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

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EDITORIAL

Health surveillance: Complex systems, complex solutions

In this issue of *Surveillance* you can read about some of the outputs of the biosecurity surveillance programmes that MPI operates. However, the articles pay less attention to the management and operation of these programmes. This is not remarkable: despite an increasing number of publications on animal health surveillance, the organisational aspects of the activities performed are comparatively poorly described.

Public health surveillance, as an independent discipline distinct from epidemiology, is considered to have come into its own in the mid-1960s, and has become established as an essential function of public health practice (Declich & Carter, 1994). The development of animal health surveillance has not been as well documented, but follows a similar path. Control of infectious livestock disease has long been regulated: for instance, as long ago as 1884 the US Department of Agriculture implemented regulatory controls to limit livestock diseases after outbreaks of contagious diseases closed some European markets to US meat (USDA-APHIS 2012). While this was not defined or understood as animal health surveillance, the direct application of epidemiological methods to ongoing control of diseases should be considered to be a precursor. Application of the term "surveillance" to animal health became more common from the mid-1980s onwards. The World Organisation for Animal Health (OIE) started using the term in the early 1990s, with Hueston (1993) being the first to present a standardised approach for assessing national surveillance and monitoring systems in the international trade of animals and animal products. The current OIE Terrestrial Animal Health Code contains a chapter on animal health surveillance that provides definitions and descriptions of types of surveillance (OIE, 2015).

The principles of health surveillance are now applied to an ever-widening scope of activities including non-communicable diseases, occupational safety, environmental and plant health, aquatic and marine health, emerging infectious diseases and pharmacosurveillance (Choi, 2012). Arguably, activities such as the monitoring of the welfare of production animals also constitutes surveillance. Since the turn of this century, globalisation of trade and the increases in human and animal movement, in combination with pandemics such as SARS and HPAI, have led to the development of "global health security". Ultimately, the partnership of the OIE, WHO and the Food and Agricultural Organisation (FAO) of the United Nations led to the emergence of the "One Health" concept and inter-agency disease intelligence systems such as the Global Early Warning and Response System (GLEWS)(Corsivi). Today, surveillance is carried out by numerous parties including multilateral organisations, government organisations, healthcare providers, stakeholder groups, industry organisations and individual producers.

Before surveillance can be initiated, consideration must be given to a number of factors related to the objectives, including specific characteristics of the disease/s for which surveillance is performed, diagnostic aspects, the structure and nature of the populations under surveillance, facilities and resources available and international sanitary and trade-driven requirements. This combination of epidemiological and situational factors will determine what and how surveillance is performed. There is a basic distinction between active (investigator-initiated) and passive (observer-initiated) surveillance. However, given the diversity of applications of health surveillance, it is not surprising that many specific types of surveillance have been developed for specific purposes, using a range of terms and concepts; a wealth of publications describing these have been published. There have been introductions of new terms, inconsistent interpretation of existing terms and disparities in the application of concepts. In the field of animal health surveillance alone, this has led to problems in the communication and interpretation of surveillance results that are surprisingly difficult to resolve (Hoinville *et al*., 2013): similar terms may refer to disparate meanings and conversely, different terms may allude to effectively the same thing.

If such a profusion of terminology impedes understanding at the planning and design stage, the organisational systems and procedures that will come into force when a surveillance system is established will likely result in an equally large diversity. Fortunately, the generic definition of health surveillance is sufficiently precise to specify three common features in the implementation: data collection, data analysis and interpretation, and communication of surveillance outputs to stakeholders. This is often referred to as the "surveillance cycle" and it implies a continuous activity. Hence, surveillance systems are run as organised and managed programmes, with defined and standardised operating procedures including a set of protocols defining what actions/responses are undertaken when prescribed or predefined thresholds are exceeded. In practice, these activities (e.g., collection of field samples and data, and laboratory diagnostics) are performed by contracted service providers so that management largely relates to such issues as work agreements, measurement of outputs against milestones, financial monitoring and reporting of the results.

Another aspect on which there is consensus in the literature is the importance of performing periodic evaluation of the system to ensure it functions effectively and meets its stated objectives. Usually, various characteristics of the system are considered in turn and the findings integrated to provide an overall view of its strengths and weaknesses and identify where improvements can be made. These characteristics include:

- the overall importance of the health event under surveillance;

- objectives and components of the system;
- the extent to which the surveillance activities meet these objectives;
- cost; and
- the overall quality of the system, which is further broken down into attributes such as simplicity, flexibility, acceptability, data quality, sensitivity and/or specificity, predictive values positive and/or negative, representativeness and timeliness (Declich and Carter, 1994; CDC, 2001).

Until recently, evaluation processes for animal health surveillance systems had not been standardised, so there were many different approaches. The systematic review of Drewe *et al*. (2012) highlighted the incompleteness of most evaluations reported in the literature. They also remarked that there was substantial inconstancy in the attributes that were assessed, and ascribed this to the range and variability of the surveillance objectives and designs under evaluation. This work was followed up with the development of a proposed evaluation tool called SuRveillance EVALuation (SERVAL)(Drewe *et al*., 2013), which effectively incorporated the attributes mentioned above into a structured framework. The RISKSUR project, an EU-funded project implemented by a consortium of institutions, produced an additional set of outputs including another systematic review of economic assessments and evaluation methods (RISKSUR, 2013a), another evaluation support tool called EVA (RISKSUR, 2013b) and a surveillance design framework (RISKSUR, 2016). Some of the review work was recently published by Calba *et al*., (2015). Where there was once a dearth of information and resources to perform evaluations, there is now an abundance.

The surveillance programmes operated by MPI cover animal, aquatic and marine, and plant and environmental health. In addition, monitoring of food safety and animal welfare, while not strictly classified as surveillance, adopts many of the same principles.

To meet the identified need for periodic evaluation of these programmes, the evaluation frameworks cited above were modified into a bespoke tool called the Surveillance evaluation Framework (SurF). This tool is flexible enough to be applied across the sectors and can be used for different levels or intensity of evaluation. Aside from ensuring regular, appropriate evaluation, the benefit of a standardised tool such as SurF is that it enables the direct comparison of different surveillance programmes. SurF has been trialled and is currently being finalised.

The future: perspectives and developments

While the technical aspects of surveillance design, operation and evaluation have been well described, an area of growing interest is optimisation: combining the outputs from the evaluation with updated information and knowledge including new techniques, changes in the industry, market and political situation and resource budgets. This logical post-evaluation step considers questions such as: is surveillance still required? Does it need to be adapted or recalibrated to be more effective; and if so, what are the options? How can the economic benefit be quantified and can efficiencies be implemented to provide measurably better value for money? What external factors, risks and opportunities impact on the surveillance activity and how can these be influenced, mitigated or maximised, respectively? There is much scope for developing such optimisation methodologies.

It is important not to lose sight of the bigger picture: the national and international contexts in which surveillance programmes are embedded. The interrelationships between the scientific and operational management of these programmes and the political drivers and decision-making processes that influence them remain somewhat nebulous. Governance or oversight is required to monitor the functioning of individual programmes; but more importantly, it should ensure that the combined portfolio of surveillance activities is an integral part of animal and public health strategies at national and regional level. These strategies incorporate non-health-related factors such as international guidelines, regulations and reporting obligations, trade and market access imperatives, domestic economic factors, public/private partnerships, involvement of stakeholder groups, access to data and dissemination of information. These factors may have a greater influence than health-related determinants on the prioritisation of surveillance needs and allocation of resources to surveillance programmes. In a fast-changing world, consideration of such external factors is required to make sure health surveillance is responsive to changes and remains fit for purpose.

