverse phylum of parasites, some of which are known to infect shellfish. APX-like cells were observed by histology in all geoducks. Using a generic apicomplexan PCR, two different APX DNA sequences were recovered. One sequence was 99 percent similar to APX, while the other could not be assigned a species-level identification. No exotic disease was identified. The notifier was informed of the findings and the investigation was closed. However, research to identify these parasites to species level and to understand this host-parasite relationship is being carried out.

Sick paua, Chatham Islands

MPI was notified via the exotic pest and disease hotline after wild-caught paua (*Haliotis iris*) from the Chatham Islands were found to have a low meat-to-shell ratio. It was suspected that this was due to localised starvation as a result of recent adverse weather, but there was concern that it could be due to *Perkinsus* infection. *P. olseni* has been observed in New Zealand before, but *P. marinus* is considered exotic. Whole paua were collected from the wild and submitted to MPI's Animal Health Laboratory for disease testing.

Gross pathology showed distinct atrophy of the gut in some of the paua. Ray's culture (*Perkinsus* testing) was negative for both species. Histopathology showed some moderate haemocyte infiltrate around the digestive gland and below the intestinal epithelium. This infiltrate is considered a non-specific reaction to various insults including environmental, pathological and toxic. There were no other indications of disease in the histology slides. Based on the lack of evidence of disease and the presence of organic matter and sand in the foregut of the paua, the signs were attributed to a period of impeded feeding and the investigation was closed.

Unusual starfish, Leigh Harbour

An experienced diver called the MPI exotic pest and disease hotline to report unusual-looking starfish in Leigh Harbour, which he had not seen before. He described them as abundant, "invasive-looking" and said they had a purple tinge. This raised particular concern as it could be a description of the northern Pacific seastar, *Asterias amurensis*, an exotic species that would have significant negative impacts on the marine environment. NIWA divers based in Whangarei and University of Auckland researchers were contacted to see if they had noticed any unusual starfish in the area but all only reported seeing the native *Coscinasterias* and *Patiriella* species.

A local dive shop was contacted with information to ask divers to report unusual starfish to MPI. Given that the NIWA divers had not noticed anything unusual and there were no further reports of unusual starfish in the area, the investigation was closed. The caller was notified that whatever he had seen, it was unlikely to be an exotic species.

Fish mortality, Mangonui

A member of the public called the MPI exotic pest and disease hotline to report a large number of small baitfish that had washed up dead at the south end of Coopers Beach the previous day. There had been no unusual weather in the area recently and there was concern that there might be a disease involved.

Asked to collect samples for disease testing, the caller returned to the beach but found that all of the fish were gone, presumably eaten by birds or washed back out to sea. Without samples, the options for further investigation were limited, but the Northland Regional Council was contacted to see if they knew anything about the case, or significant events that might have happened in the area. They had no knowledge of the event.

Specialists at MPI were contacted to ascertain whether there had been any ichthyotoxic algal blooms in the area. *Alexandrium pacificum* was the only ichthyotoxic species present at the time, but not at concentrations high enough to cause fish mortalities. The nearest samples had been taken from Mangonui wharf, which is close to Coopers Beach but in an estuary so the environmental conditions would be different.

No further mortalities were reported in the following weeks. The mortality was attributed to a one-off event, perhaps related to predation pressure on the baitfish forcing them into the shallows and becoming stranded by the tide. The investigation was closed.

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Plant health surveillance and incursion investigation report

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. Investigators and scientists are based in Auckland, Wellington, Rotorua and Christchurch. These teams provide field investigation, diagnostic testing and technical expertise to detect and report new pests and diseases affecting plants and the environment. They support surveillance and response functions, including carrying out research and development.

Incursion Investigators (IIs) received 309 plant and environment notifications (**Figure 1**) during the 3-month period from October to December 2018, a slight increase compared to the same quarter in 2017 (297). With a 32 percent increase in cases that required investigation (207) compared to the same quarter in 2017 (140), it was a particularly busy period. The investigators immediately stood down 80 cases where the presence of biological risk was able to be ruled out. The remaining 22 notifications were redirected to other agencies who were responsible for the management of the pest concerned.