Conclusion

Health surveillance has developed rapidly as a discipline and continues to expand, accompanied by new challenges, opportunities and solutions. Recent work has contributed to more effective systems and frameworks for the design and evaluation of surveillance programmes, which contribute to their effectiveness and transparency. The SurF tool will enable these outputs to be applied to New Zealand's surveillance programmes to ensure they are effective and fit for purpose. There is growing recognition that improvements in communication between technical health professionals and non-technical decision-makers and managers will improve health surveillance. Health surveillance systems must remain responsive to change. Their interdisciplinary and multi-faceted nature means that they fulfil the criteria for complex adaptive systems. Further evolution of these ideas and concepts and their implementation will hopefully lead towards integration and standardisation of such systems. To an increasing extent, this is putting One Health into practice.

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Davainea proglottina: a potentially underestimated but significant parasite of free-range poultry

A call to the MPI exotic pest and disease hotline triggered an investigation that identified Davainea proglottina, known as the minute tapeworm or the small chicken tapeworm, as the cause of significant mortality in a backyard chicken flock. Here we report on this potentially underestimated poultry parasite that poses a diagnostic and control challenge for backyard poultry flocks and the increasing numbers of free-range commercial egg-laying operations. Of further significance is that a clinical manifestation of *D. proglottina* infection can resemble that of neurotropic Newcastle disease (ND), an exotic disease in New Zealand.

A medical professional called the MPI exotic pest and disease hotline to report high mortality in a chicken flock whose owner had been diagnosed with influenza. An Incursion Investigator interviewed the flock owner. It transpired that 32 chickens in a flock of 60 (53 percent) had died, mostly at eight to 16 weeks of age and over a six-month period. Typically an affected bird lost weight, "dropped a wing", became recumbent and eventually died. Recumbent chickens still tried to eat despite having difficulty swallowing. Egg production ceased in the flock. Based on the epidemiology and clinical signs (there were no neurological signs in this case), the potential exotic diagnoses of avian influenza and Newcastle disease were ruled out.

The owner's influenza was subsequently typed and confirmed as regular seasonal influenza A, subtype H3N2. Nevertheless, given the high mortality rate in the flock, the investigation proceeded in order to determine the cause.

The flock owner submitted two moribund chickens to a commercial pathology laboratory. Both birds were in poor body condition, with moderate-to-marked atrophy of the pectoral musculature and a lack of abdominal fat. Histopathological findings were most consistent with parasitism but one bird also displayed signs of a terminal septicaemia. Consequently the Incursion Investigator arranged a visit to the property with a poultry specialist.

The affected flock consisted of a mixture of breeds acquired over a two-year period. At the beginning of the outbreak there had been 60 birds ranging in age from one week to three years. The chickens were closed in two coops at night and free-ranged during the day. They were fed a commercial laying-hen-and-pullet feed once a day (in the morning). The flock owner had recently started adding flubendazole dewormer (Flubenol, Janssen Pharmaceuticals) to the diet at three-weekly intervals.

The remaining 30 birds were examined in their coops. Seven birds were handled. Except for a number in poor body condition, there were no obvious signs of ill-health in the flock. The poor egg production could be partly attributed to the time of year.

Three chickens in poor body condition were humanely culled by cervical dislocation. Post-mortem findings included variable amounts of feed in the crops, and enlarged gall bladders. Small spleens suggested the involvement of a non-infectious agent. The duodena were somewhat enlarged and two birds had duodenal haemorrhages. Mucosal scrapings contained large numbers of motile, immature segments of *D. proglottina*. Scolices were less readily visible. In one bird, hairworms (*Capillaria* spp.) were present in crop scrapings. It was concluded that *D. proglottina* was responsible for the clinical signs and mortality rate observed in this flock and the investigation was closed.

Epidemiology and pathogenesis

D. proglottina is a very small (0.5–2 mm) tapeworm affecting the duodenum and upper jejunum of chickens, other gallinaceous birds and pigeons (Soulsby, 1968). The adult tapeworm has four to nine segments and the head (scolex) has four suckers surrounded by rings of hooks, with the mouth also ringed by hooks (**Figure 1**). It has an indirect

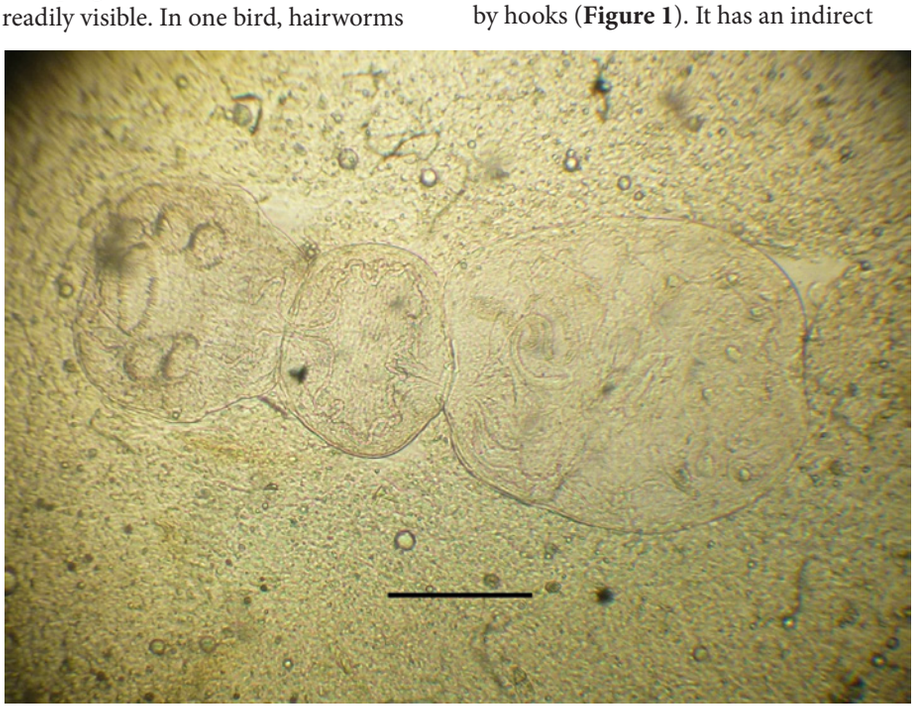

Figure 1: *Davainea proglottina*: scolex and first two segments (scale bar = 100 µ).
Photo: Neil Christensen, Avivet