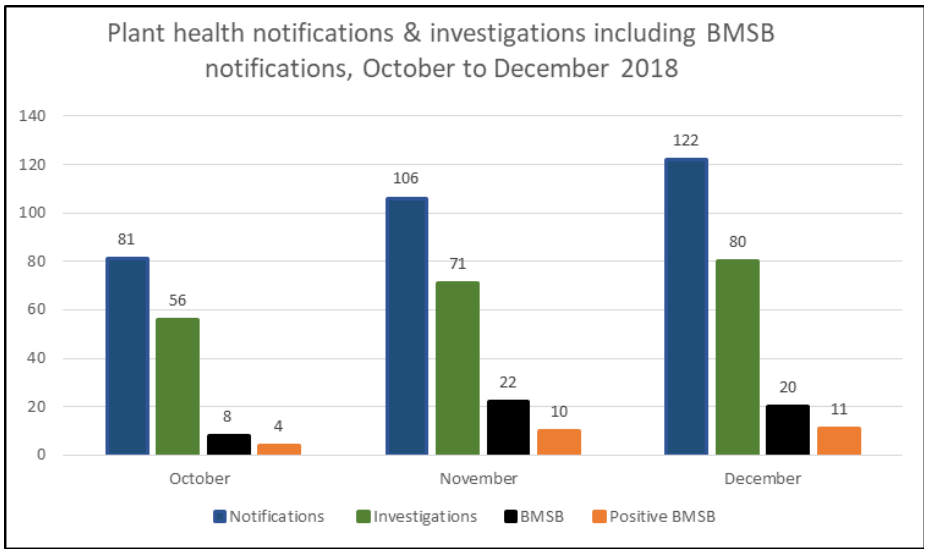

Figure 1: Plant health notifications and investigations and notifications of suspected and confirmed BMSB cases managed by incursion investigators, October to December 2018

Brown marmorated stink bug (BMSB)

This quarter the plant health team received 50 notifications (**Figure 1**) of suspected BMSB, almost double that received for the same period in 2017 (27). Notifications of suspected BMSB are given high priority as this pest is not present in New Zealand and is an Unwanted Organism under the Biosecurity Act 1993. *Halyomorpha halys* (Hemiptera: Pentatomidae) is an agricultural pest native to Asia, notably found in China, Japan and Korea. It has invaded the US and Europe and could successfully establish in New Zealand.

BMSB poses a major threat to New Zealand's primary industries, feeding on more than 300 host plants including citrus, pip fruit, stone fruit,

TABLE 1: CONFIRMED BMSB FINDS, OCTOBER TO DECEMBER 2018

	STATUS	CROSBY DISTRICT
In suitcase from Switzerland	Live	Auckland
In hotel room, occupants from US	Live	Auckland
In boxes of metal ware from China (live YSSB)	Dead	Auckland
On bedroom curtain, after resident visited the US	Live	Auckland
In garage, associated with pram from the US	Live	Auckland
On home appliances from Italy	Live	Auckland
In steamroller from Belgium	Dead	Auckland
On concert gear from the US	Dead	Auckland
Inside home, after visiting Austria	Live	Auckland
In luggage from Italy	Live	Auckland
On wine barrel from Italy	Live	Auckland
In flat-pack goods from Asia	Live	Auckland
In house, not associated with imported goods	Live	Bay of Plenty
In apartment occupied by visitors from US	Live	Bay of Plenty
In printing equipment from Italy	Live	Wairarapa
In imported goods from Italy	Live	Wellington
In clothing from Canada	Live	Wellington
In FedEx package from Michigan, US	Live	Central Otago
On new car imported from US	Live	Nelson
Associated with luggage from Switzerland	Live	Mid Canterbury
On "non-risk" goods from China	Live	Mid Canterbury
In hotel room occupied by visitor from US	Live	Mid Canterbury
In shoes purchased from US	Live	Dunedin
In a hotel room occupied by visitors from US	Live	Otago Lakes
On clothing from Japan	Live	Southland

berries, grapes, asparagus, soybeans, sweetcorn and ornamentals including honeysuckle, hibiscus, maple, cypress and roses. The adults generally feed on mature and immature fruit, while the nymphs feed on leaves, stems and fruit. Insect-feeding severely disfigures fruit crops, rendering them unmarketable, whereas the damage to woody ornamentals is reported as cosmetic only.