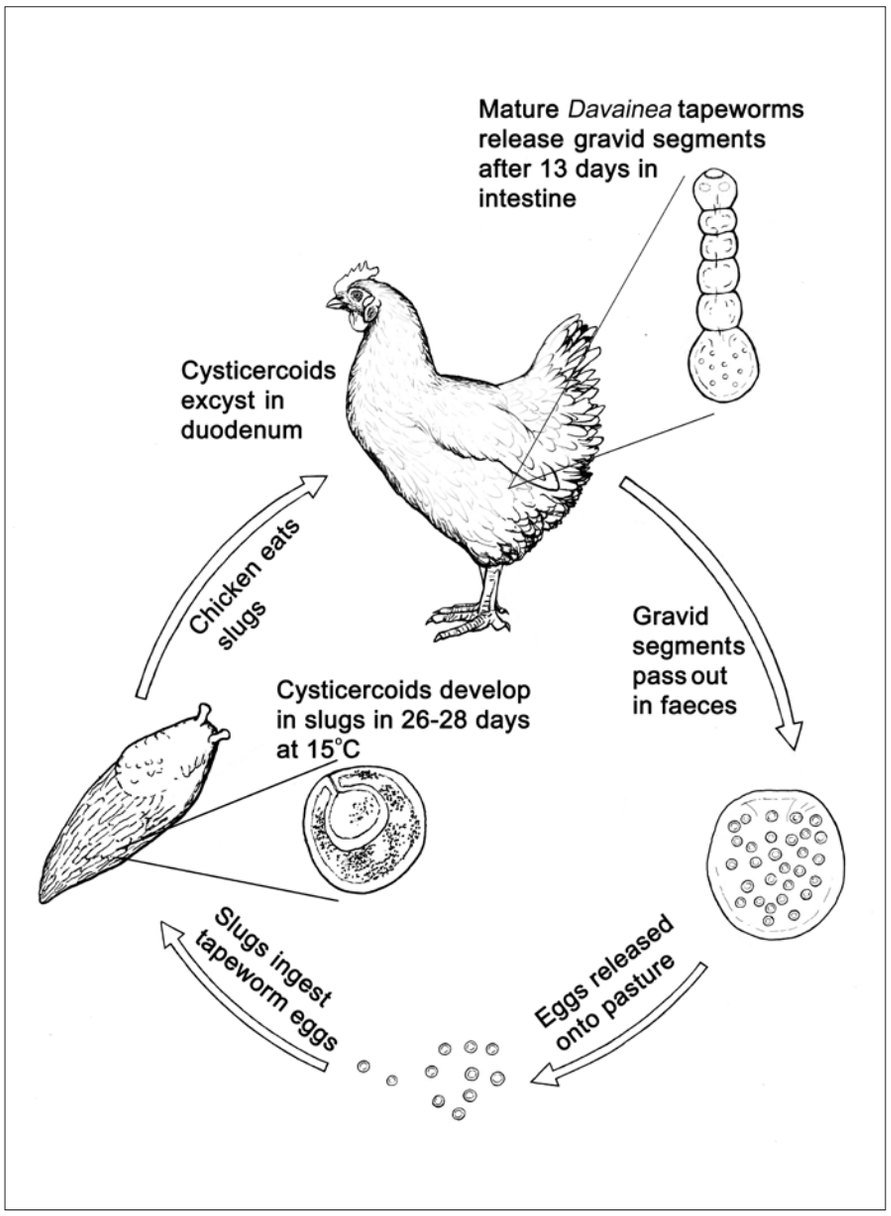

Figure 2: Life cycle of *D. proglottina*

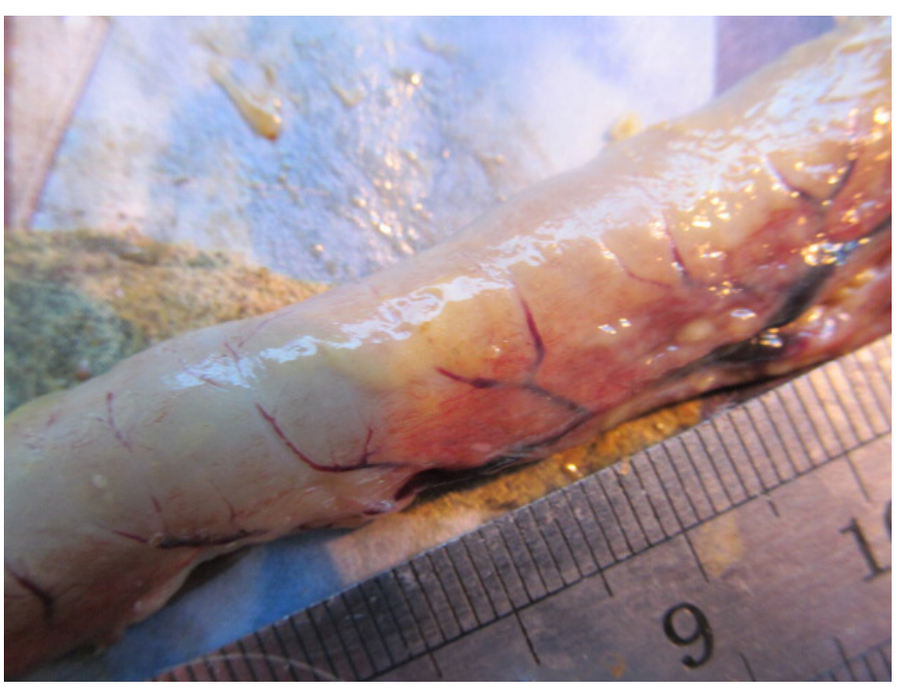

Figure 3: The scolices of the worms burrow deeply into the intestinal mucosa and give rise to a marked enteritis. Photo: Neil Christensen, Avivet

life cycle (**Figure 2**) with several species of slugs of the genera *Cepoea, Limax, Agrilimax* and *Arion* acting as intermediate hosts (Abbou, 1958a). After release of the gravid segments and eggs in the birds' faeces, the embryos (oncospheres) are taken up by the intermediate hosts. A cysticercoid develops in the intermediate host in about 13 days at a temperature of 26°C, or in 26–28 days at 15°C. Chickens become infected by ingesting infected slugs, and the adult stage is reached in 10–13 days (Abbou, 1958b). Slugs of the families Agriolimacidae (e.g., *Deroceras panormitanium*) and Limacidae (genus indeterminate) have been recovered from properties where birds have become infected with *D. proglottina* in New Zealand.

Despite its size *D. proglottina* is one of the more pathogenic cestodes in poultry, especially in young birds (McDougald, 2013) and particularly when it occurs in large numbers (Permin & Hansen, 1998). The scolices of the worms burrow deeply into the mucosa and give rise to a marked enteritis that can penetrate to the serosa (**Figure 3**).

Clinical signs and diagnosis

The clinical signs of davaineasis include dull plumage, slow movements, reduced weight gain, emaciation, paralysis and death. Sometimes there is penetration of the intestine, leading to peritonitis. The infection can subsequently spread to involve the head and sinuses, and present as torticollis, thus mimicking neurotropic Newcastle disease. This association between davaineasis and nervous signs has previously been observed (Soulsby, 1968).

Diagnosis can be difficult. Because the small worm autolyses quickly, infections with *Davainea* may have been underdiagnosed when pathologists have been presented with birds that have been dead for 24 hours or more. Careful examination of the intestines is necessary whenever birds present with ill-thrift and emaciation, egg peritonitis in the absence of obvious vent pecking, inflammation of the serosal surface of the duodenum, or torticollis. Duodenal mucosa scrapings should be examined under low-power magnification (4x and 10x objectives).

Treatment and control

Praziquantel is the drug of choice but it is not licensed for use in poultry in

New Zealand. The recommended dose is 7.5 mg/kg. In-feed formulations of 5 percent flubendazole are available and registered for use against other tapeworms, but efficacy against *D. proglottina* is not claimed. Further work is required on the scope for using other anthelminthics, or higher doses of flubendazol.

Suppression of any secondary bacterial infections resulting from penetrating ulcers or serositis can be attempted using antibiotics that have a zero egg-withholding period.

D. proglottina control should be directed at the intermediate host. The new low-toxicity iron chelate molluscicides such as Multiguard® (Multicrop) or Dusk® (Orion) are an option. Sheep can be used to graze the range, sweeping it of intermediate hosts. Keeping the grass short also creates a less favourable environment for the intermediate hosts.

Conclusion

During 2013–2015 *D. proglottina* has been diagnosed in seven commercial or backyard poultry flocks from Kaipara to Marlborough, in environments that are favourable to the intermediate hosts. In commercial free-range layer flocks, mortality rates of 40 percent and hen-day egg production declines to 40 percent have been observed, identifying the pathogen as a threat to the viability of free-range egg production (Christensen, 2016). No licensed treatments are available but there are management options to limit the parasite's impact.

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Investigation of bee mortality in a beekeeper operation in the Coromandel district

Introduction

The Ministry for Primary Industries (MPI) was notified during October 2014 of a bee mortality event in a beekeeper operation in the Coromandel district. Early in the ensuing investigation a Plant and Food Research scientist concluded that the event described did not fit with known exotic causes of bee mortality (European foulbrood and bee tracheal mite). This was confirmed by an Apiary Officer (AO) but after interviewing the beekeeper the AO determined that the impact of the event was sufficient to warrant further investigation.

None of the major exotic bee pathogens were detected but the trypanosome *Lotmaria passim* was identified. (Until recently this was classified incorrectly as *Crithida mellificae*, a honey bee parasite not previously detected in New Zealand.) First found in Australia in the 1960s, it has only recently been suggested to be associated with winter losses in bees. The present investigation did not show an association with bee losses, but only a few colonies were tested. There was no correlation found for the level of agents *L. passim* and *N. ceranae* present in affected colonies, but these analyses do not exclude that possibility. Testing live bees remaining in the affected colony, rather than the bees that had left, may have concealed a true association. In addition, the levels of these agents at the time that losses are occurring is not necessarily relevant. Reports in the literature show the levels of these agents in summer are associated with bee losses occurring in winter.

L. passim has subsequently been identified in beekeeper operations outside of the Coromandel district, suggesting it could have been here for some time but only detected recently through new molecular diagnostic methods.

Background

The causes of bee mortality can be challenging to investigate because it is impossible to assess the significance of all the potential agents found. Interactions among these agents can make the causal web of disease very complex. In addition, clinical signs of disease in bees tend to be so generalised that interpreting clinical signs and their significance in a specific colony can be problematic. There are also limited diagnostic methods for understanding disease in bees. Where multiple mortality events occur within separate beekeeper operations it can be hard to tell whether these are related to the same event or unrelated events. Multiple beekeeper operations are affected by diverse factors including climate, varroa infection and feed availability.

To address these difficulties, MPI established a working group comprising several parties, including the affected beekeeper, AsureQuality, Plant and Food Research, and other known bee experts.

Initial investigations

As part of the investigation, a bee scientist made an initial visit to the beekeeper operation and examined bees and colonies from five affected apiaries. Generally the hives had sufficient honey and most had stored pollen reserves. There was evidence of endemic disease but no indication that infection levels were higher than normal. Several cells in one hive had chalk brood; three hives had larval clinical signs consistent with sacbrood virus in unused comb; and two bees were seen with deformed wings possibly caused by deformed wing virus (DWV). There was no indication of clinical American foulbrood (AFB); nor were varroa or small hive beetle (both exotic to New Zealand) observed. All colonies examined had laying queens.

In the hives from affected apiaries, bee numbers had declined from an estimate of 10 000–20 000 to 1000–2000 over a period of one month and the brood area had shrunk from 4–5 frames to about half a frame. There were large numbers of eggs present, compared with the number of larvae and capped cells. Although no measurements were taken, there appeared to be very low conversion rates from eggs to pupae: possibly as low as 5 percent, when 80–100 percent would be expected. A further observation was that there was little larval food with the larvae. There did not appear to be any health issue with the queen where it had been removed from the weak hives and placed in unaffected colonies (n = 10). When five of these colonies were combined with healthy colonies, with a queen excluder between them, they quickly recovered without further heath issues.

After investigation had commenced on this beekeeping operation, it was noticed that similar effects had been noted by five other commercial beekeepers on the Coromandel Peninsula.

Although this presentation did not fit with that of known exotic agents, bee samples were collected during October 2014 from 10 of the worst-affected colonies and tested. Samples were negative by PCR for European foulbrood (EFB), and tracheal mite. In addition, quantitative PCR (qPCR) was used to determine the levels of DWV, black queen cell virus (BQCV), chronic bee paralysis virus (CBPV) and members of the Dicistroviridae family (DF). This family includes Kashmir bee virus (KBV), acute paralysis and Israeli-associated paralysis viruses (IAPVs); IAPVs are not known to be present in New Zealand (McFadden *et al.* 2014). Tests were negative by qPCR for CBPV but positive for KBV (confirmed by DNA sequencing). Samples had only low to moderate levels of DWV and BQCV, suggesting these were not major pathogens in affected colonies. However, when interpreting these results we need to be aware that bees making up the high percentage that deserted their colonies could not be tested; furthermore, only live bees were tested.

Investigations of the significance of *Nosema*

Spore counts were determined for *Nosema* spp. and some of these counts were extremely high: about 100 million per bee. Quantitative PCR was used to confirm these levels and differentiate between counts for *N. apis* and *N. ceranae*. In order to understand the significance of this infection, in mid-

December 2014 surviving bees were collected and tested from 33 hives in a number of apiaries within the beekeeper operation. Samples were grouped by colony status, which was based on observed clinical signs, each group being categorised as "affected", "unaffected" or "unknown". Samples were also collected from 10 unaffected colonies in another beekeeper operation in the same general area; while these were considered to be "negative controls", all colonies in the Coromandel area could have been affected.

Nosema tests were generally positive for both *N. apis* and *N. ceranae* regardless of colony status (**Figures 1 & 2**). Linear regression was used to compare the PCR Cq values (amount of genetic material of the agent: typically, the lower the Cq number the more target organisms present in the bees), based on group status, with Cq normalised by natural logarithm transformation. The groups within the affected beekeeper operation did not have significantly higher *N. apis* levels than the negative control group. *N. ceranae* levels were greater in the affected beekeeper operation than the negative control group ($p < 0.01$) but not significantly different between status groups within the affected beekeeper operation.

Logistic regression was carried out to identify interaction between the levels of *N. apis* and *N. ceranae*, with the response variable coded dichotomously as affected or unaffected; the latter category was expanded to include "negative controls". There was no significant interaction between status and the Cq of *N. apis* and *N. ceranae*.

While the study of *Nosema* was not sufficiently comprehensive to answer all questions, data showed that the presence of the syndrome (based on beekeeper assessment of colony status) in the affected beekeeping operation was not associated with the levels of *Nosema* spp. at the time of testing. It is possible that there was a lag between measurement of *Nosema* spp. by qPCR and the development of clinical signs observed. If this were the case "unaffected" and "unknowns" could have had the agents present but no clinical signs at the time of sampling. If so, a true association may not have been detected because of the timing of sampling.