BMSB overwinter in large groups in dark, narrow spaces in a wide range of places and on inanimate objects such as used vehicles, containers, farm machinery and personal belongings of travellers, all of which provide potential pathways of entry. The risk season for BMSB interceptions at the border and post-border detections in New Zealand is from September to April inclusive, corresponding with the period in the northern hemisphere when daylength shortens, the weather cools and the insects find places to hibernate. New Zealand's multi-layered biosecurity system has a number of measures to reduce the risk of introducing exotic pests and since zero risk is not possible, the IIs are the first responders to post-border detections and reports of suspect exotic pests. Information on how to recognise and protect New Zealand is accessible on MPI's website and is provided to persons involved with BMSB detections by IIs, who also ask New Zealanders to remain vigilant when dealing with goods that originate from countries known to have BMSB. For more information see https://www.biosecurity.govt.nz/protection-and-response/responding/alerts/brown-marmorated-stink-bug/

Table 1 shows the 25 positive BMSB investigations for this quarter, based on the Crosby boundary definitions recording specimen localities (Crosby et al. 1976). A find of one yellow-spotted stink bug (YSSB, *Erthesina fullo*) incorrectly reported as BMSB is also included. These cases provide an insight into the work undertaken by Incursion Investigators when stink bugs are reported. They highlight the increasing risk associated with travel to New Zealand from countries known to have BMSB, and the importance of general public awareness. The first line of contact is via the exotic pest & disease hotline, 0800 80 99 66. MPI has also implemented BMSB awareness campaigns at international airports, and feedback from notifier's suggests this is effective.

MPI implemented a pilot BMSB surveillance project in October, to run until April 2019, using lures and traps at Transitional Facilities (TFs) where three or more BMSB had been detected in the last 3 years, and in TFs that import high volumes of risk goods such as used cars and machinery from countries with known BMSB populations. Trials completed in the US and survey results from Chile have shown that the lures do not attract BMSB directly to the actual trap, but instead to vegetation nearby. The pilot surveillance provides an opportunity to test the efficacy of the traps and to obtain surveillance data to support the use of practical tools under New Zealand conditions to attempt to reduce the risk of BMSB establishing here undetected.

In some of the cases reported below, traps were deployed. The decision whether to deploy traps or not is unique to each investigation and is based on information gathered from the site inspection, such as possible residual risk and likely outcome related to that risk and, the location and environment of the find.

Following are some examples of BMSB investigations.

BMSB in hotel room of US visitor

MPI was notified via the public enquiry email service, of a suspect BMSB found in a Christchurch hotel room. Photos provided resulted in an immediate response by the Incursion Investigation team. The duty II and a Quarantine Officer (QO) visited the hotel to collect the specimen and completed an inspection of the room and surrounding garden. Another BMSB was found on a curtain inside the room, so an agitation spray was used to entice insect movement. No more BMSB were found in the room or on vegetation outside. MPI's Plant Health & Environment Laboratory (PHEL) entomologists confirmed both bugs were unmated, non-reproductive females. Surveillance traps were deployed in the area and monitored fortnightly for 12 weeks, with no BMSB trapped.

BMSB in goods imported from Italy

In Lower Hutt, staff at a traffic technology company found three live BMSB in two wooden crates from Italy that contained LED message boards. The specimens were subsequently confirmed by PHEL as two males and one unmated female. The staff had taken precautions to contain any other insects before calling the exotic pest and disease hotline. They shrinkwrapped the message boards and kept all the packaging material in a waste bin pending inspection by MPI staff, who found no more bugs. One of the crates had been damaged either in transit or during unpacking and the investigator thought that may have been how the bugs escaped. All goods associated with this detection were fumigated using methyl bromide at a nearby Transitional Facility (TF). After fumigation, a QO inspected the goods and found two dead BMSB and one dead exotic non-regulated spider. A BMSB detector dog and dog handler inspected the warehouse, vegetation in the vicinity and the Wellington freight depot; no further BMSB were found.

BMSB on clothing from Japan

Two suspect live BMSB were found in a rain jacket in the notifier's car at her Mossburn home. The notifier, who was aware of the biosecurity risk, thought she might have accidentally brought the bugs into New Zealand in the jacket after returning from a 5-month trip to Japan. The two bugs had actually been caught and killed 3 weeks before being reported, so no specimens were available for identification. However, she had taken very good photos from which the PHEL entomologists were confident the bugs were *Halyomorpha halys*. The II arranged an inspection of the notifier's personal belongings, house and home garden, and no BMSB were found. Further surveillance for 12 weeks was undertaken using strategically placed traps that were checked fortnightly. No BMSB were trapped and the investigator concluded the BMSB were hitchhikers.

BMSB in flat-pack goods from Asia

A live unmated BMSB was caught inside an Auckland house by the owner after she heard it fly into an internal door. On the previous day she had purchased and unpacked wooden flat-pack furniture and

soft furnishings that had originated in Malaysia, China and Vietnam. After she notified the II, a site inspection by a QO was immediately arranged, along with surveillance of the house, section and vegetation by the BMSB detector dog and handler. No bugs were found. The storage area of the shop where the furniture had been purchased was inspected and no BMSB were found. In the absence of any further finds, it was concluded that this was another single hitchhiker associated with items that came from China.