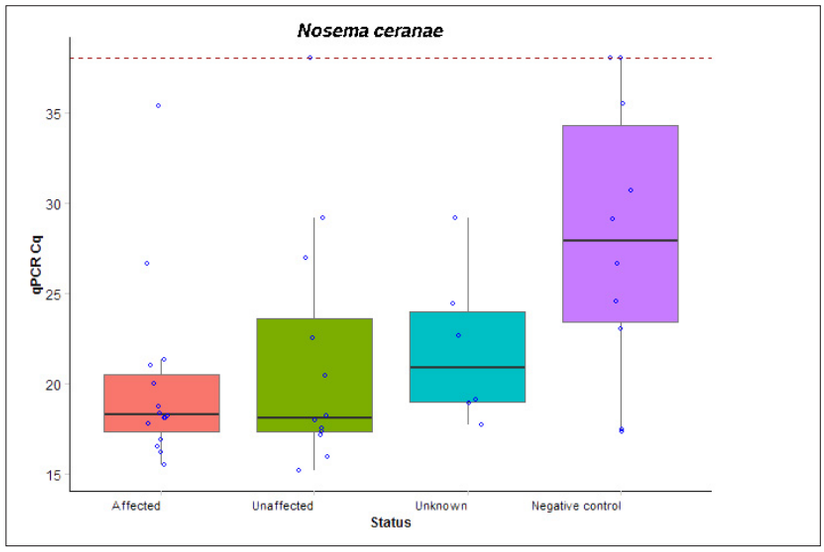

Figure 1

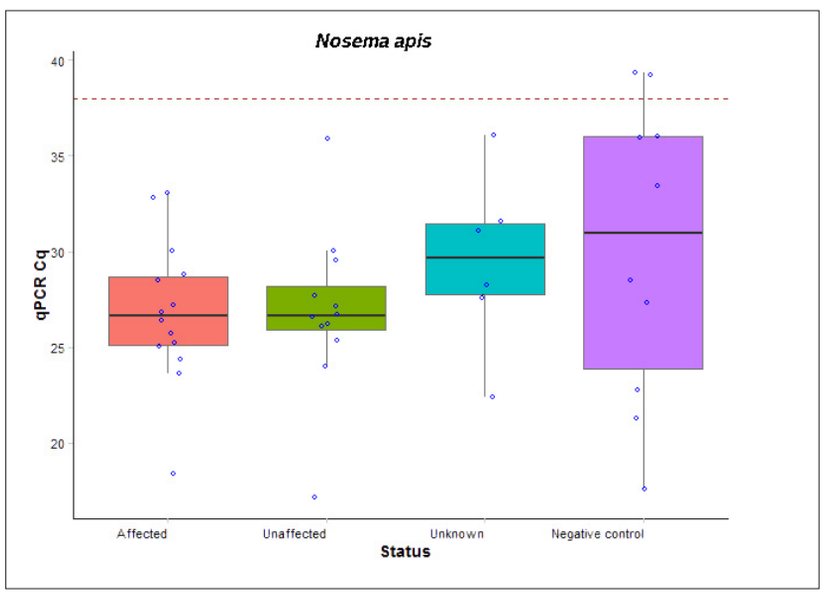

Figure 2

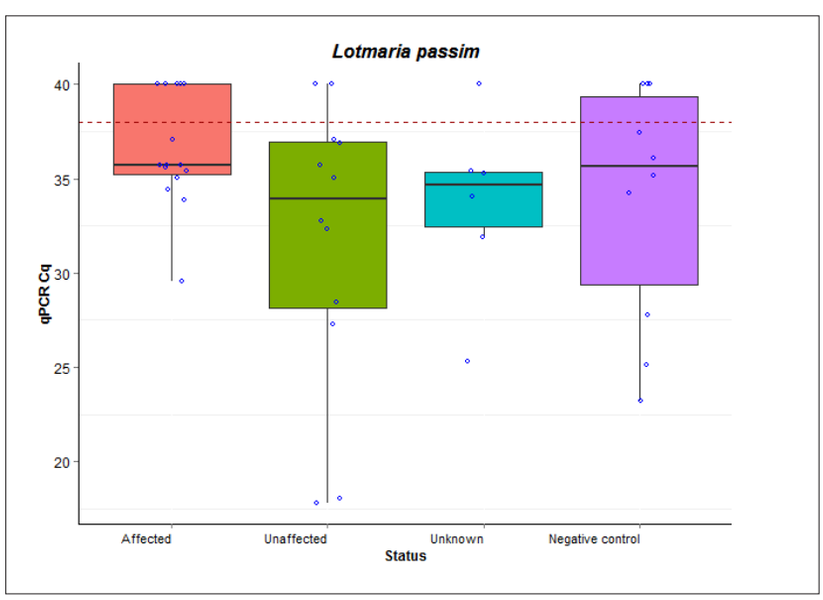

Figure 3

Boxplots of the amount of genetic material for a number of bee pathogens: *Nosema ceranae* (Figure 1), *Nosema apis* (Figure 2) and *Lotmaria passim* (Figure 3) present in bee samples collected from colonies classified as "affected", "unaffected", "unknown" and as a "negative control". The higher the Cq value the less the amount of the target organism present in the bee samples. A box plot provides the minimum, maximum and the interquartile range of Cq values. The negative control consisted of colonies from apiaries from another beekeeper that were not affected.

There is insufficient information to interpret the difference in *N. ceranae* levels between the affected beekeeing operation and the negative control colonies; although the levels were different, the negative controls were still generally strongly positive. The R^2 value for the regression was 16 percent, indicating that a number of other factors other than *N. ceranae* levels must have explained the difference in colony status.

Testing for other agents

Further testing was carried out for other possible agents, based on the hypothesis that the cause of the disease was multifactorial. This investigation centred around two agents that have been associated with winter bee losses and where a synergistic effect with *N. ceranae* has been reported. These were the insecticide fiprinol (Aufauvre *et al.,* 2012) and the trypanosomid parasite *L. passim* (*Crithida mellificae*; Ravoet *et al.,* 2013). Fiprinol was not detected in toxicological tests at Hills Laboratories, Hamilton.

According to Schwarz *et al.* 2015, reports of *C. mellificae* in honey bees are the result of misclassification and the species is in fact *Lotmaria passim,* which is globally the predominant trypanosome parasite of honey bees. This species has been known in Australian bees since 1967, but has only recently been implicated as pathogenic. It has been detected in many countries including North America, Asia and Europe. There have been no reports in New Zealand, but until recently no PCR assay was available, so detection does not necessarily signify a new incursion.

The impact of *N. ceranae* in winter increases with levels of *L. passim* (*C. mellificae*) and *N. ceranae* in summer (Ravoet *et al.,* 2013). Ravoet *et al.* (2013) speculated that variable impact of *N. ceranae* may be the result of factors such as *L. passim*. A related agent, *Crithidia bombi,* has for some years been known to infect bumble bees (Brown *et al.,* 2000).

The same 43 colonies that had been used to examine the relationship between *Nosema* spp. and colony status were tested for the presence of *L. passim*. Of these, 74 percent were positive for *L. passim* although in most cases there was a high Cq value, i.e., a low level of target organisms in the bees (**Figure 3**). Linear regression was again used to compare the Cq values between colony groups. The Cq (log transformed) of the "unaffected" colonies was lower (higher amount of target organism in the bees) than that for other groups. Thus higher levels of *L. passim* at the time of testing were not associated with disease. Logistic regression was carried out to identify interaction between the levels of *N. ceranae* and *L. passim* and colony status. No interaction was observed using this analysis.

Conclusions

Further research is necessary to understand the significance of mortality events such as this, using a longitudinal design of multiple beekeeper operations. To understand the complex interactions between agents and the effect of the lag between exposure and development of clinical signs would require multiple tests over several seasons.

Landcare Research, in conjunction with the bee industry and MPI, is carrying out a survey of 250 of the biggest beekeeper operations in New Zealand to find out more about colony losses over the past 12 months. Further work will also be carried out to determine what bee pathogens are present. A key issue is establishing a baseline of what level of bee losses is normal, and determining the associated risk factors in beekeeper operations where mortalities occur. Apiary samples will be tested for exotic bee diseases and the extent to which the bees are infested with endemic *Varroa* mites and *Nosema* spores. This research will be conducted in 2016.

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Quarterly report of diagnostic cases: October to December 2015

New Zealand Veterinary Pathology

Bovine

A property in the Waikato had two milking cows that appeared pale. Their hematocrits were 0.14 and 0.12 (reference range 0.24–0.40). *Theileria* spp. organisms were seen in their blood smears, confirming ***Theileria orientalis*** **infection**. A number of other clinically significant cases of *Theileria* were also seen in the Waikato during this quarter, confirming the importance of this organism.

A cow in the Waikato had evidence of anaemia and haemorrhage. A coagulopathy was suspected. The prothrombin time of this cow was markedly elevated, at 58.4 seconds (reference range 16–24), consistent with a primary coagulopathy such as **anticoagulant rodenticide toxicity**.

A dairy herd in the Horowhenua had three animals that were losing weight and scouring despite eating well. A Johne's ELISA test was positive on all three, suggesting that **Johne's disease** is a significant problem in this herd.

A herd in Marlborough had several calves aged one to four weeks with marked scour. All three tested were positive to a rotavirus antigen test. Testing was negative for coronavirus, coccidia and *Salmonella*. **Rotaviral diarrhoea** was diagnosed.

Milk from five Kaipara dairy cows was submitted for mastitis culture. Four samples yielded pure cultures of ***Serratia marcescens***, an uncommon minor environmental mastitis pathogen. *Serratia* can be poorly responsive to antibiotics and frequently exhibits extensive resistance to cephalosporins, penicillins and tetracyclines.