BMSB in shoes purchased from the US

AsureQuality notified MPI that an Oamaru resident had found many bugs in a pair of shoes purchased online from West Virginia in the US. On finding the insects the man sensibly took the package to the bathroom and shook the shoes over the bathtub. Insects fell out and he placed them in a bag and put them into his freezer until a QO collected them. The shoes were washed and inspected to ensure no insects were remaining. Photos provided to the II were initially identified by PHEL as likely to be of BMSB. When the specimens were received by PHEL it was confirmed that 17 were male and nine were female BMSB. In total 31 insects were found, of which 26 were thought to have been alive when the package was opened. The investigator determined there was no further risk and the investigation was closed.

Investigation positive; establishment prevented through urgent measures

These investigations found organisms not known to be present in New Zealand and in circumstances enabling treatments to be applied to mitigate the biosecurity risk. They typically involved imported goods and containers.

Seed imports

Seed imports are governed by Import Health Standards (IHS), documents issued under section 24A of the Biosecurity Act 1993 setting out requirements that must be met before importing risk goods. Every week the Plant Health team is notified by concerned citizens, Facebook posts and Trademe sales, of seeds being imported illegally via ecommerce. Naive customers are often duped by online offers of weird and wonderful (and cheap) seeds; two recent cases involved "rainbow corn" and "blue strawberry" seeds from China. Unlikely to ever deliver on their promises, these seeds also did not comply with the IHS Seeds for Sowing 155.02.05 so they were destroyed.

There were 18 seed-related notifications this quarter, including:

- bamboo, maize, strawberry, basil and mint seeds purchased from the online shopping company Wish;
- Mexican sour gherkin seeds offered for sale on Trademe;
- packets of carrot seeds as a gift with equestrian jackets ordered from the US;
- cantaloupe, watermelon and radish seeds given to a New Zealand resident by an American traveller;
- lily, capsicum, blackberry and chilli seeds purchased from Aliexpress for a backyard business; and
- pumpkin seeds infested with live insects.

MPI receives excellent intelligence and co-operation from Trademe administrators when breaches of import standards like these are picked up. Trademe is upfront about how and when they share data with third parties. An annual transparency report is available on their website and it details requests they have received and their responses. Public awareness in essential to curtail breaches of IHS, and educational material is provided to offenders.

Seeds mislabelled to imply local origin

Thinking they were buying seeds from New Zealand, a home gardener purchased seeds online from a website with a New Zealand domain name, but the seeds were dispatched from Greece. Contact details on the website were for a business in Invercargill that turned out to have no association with the seller, or any knowledge that its contact details were being used. The seeds received were flame tree (*Delonix regia)* and sensitive plant (*Mimosa pudica*) (Fabales: Fabaceae), and Venus fly trap (*Dionaea muscipula*) (Caryophyllales: Droseraceae). These seeds are all listed as Basic on the MPI Plant Biosecurity Index and were posted to MPI, where they passed inspection and were returned. A search of the website revealed non-basic seeds listed for sale, including species that require post-entry quarantine. The website administrators removed all non-basic listings and the fake contact details as requested by MPI. The domain name provider was also notified of the investigation to take action.

Imported flowers destroyed

The reluctance of a US exporter to provide the correct documentation for imported plant material did not end well for a Queenstown bride. A consignment of preserved roses was sent from the US to Queenstown for a wedding. The flowers were unusual in that they looked like fresh flowers but had been freeze-dried, bleached, dyed and then treated to make them look fresh. The consignment was stopped at the border and a declaration of treatment was requested from the exporter. Instead the exporter sent a second consignment, declared this time as "artificial flowers", and this consignment was released. Later, Queenstown border-clearance staff made the connection between the two consignments and the Incursion Investigation team was notified. The falsely declared "artificial" flowers were seized, inspected and held pending documentation from the manufacturer. Considering the time constraints around the event, the investigator contacted the importer directly to facilitate clearance. However, the manufacturer declined to provide the necessary information, stating that this would require disclosing their proprietary process, so both flower consignments had to be destroyed.

Imported seeds breached IHS

An II was notified by the International Mail Centre (IMC) of a hobbyist gardener who had received 11 packets of incorrectly declared seeds that the IMC had intercepted for destruction. The importer had been flagged as a repeat offender and was visited by the investigator. The hobbyist voluntarily surrendered a number of other seeds that had been imported and were in breach of the IHS. The hobbyist's willingness to comply meant legal action was not taken.