Two dairy heifers in the Waipa district presented with spontaneous fractures of the humerus. Four other animals from the same property had also been affected earlier in the season. Liver copper levels for both animals were < 45 µmol/kg (reference range 95–2000), confirming **copper deficiency**. Osteoporosis is an occasional feature of copper deficiency in animals. Copper forms part of lysyl oxidase, an enzyme essential to the cross-linking of collagen and elastin. Failure of cross-linking can cause abnormal mineralisation of the bone and result in osteoporosis. Spontaneous fractures in heifers caused by copper deficiency are not uncommon in New Zealand but the incidence appears higher in some years than others. This presentation has been less common in 2015 than in previous years.

A 400-cow dairy herd in Marlborough reported five sick cows and one animal died. Samples were submitted from cows exhibiting weakness, pale or muddy mucous membranes, wasting and decreased milk production. Haematology from three animals revealed a mild to moderate anaemia, with haemocrits ranging from 0.11 to 0.20 (reference range 0.24–0.4). Two of the animals had *Theileria* spp. organisms visible on the blood smear. PCR confirmed the presence of ***Theileria orientalis*** **Ikeda** in all three animals. Large numbers of ticks were observed on this property by the attending veterinarian.

A cow from a dairy herd in the Waikato exhibited evidence of bovine digital dermatitis. Biopsy of an interdigital papillomatous proliferation revealed marked papillomatous epidermal hyperplasia with orthokeratotic and parakeratotic hyperkeratosis. Warthin-Starry stains of histologic sections revealed the presence of large clumps and sheaves of slender, silver-staining bacilli, consistent with spirochetes, within the keratinocytes forming the hyperplastic fronds of epidermis. These changes are typical of **bovine digital dermatitis**.

A one-year-old bull from a property in the Western Bay of Plenty exhibited marked weight loss and scouring. The bull had been drenched five weeks earlier. A few other animals in the same mob were exhibiting similar clinical signs. The faecal egg count from the bull was very high, at 3250 eggs/g. ***Yersinia pseudotuberculosis*** was isolated from the faeces. **Yersiniosis** complicated by **gastrointestinal parasitism** was diagnosed.

A dairy herd in the Western Bay of Plenty had three animals exhibiting clinical signs of anaemia and haemolysis, including pale mucous membranes and icterus. Haematocrits for all three animals tested ranged from 0.08 to 0.21 (reference range 0.24–0.40). Organisms consistent with ***Theileria orientalis*** were noted on blood smears from two of the animals.

A group of suckler beef calves failed to thrive and one animal died. Samples from three animals were submitted. The calves had pale mucous membranes and no evidence of diarrhoea. Two of them had moderate to marked anaemia, with haematocrits of 0.13 and 0.14 (reference range 0.2–0.40). Organisms consistent with ***Theileria orientalis*** were noted on the blood smears.

A dairy cow in the Waikato presented with acute onset of blindness. There was bleeding into the anterior chamber of both eyes. Examination of a fresh blood smear revealed that almost no platelets were present. The cow was also anaemic, with a haematocrit of 0.22 (reference range 0.24–0.40). The intraocular haemorrhage was considered the result of an **immune-mediated thrombocytopaenia**.

A group of calves in the Hauraki district exhibited coughing with diarrhoea. Serum chemistry on one animal showed a marked hyponatraemia and hypochloraemia, both of which are changes that may occur in conjunction with severe diarrhoea. Faecal culture revealed the presence of ***Yersinia pseudotuberculosis*** in two separate samples. Faecal egg counts were not significant. **Enteric yersiniosis** was diagnosed.

Ovine

A 14-month-old pet sheep had been regularly wormed and was in excellent condition when home-killed. It had been moved to a new property six months previously. At slaughter, numerous

small white lesions were visible on the capsular surface of the liver. Histologic examination revealed the presence of subcapsular hepatic granulomas with intralesional cestodes. A presumptive diagnosis of ***Cysticercus ovis*** (the encysted form of sheep measles, *Taenia ovis*) was made. Advice was issued regarding the transmission of *T. ovis* and the need to de-worm any dogs coming into contact with sheep.

Four Merino wethers from a property in Marlborough exhibited progressive hindlimb ataxia and paresis. The animals were euthanased and histologic examination of the brainstem and spinal cord revealed mild to moderate multifocal axonal degeneration and swelling, consistent with **segmental axonopathy of Merino sheep** (Jolly *et al.*, 2006).

Equine

A Thoroughbred foal in the Waikato presented initially with a septic arthritis affecting the femoropatellar joint, which was treated with antibiotics. The foal appeared to improve clinically but remained lame after initial treatment. Cytologic examination of joint fluid revealed the presence of severe suppurative inflammation with degenerate neutrophils, and there were bacteria in the neutrophil cytoplasm. Culture of the joint fluid revealed the presence of ***Salmonella*** spp. (further typing was not complete at the time of writing.) **Septic arthritis** caused by ***Salmonella* infection** was diagnosed.

A 24-year-old gelding in Wanganui had a sudden onset of oedema over a 4–5-day period before submission, with swelling on the brisket and ventral abdomen and above the eyes. There were also large wheals on the side of the neck. The horse was still eating well, though the mouth appeared inflamed. Biochemistry revealed a marked hyperproteinaemia, with a total protein of 92 g/L (reference range 53–73) and a marked hyperglobulinemia of 72 g/L (reference range 20–41). Serum protein electrophoresis revealed that the hyperglobulinemia was due to a monoclonal gammopathy. A CBC revealed a marked lymphocytosis (14.20×10^9 cells/L; reference range $1.5–6.3 \times 10^9$), with moderate numbers of medium-sized to large lymphocytes present. **Lymphoma** with a **monoclonal gammopathy** was diagnosed.

Porcine

The owner of a South Wairarapa property raising small numbers of pigs had lost seven piglets over the past few months. One piglet exhibited nervous signs and was euthanased for postmortem by the submitting veterinarian. Gross abnormalities were limited to a mottled capsular surface of the kidney and a haemorrhagic cerebellum. Histology revealed a number of lesions including vasculitis, thrombosis and encephalomalacia in the cerebellum, an acute ulcerative colitis consistent with *Balantidium coli*, and marked splenic lymphoid depletion with histiocytic proliferation. Histiocytes in the spleen contained large botryoid intracytoplasmic inclusion bodies consistent with circoviral inclusions. There was also suppurative pneumonia and interstitial nephritis. **Porcine circovirus-2** infection was diagnosed, accompanied by secondary **colitis** caused by ***Balantidium coli***. The cerebellar lesion was likely a direct result of the vasculitis that may be induced by PCV-2 (Seeliger *et al.,* 2007).

Lagomorph

A feral rabbit was found dead in Auckland. Necropsy revealed multifocal white patches present through the hepatic parenchyma. Histology revealed a severe cholangiohepatitis with biliary ductular intraepithelial and intraluminal coccidia. **Hepatic coccidiosis** caused by ***Eimeria stiedae*** was diagnosed.

Avian

Two lovebirds (*Agapornis* sp.) from a collection in Auckland died, with evidence of poor body condition, shivering and heterophilia. The gross postmortem was unremarkable with both birds, but histology revealed that the liver and spleen contained numerous histiocytic infiltrates, which contained numerous acid-fast bacilli. **Systemic mycobacteriosis** was diagnosed.

Another lovebird from the same collection lost weight and was found dead. Histology revealed a marked granulomatous splenitis with intralesional acid-fast bacteria. The liver of this bird appeared normal. The proventriculus also contained numerous very large structures consistent with ***Macrorhabdus onithogaster*** (Megabacteria). **Mycobacteriosis** and **megabacteriosis** were diagnosed.

Five ducklings on a property in Wellington died with clinical signs of respiratory distress, wheezing, coughing and oculonasal discharge. The DNA of ***Chlamydiophyla*** spp. was detected in four of the five animals tested by PCR. **Psittacosis** was diagnosed and relevant public health authorities were notified.