Money plants seized

A Trademe auction for seeds of the Chinese money plant, *Pilea peperomioides* (Rosales: Urticaceae), was reported to MPI. As there is no evidence of the plant being present in NZ prior to 29 July 1998, *P. peperomioides* is deemed a "new to New Zealand organism" under the Hazardous Substances and New Organisms Act 1996 and therefore,

cannot be traded. Trademe was contacted and withdrew the auction. An Incursion Investigator visited the seller at an Auckland property and seized three plants. Information obtained during the investigation resulted in the seizures of 41 plants from a Whangarei property. A third property was visited and in total the investigation resulted in the seizure of 49 plants, which were destroyed.

Banana spider hitchhikes on oil barrels

A suspect huntsman spider was found under a desk at a Christchurch TF that imports automotive oil in large barrels and plastic containers from the US, Australia and Spain. The spider was confirmed by a PHEL entomologist to be the banana spider, *Heteropoda venatoria* (Araneae: Sparassidae), native to tropical regions of the world and present in some subtropical areas as an introduced species, including Australia and the US. Inspection of the area yielded no further specimens and the investigator concluded it was a lone hitchhiker, most likely on goods from Australia or the US.

Irradiated mango seed weevil from Australia

Live insects described as brown and oval in shape were found inside a mango seed from Australia. The description was consistent with the mango seed weevil, *Sternochetus mangiferae* (Coleoptera: Curculionidae), which is a common interception in mangoes, including from Australia. The mango, purchased in Tauranga, had a sticker on it stating the fruit had been irradiated prior to export. Irradiation does not necessarily kill insects, but makes them sterile. Mango seed weevils require mango fruit to complete their life cycle, and since in New Zealand mangoes are not grown commercially, and the fruit had been irradiated, the biosecurity risk was considered to be low. As an added safeguard the notifier was asked to place the weevils in the freezer for 24 hours.

Investigation positive; urgent measures limit harm

These investigations resulted in detection of organisms that are not known to be present in New Zealand and in circumstances enabling treatments to be applied to all retrievable items, usually recent imports. There may be some residual risk associated with items that cannot be retrieved.

Exotic ants from Singapore

During a routine vehicle compliance check at a vehicle testing facility in Nelson, a staff member noticed many small ants inside the rear boot rubber of a vehicle that had recently been imported from Singapore. The ants moved deeper into the vehicle when they were disturbed. Staff sprayed a ring of insecticide on the ground around the vehicle to deter the ants from escaping. That afternoon, a QO visited the site, collected the ants and submitted a sample to PHEL, who confirmed *Tapinoma minutum* (Hymenoptera: Formicidae), an exotic species not present in New Zealand. The entomologist found both larvae and workers in the sample, implying a colony was present. Although *T. minutum* is not on any pest alerts, a study by Nafus (1993) indicates that *T. minutum* preys on eggs and larvae of two butterfly species endemic to Micronesia, resulting in reduced populations. An MPI contractor inspected the facility, laid bait and ant traps and treated the exterior area with insecticide. The vehicle was fumigated to mitigate the risk of any undetected exotic ants.

Exotic ants in containers

In two other investigations involving exotic ants, PHEL confirmed that western carpenter ants, *Camponotus modoc* (Hymenoptera: Formicidae) were present in a container used to import western red cedar from Canada; and crazy ants, *Paratrechina longicornis* (Hymenoptera: Formicidae), were collected from debris and leaves in a container from Taiwan. In the latter case, PHEL identified one queen and many larvae and workers. Both containers were fumigated and the usual urgent measures carried out. On-going surveillance of the three sites detected no further ants, indicating that the urgent measures had mitigated the biosecurity risk.

Jumping spider in grapes

In December, supermarket staff in Kaiapoi found a single live spider inside a bag of green grapes imported from California. The staff checked all their grapes for spiders before putting them out for sale, and found no further spiders. The spider was identified by PHEL as *Phidippus audax* (Araneae: Salticidae), one of the most common jumping spiders in North America. Although not present in New Zealand, it is not a significant biosecurity or human health concern. The Incursion Investigator informed the supermarkets head office staff of the detection and requested that they instruct staff to be watchful for spiders in any remaining grapes from the consignment. Head office staff advised that the grapes had been distributed to many stores throughout New Zealand and some almost a week prior. Five days after the initial detection a second *P. audax* was found in grapes at a Fielding store, suspected to be from the same consignment. Grapes are a rapid-turnover commodity so a product withdrawal was not considered practical. The II raised the issue with staff who deal with imported fresh produce, to raise their awareness.

Investigation positive; no action taken

These investigations revealed organisms that were not previously known to be present in New Zealand, and while they were investigated, no further action was taken. Reasons for taking no action vary according to each case: often a risk assessment indicates that an apparently "new" to New Zealand or newly described indigenous organism is actually well established or has a wide distribution and is unlikely to pose a new risk to economic, environmental, social and cultural values. In some cases the organism is an established and known pest managed under a pest-management programme by MPI and or regional and local authorities.

New to NZ rush fungus

A new to New Zealand fungus, *Stagonospora pseudoperfecta* (Pleosporales: Massarinaceae), was isolated from *Juncus* sp. (rush) collected during a High Risk Site Surveillance (HRSS) inspection in Takanini, Auckland. This species was originally found and described from dead leaves of *Typha latifolia* in Japan and little is known about its biology. While there are known plant pathogenic species in this genus, many other species are also endophytic and beneficial. This fungus was unlikely to be a biosecurity concern and the investigation was closed.

New to NZ peppermint tree fungus

Pseudosydowia eucalypti (Dothideales: Saccotheciaceae), a fungus previously

not considered to be present in New Zealand, was found in *Agonis flexuosa* (peppermint tree) collected during an HRSS inspection in Tauranga. This fungus has been associated with leaf spots on *Eucalyptus* spp. in Australia, Portugal and South Africa. The little published information on its biology and impact suggests that it is unlikely to be a significant pathogen. Based on currently available literature, it is unlikely to be a biosecurity concern and the investigation was closed.

New to NZ grapevine virus

The Crown Research Institute, Plant and Food Research (PFR), reported an organism suspected to be a new sequence for Grapevine virus D (GVD; no GVD full-length sequence is currently available publicly) and a second, new-to-science vitivirus on grapevines. From a sample of a Chardonnay grapevine in the NZ Winegrowers grapevine collection at Lincoln, the virus GVD was previously detected for the first time in New Zealand by MPI. From the same plant, PFR detected what was suspected to be a second vitivirus. From Next Generation Sequencing (NGS) data, two contiguous sequences covering the same region of the genome were assembled. They were found to be only 50% identical to each other at the nucleotide sequence over 1300 bp. Both contigs have Grapevine virus E (GVE) as the closest relative on GeneBank; Contig 1 has 72 percent and Contig 2 has 41 percent amino-acid sequence identity across the N terminus of the movement protein. PFR believes that the Contig 1 was GVD but because there was no available sequence online for the movement protein of GVD it cannot yet confirm this theory. Contig 2 represents a putative novel virus of the same vitivirus genus.

Vitiviruses are common in grapevines, are most commonly represented in infected grapes by GVA, and can be responsible for graft union diseases. They are vectored by mealybugs when in the presence of a member of the Closteroviridae such as Grapevine leafroll-associated virus 3 (GLRaV-3). Two additional Chardonnay plants from the same location were found to have NGS continuing sequence of the same novel virus. These grapevines are in a historic collection that existed in New Zealand before the HSNO Act came into force for new organisms on 29 July 1998, so these suspected viruses are unlikely to be new to New Zealand. Testing by PFR to confirm their findings is intended by undertaking PCR and sequencing of the contigs and to sequence the total genomes of the suspected GVD and the second vitivirus. The results of this work will be communicated to PHEL. New Zealand Winegrowers was informed of this work and will be advised of the results when available. This investigation will be updated when PFR advises additional information.

Australian organics on Trademe

MPI was notified that an Australian company was selling packets of seed and mushroom spawn on Trademe. The listings were removed and Trademe advised the spawn had not been sold previously by this trader. Working with Trademe, the II was able to contact the trader directly. An educational letter was sent to the trader requesting that these items not be listed on Trademe in future as this breaches the Biosecurity Act. The trader replied advising that since being made aware of the biosecurity issues involved in trading certain products, listings of an organic nature had been removed and guidelines had been put in place to ensure they would not be listed in the future.

Beetle larva in garlic

A single live larva was found inside a bulb of garlic imported from the US that had been purchased from a supermarket in Christchurch. The larva was identified by genetic sequencing as the dried fruit beetle, *Carpophilus obsoletus* (Coleoptera: Nitidulidae), an unwanted regulated organism. Tracing of the garlic revealed that all stock from the consignment had been sold. Since there was no stock left to treat and only one larva had been found, the investigation was closed. *Carpophilus* spp. are considered a minor pest of fresh produce and stored products and are found in the US, the Caribbean, Malaysia, Singapore, Taiwan, Japan and Indonesia.