A group of penguins held in captivity had poor hatchability. An egg was submitted for post-mortem examination. The chick in the egg had significant gross abnormalities: no upper beak and cranial skull, and a single enlarged eye in the centre of the forehead (synopthalmos). It was also malpositioned, with the head and beak under the right foot. **Congenital malformation** would have caused the malposition and inability to hatch. The causes of congenital malformations can include genetic influences and environmental problems such as inappropriate temperature for incubation, or heavy metal exposure.

Canine

A three-month-old Foxhound from a kennel in Auckland had bloody diarrhoea. ***Giardia*** antigen testing of faeces was positive, as was a culture for ***Campylobacter jejuni***. **Giardiasis** complicated by **campylobacteriosis** was diagnosed.

A six-year-old dog in the Tasman district presented with black soft/runny faeces for four days and had been out of sorts for about a month. Testing for parasites (including faecal egg count, *Giardia* and *Cryptosporidium*) was negative, but ***Campylobacter upsaliensis*** was isolated from the faeces. *C. upsaliensis* can be isolated from the faeces of both diarrhoeic and nondiarrhoeic dogs, so its significance in this case is unknown. However, it is considered potentially important as a cause of diarrhoea in humans, so its zoonotic potential cannot be ignored (Parsons *et al.*, 2010).

Feline

An 11-year-old purebred cat in Auckland had a two-month history of ulcerated skin lesions on the dorsal hindquarters and face. Skin biopsies of the nose and flank revealed a severe ulcerative pylogranulomatous dermatitis with intralesional yeasts, consistent with ***Cryptococcus*** spp. infection. Additional testing for feline leukaemia virus and feline immunodeficiency virus in this cat was negative.

Zoo animals

A Hawksbill sea turtle (*Eretmochelys imbricata*) in a collection in Auckland died with few premonitory signs. Histologic examination revealed a marked subacute necrotising pneumonia with intralesional fungal hyphae, consistent with *Aspergillus* spp. Similar lesions were also present in the spleen, liver and kidney. **Systemic fungal infection** (likely ***Aspergillus*** spp.) was diagnosed. Within the adrenal gland there were fragments of trematode eggs (***Spirorchis*** spp.) surrounded by multinucleate macrophages. Spirorchidiasis is common in sea turtles and is thought to have a life cycle similar to schistosomiasis, i.e., with a mollusc intermediate host. Spirorchids are thought to contribute to a loss in body condition and may predispose to secondary bacterial or fungal infections.

A Macaque (*Macaca* sp.) from a zoological collection in Auckland was necropsied after dying under anaesthetic. Histology revealed a number of age-related changes, including a **chronic cardiomyopathy, atrioventricular valvular endocardiosis** and **aortic arteriosclerosis**. There was also a moderate subacute **cholangiohepatitis** with **cholelithiasis**. Superimposed upon these chronic age-related changes there was also evidence of an **acute aspiration pneumonia**.

Gribbles Veterinary Pathology

Bovine

Three Hereford cross beef calves in Northland aged four to five months died suddenly and two more were found recumbent. After rising, these calves exhibited strange behaviour, including gnawing at the fence. The next day they were found dead. Exploration of the paddock revealed a lead battery was present, although the paddock had been grazed for years with no prior problems. Histopathology on the cerebrum, hippocampus and thalamus revealed acute laminar neuronal necrosis with mild lymphoplasmacytic, histiocytic and eosinophilic meningitis. Blood lead concentrations of two of the animals were elevated, at 0.75 and 1.00 mg/L (reference range 0–0.3), confirming a diagnosis of **lead toxicity**.

Seven dairy cows from the Bay of Islands were examined because they were displaying decreased milk production and chronic facial eczema was suspected. GGT and GDH levels in all the cows were within or below the reference intervals. In serum samples from six of the seven cows antibodies to ***Mycobacterium paratuberculosis*** were detected by ELISA, confirming a diagnosis of **Johne's disease**.

Biopsies were received from a two-year-old Friesian heifer from Golden Bay that had bilateral swellings above both eyes. Fine-needle aspiration had been unrewarding. Biopsies for histopathology showed both lesions consisted of granulomatous inflammation with caseation and acid-fast bacteria present, consistent with **skin tuberculosis**.

A biopsy was received from a growth on the nostril of a one-year-old Friesian cross heifer from a mid-Canterbury farm. Histologically the biopsy had pyogranulomatous inflammation with club colonies, consistent with **actinobacillosis**.

Cases of **bovine neonatal pancytopenia** were seen this spring on two farms in South Canterbury. These cases occurred despite the withdrawal in 2011 of a BVD vaccine that was considered to have been responsible for this problem. On one farm, the farmer had identified cases in the past and recognised this problem immediately. On the other farm the problem had not been recognised before. In all cases the affected calves had hypoplastic bone marrow with a marked loss of haematopoietic cells. Some of the calves also had bacterial infections with a notable absence of an inflammatory response to the bacteria.

In a group of 20 dairy heifers on a North Canterbury farm, six calves with enlarged thyroids were born over a four-day period. Histological examination of one thyroid showed irregular-shaped follicles with infolding of the epithelium. Most follicles lacked colloid and those with colloid had only small amounts, consistent with a diagnosis of **goitre**.

A two-week-old Angus heifer calf was found dead on a Southland beef farm after a brief episode of coughing, epistaxis, rectal bleeding and terminal opisthotonus. Necropsy revealed haemorrhages throughout the carcass. A fixed sample of sternal bone marrow was markedly hypocellular, consistent with a diagnosis of **bovine neonatal pancytopaenia (BNP)**. There were no more cases on this farm. Four years previously there had been six cases of BNP in neonatal calves on the same farm. The dam of the heifer calf that died this year had some years previously produced an affected calf, but then had normal calves in subsequent calvings.

On a Southland dairy farm four 2-year-old heifers were found recumbent, with humeral fractures. They were euthanased and a liver sample was taken from each for copper assay. All had liver copper concentrations from 34 to 45 µmol/kg (adequate > 95), consistent with a diagnosis of **copper-deficiency-induced osteopenia.**

In mid-October an adult cow was found recumbent and dehydrated with severe diarrhoea on a Southland dairy farm. ***Salmonella*** **Brandenburg** was cultured from the faeces. Although this bacterium is occasionally associated with diarrhoea in calves and abortion in cows in Otago and Southland, it has not previously been isolated from adult cattle with diarrhoea.

Six-week-old unweaned calves started wasting and dying on a Southland dairy farm two weeks after a **rotavirus** outbreak. Necropsy of two calves revealed severe but nonspecific lesions of a **subacute nephrotoxicosis**. Over a month, about 20 calves out of 200 died after a short period of anorexia and weight loss. Further questioning revealed that during the rotavirus outbreak the farmer had purchased a large drum of concentrated disinfectant. This had been used to disinfect the calf feeders but they had not been thoroughly washed out afterwards, leading to a toxic nephropathy.

An outbreak of nervous signs in three-month-old calves caused by ***Chlamydophilia pecorum*** occurred on a Southland dairy farm. In the affected mob of 100 calves, 13 showed clinical signs of circling, opisthotonus, nystagmus and ataxia when disturbed. Two were found dead. Histopathological examination of fixed brain from one calf revealed severe nonsuppurative meningoencephalitis consistent with *C. pecorum* infection. This was later confirmed by PCR testing.

In December there were outbreaks of ***Pasteurella multocida*** serositis in mobs of three-month-old dairy calves on two Southland farms. In one case the

calves started dying shortly after being transferred to a grazier. In both cases only small numbers of animals were affected. Necropsies all showed excess proteinaceous fluid in the body cavity and pericardial sac. *P. multocida* was cultured from this fluid.

Polioencephalomalacia was diagnosed on three Southland farms during this quarter. Small numbers of young calves were affected and showed nervous signs including recumbency, depression, nystagmus and tooth-grinding. The diagnosis was confirmed by histopathology of the brain.