Investigation for high impact pests: negative

These investigations resulted from reports of suspected high-impact pests or diseases that were proven to be "not present".

Delphinium fungus ruled out

Seedling delphinium plants (*Delphinium elatum*) of five different named varieties were submitted to MPI by a specialist producer and breeder of delphinium plants, as they displayed unusual virus-like signs. Testing by PHEL using herbaceous indexing, transmission electron microscopy and molecular DNA techniques found evidence of a virus, but tests specific for all viruses known to infect delphiniums were negative. Although initial results suggested an undescribed Caulimovirus species was present, further testing concluded it not to be a virus but a DNA sequence (part of a Caulimovirus genome) contained within the delphinium genome. It is known that viral fragments (DNA sequences) are sometimes ancestrally integrated into plant-host DNA, and hence passed from one generation to the next. As no functional virus was found, it is likely one or a combination of abiotic factors were responsible for the signs seen. Information was provided on potential seedling trials the breeder might undertake to test abiotic variables (e.g. potting mix, nutrition, temperature), to identify the cause. An abiotic cause is more consistent with symptoms of leaf variegation or yellowing observed in the seedlings, than with leaf mosaic or vein-clearing signs that typically result from Caulimovirus infection. Regardless, the presence of an exotic species was ruled out, and no further action by MPI was warranted.

Tomato hybrid not new to NZ

MPI was notified that an imported hybrid tomato rootstock, *Solanum lycopersicum* x *S. habrochaites*, was a suspect new organism to New Zealand under the HSNO Act. An investigation was opened as one consignment had previously been released and planted and a second consignment had been stopped at the border by MPI because of incorrect import documentation. The rootstock, usually imported as pelleted seeds, is grown for grafting predominantly tomatoes and eggplants under controlled conditions in greenhouses, producing high-value plants. A small number of importers distribute the rootstock to a small group of tomato growers across New Zealand. Biosecurity clearance of a hybrid species requires that both parents are listed on the PBI and meet IHS. In this case, *S. lycopersicum* was listed but

S. habrochaites was not. *S. habrochaites* (syn. *Lycopersicon hirsutum* f. typicum, *L. agrimoniifolium*, *L. hirsutum* var. agrimoniifolium) or wild hairy tomato is a sprawling perennial vine that is cold-tolerant and very resistant to powdery mildews, early blight, bacterial speck, root-knot nematodes and two species of red spider mite.

Further tracing revealed that since at least 2008 the hybrid had unintentionally been imported into New Zealand with incorrect phytosanitary certificates. While preparing a determination application to the Environmental Protection Agency (EPA), historic evidence provided by a tomato exporter showed that the hybrid had been used for tomato breeding in New Zealand long before the HSNO Act came into force. Based on that evidence, and bearing in mind that *S. habrochaites* presented no additional pathogen risk, EPA determined that the hybrids were not new to New Zealand organisms. Hybrid *S. habrochaites x S. lycopersicon* was added to the PBI (Seeds for sowing 'see 155.02.05 under *Solanum lycopersicum*'), allowing its importation to New Zealand. The consignment was released and no further action warranted.

Local beetles in imported timber

Live beetles were reported emerging from imported timber during construction of a deck on a Christchurch residential property. Investigation revealed the timber had been grown in Peru and imported only 3 weeks prior. Specimens were identified by PHEL as *Hylurgus ligniperda* (Coleoptera: Scolytidae), an exotic bark beetle that has been present in New Zealand since the 1970s and is known to colonise recently dead *Pinus radiata*. The investigation concluded that the adult beetles had originated from a nearby recently-harvested stand of *P. radiata* and not the decking timber. Subsequent discussion with a Scion entomologist concluded that the relatively large number of adult beetles seen could be explained by proximity to the recent harvest area, and also by high spring temperatures coinciding with the first seasonal flight of *H. ligniperda* adults. It was thought the adults may have been attracted to the timber stain being used on the deck.

Cocktail mixer with lemon from China

An MPI employee purchased a cocktail mixer containing citrus slices from a supermarket and suspected the product did not meet IHS requirements. Dried citrus products require either a phytosanitary certificate to confirm the country of origin is free of citrus canker prior to inspection by MPI, or a heat-treatment certificate. Neither of these documents could be immediately provided so the products were withdrawn from shelves and held by the supermarket until further investigation and risk assessment could be completed. Import documents correctly declared the cocktail mixers but failed to mention the citrus. Discussion with the exporter in Australia revealed that the dried citrus originated from China and had been heat treated prior to arrival in New Zealand. A conforming heat treatment was presented to MPI and the cocktail mixers were cleared for sale. The importer and customs agent involved were asked in future to check the components of the mixers to ensure accurate documentation was provided to MPI.

Negative and inconclusive investigations

There were 78 notifications involving organisms that posed no biological risk and all these investigations were closed. Common New Zealand organisms often fall into this category and in this quarter included spiders, stored product pests such as weevils; ladybirds, cockroaches, wasps and dragonflies. Reporting by the general public is appreciated and demonstrates their concern for our environment.

Ten investigations were inconclusive and stood down because the results or absence of results did not determine the presence or absence of a biological risk and further investigation was not warranted. Such investigations can result from confusion of the origin of a pest or risk good, or because there was no pest present in the first place. Examples include slugs found in "Shanghai" bok choy that turned out to be locally grown, and spider webs in grapes from the US and Australia on which no spiders were present

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PEST WATCH: 23 October – 1 February 2019

Biosecurity is about managing risks: protecting New Zealand from exotic pests and diseases that could harm our natural resources and primary industries. MPI's Diagnostics and Surveillance Directorate (DSD) devotes much of its time to ensuring that new organism records come to its attention, and to following up as appropriate.

This information was collected from 25 July to 22 October 2018. The plant information is held in the MPI Plant Pest Information Network (PPIN) database. Wherever possible, common names have been included. Records in this format were previously published in the now discontinued magazine *Biosecurity*.

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

Validated new to New Zealand reports

Type	Organism	Host	Location	Submitted by	Comments
bacterium	*Corynebacterium ilicis* no common name	*Entelea arborescens* whau	Auckland	General Surveillance (Landcare Research)	The significance of this bacterium on this plant is unknown.
chromistid	*Phytophthora pseudocryptogea* no common name	*Pinus radiata* radiata pine	Auckland	General Surveillance (SCION)	This species has been present in New Zealand for more than four decades. It has recently been given the new species name.
chromistid	*Plasmopara destructor* no common name	*Impatiens walleriana* impatiens, busy lizzie	Auckland	General Surveillance (PHEL)	This is a member of the *P. obducens* species complex.
fungus	*Aspergillus proliferans* no common name	*Agrostis capillaris* browntop	Mid Canterbury	General Surveillance (PHEL)	This fungus was isolated from *Agrostis capillaris* (common bent) seed.
fungus	*Eutypella citricola* no common name	*Vitis* sp. grapevine	Marlborough	General Surveillance (Landcare Research)	This fungus was initially identified as *E. vitis.*
fungus	*Exophiala oligosperma* no common name	Inanimate	Westland	General Surveillance (AsureQuality)	This is a saprobic fungus and has been recorded on immunocompromised patients.
fungus	*Pseudosydowia eucalypti* no common name	*Agonis flexuosa* agonis, peppermint myrtle	Bay of Plenty	High Risk Site Survey (PHEL)	This fungus has been associated with leaf spots on Eucalyptus spp. in Australia and countries in Africa, Asia, and Europe.
fungus	*Scedosporium minutisporum* no common name	Inanimate	Westland	General Surveillance (AsureQuality)	A saprobic fungus that has been recorded on immunocompromised patients.
fungus	*Seiridium* sp. no common name	*Pinus pinaster* cluster pine	Bay of Plenty	General Surveillance (PHEL)	Likely a species new to science.
fungus	*Stagonospora pseudoperfecta* no common name	*Juncus* sp. rush	Auckland	High Risk Site Survey (PHEL)	Described in 2015.
fungus	*Stagonosporopsis valerianellae* no common name	*Valerianella locusta* corn salad	Mid Canterbury	General Surveillance (AsureQuality)	Also commonly known as *Phoma valerianellae*; infects members of the family Valerianaceae.
insect	*Macarostola ida* leaf-miner moth	*Eucalyptus* sp. gum tree	Auckland	General Surveillance (S Thorpe)	Larvae create leaf mines.
insect	*Mesopsocus immunis* bark louse	*Malus* sp. apple	Mid Canterbury	General Surveillance (PHEL)	Submitted from a fruit-fly surveillance trap.
insect	*Peltoschema* sp. leaf beetle	*Acacia longifolia* Sydney golden wattle	Auckland	General Surveillance (S Thorpe)	Belongs to a species complex that includes *P. orphana, P. suturalis* and a number of other undescribed species.
insect	*Pulvinaria torreyae* soft scale	*Cephalotaxus harringtonia* plum yew	Auckland	General Surveillance (PHEL)	Record from 2016.

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

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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
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Tel: 04 526 5600

Investigation and Diagnostic Centre – Tamaki
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