Six bull calves in Rotorua had a two-month history of diarrhoea. There had been no response to anthelmintic treatment or trace-element supplementation. Coughing was also noted on examination. In all cases the total protein, albumin, bilirubin, GGT and GDH were within reference intervals. Two calves had mild hypoglobulinemia (27 and 26 g/L; reference range 28–53). Faecal parasitology revealed low numbers of coccidial oocysts but was negative for strongyle nematodes and *Dictyocaulus*. Pooled faecal culture isolated a light growth of ***Yersinia pseudotuberculosis***, confirming a diagnosis of **yersiniosis**.

An 18-month-old Jersey/Friesian cross bull in the Waikato had been noticed with worsening ataxia over the previous year. When resting the bull held its head forward and elevated. When it was mustered, the degree of ataxia would rapidly increase until the animal began circling and fell. The bull was euthanased and the head and cervical spinal cord removed and dissected. An area of **suppurative osteomyelitis** was identified in the lateral aspect of the 5th cervical vertebra. Regions of bone degeneration were visible on the atlas, where the dens of the axis had been abnormally rubbing on the bone. In addition, regions of arthritis were visible in the fourth and fifth cervical vertebrae, thought to be related to abnormal carriage of the head and neck related to the pain of the infection and bone necrosis.

While pregnancy scanning on a beef cow breeding property in the Rangitikei, a dying fetus was identified. The aborting cow was sent to a meat plant for slaughter and the entire uterus, fetus and membranes were collected for analysis. The uterus contained about 400 ml of amniotic fluid and a 40 mm dead embryo. ***Ureaplasma diversum*** was detected by PCR in a sample of fluid collected from the embryo, and histopathology found evidence of necrosis in the fetus.

Spontaneous humerus fractures had been occurring in milking Friesian cattle on a Rotorua dairy farm. Liver biopsies from six cows aged two and three years were analysed for copper. The levels were 23, 370, 210, < 15, < 15 and < 15 µmol/kg (adequate range 95–3000), confirming that **copper deficiency** was an issue on the farm and a likely reason for the bone fractures.

An adult Friesian cow from a 280-cow dairy herd in Taranaki was presented for veterinary attention because of severe diarrhoea with dysentery. A pure culture of ***Salmonella*** **Bovismorbificans** was isolated from a faecal sample.

A group of 12 five-month-old Jersey and Friesian bull calves on a Rangitikei bull beef grazing property were the poorest growing from a group of 500. They were examined and large numbers of ticks were found. Whole blood samples from one affected calf confirmed anaemia (packed cell volume 0.16; reference range 0.24–0.46). The red blood cell concentration was 4.1 x 10^{12}/L (reference range 5–7.7 x 10^{12}) and haemoglobin 51 g/L (reference range 80–140). *Theileria* organisms were visible in the red blood cells so the anaemia may have been due to blood loss from the high tick load, plus haemolysis from ***Theileria*** infection. All the calves had diarrhoea, so a faecal sample was cultured. A heavy growth of ***Yersinia pseudotuberculosis*** was isolated, confirming that multiple aetiologies were contributing to ill-thrift in the mob.

A group of ninety 11-month-old Friesian cross dairy heifers on a Manawatu dairy farm had failed to thrive, and **bovine viral diarrhoea virus** (BVDv) infection had been confirmed in one heifer that had weight loss and diarrhoea. The entire mob was then sampled and tested for BVDv by PCR and 21 more virus-positive animals were found. The affected animals were drafted off, kept separate and blood-sampled again a month later. During that period two of the animals had died. The remaining 19 all tested positive for BVDv again, confirming that they were persistently infected.

Several cases of **perforated abomasal ulcer** were diagnosed in dairy heifer calves from three farms in the lower North Island. The calves ranged from four to eight weeks of age. On one farm, three calves out of 50 died. Mortality rates were not provided for the other farms. In all cases there was a history of disbudding under sedation 10–20 days before death. The only difference in treatment protocol from previous years was the addition of injectable ketoprofen 15 percent for pain relief. Gross postmortem on calves that died or were euthanased consistently revealed ulceration of the pyloric mucosa, with perforation and fibrinosuppurative peritonitis. The presence of granulation tissue suggested chronicity of days to weeks.

Numerous potential risk factors for bovine abomasal ulcers have been investigated over the years but the aetiology remains elusive. Factors that may play a role include environmental and nutritional stress, hyperacidity, extended feeding interval, increasing dietary roughage, gastroenteric infections, copper and vitamin E deficiencies, and trichobezoars (Navarre & Belknap, 2000). In people, it is recognised that ketoprofen carries a higher risk of bleeding peptic ulcers than most other classes of NSAID (Langman *et al.,* 1994). Whether this is also the case in ruminants warrants further investigation.

Two Friesian calves aged four months died suddenly and three more became acutely unwell over a two-week period. The calves were from a mob of heifer replacement calves on a Taranaki dairy farm. The sick calves developed tachypnoea and diarrhoea. Faecal samples from two were negative for *Salmonella* spp. and nematode eggs. Histological examination of multiple tissues revealed lung lesions characterised by diffuse pulmonary oedema and emphysema with hyaline membranes and lobular collapse. These changes were compatible with **acute bovine pulmonary oedema** and **emphysema.** The most frequently recognised cause of these signs is L-tryptophan in lush pasture, which is converted to the pneumotoxic 3-methylindole in the rumen.

Marked splenic enlargement was noted at slaughter of a line of 18 two-year-old beef bulls from a Central Hawke's Bay farm. The spleens ranged from two

to three times normal size and had a uniform, meaty texture on cut surfaces. There was no history of illness and no other abnormalities were detected on inspection of the carcasses. Histological sections of several spleens showed extensive extramedullary haematopoiesis. ***Theileria orientalis* Ikeda** was detected by PCR on pooled spleen samples, confirming the presumptive diagnosis of **theileriosis**.

Ovine

A large outbreak of **parapox (scabby mouth)** was diagnosed in a mob of 150 orphan lambs being reared in a shed on a Southland sheep farm. Sixty lambs developed severe mouth and lip lesions, which led to 25 deaths through inanition. The diagnosis was confirmed by histopathology of typical lesions. This virus was known to be endemic on this farm but the initiating cause could not be determined.

Mycoplasma ovis (formerly *Eperythrozoon ovis)* was confirmed as the cause of anaemia in ram hoggets on irrigated pasture in Central Otago. Three were found dead and others were seen to be weak in one mob of 85, while other mobs were unaffected. The diagnosis was confirmed by a combination of necropsy findings and examination of blood films.

Fifty lambs aged five weeks were being hand-reared on a grass paddock on an Otago sheep farm. Ten died and 20 were affected with severe diarrhoea over a two-week period. Large numbers of coccidial oocysts were identified in the faecal contents of one dead lamb, confirming the cause of the deaths as **acute coccidiosis**.

Hoggets in a mob of 50 with lambs at foot were grazing lucerne on a Central Otago farm. Over a two-day period five lambs were found dead and a further 15 had difficulty walking, with a stiff gait and tending to fall over when made to run. A necropsy of one dead, well-conditioned lamb revealed pale muscles in all four legs. Histopathological examination of fixed sections of affected muscle, and a liver selenium test of 530 nmol/kg (adequate concentration > 850) confirmed a **nutritional myopathy (white muscle disease)**.

Four-month-old unweaned lambs were yarded for drenching in mid-December on a Southland sheep farm. Several were noticed in sternal recumbency, while others were lame or weak and unable to walk very far although still bright and alert. Necropsy of two severely affected lambs showed no gross lesions. Histopathological examination of the hindbrain found vacuolation of the white matter (spinal cord was not examined). With the history and histological findings, copper deficiency was suspected as the cause. The liver copper concentrations in two lambs were 39 and 42 µmol/kg (adequate > 65), confirming **copper deficiency** and a diagnosis of **enzootic ataxia**.

Seven recently lambed, mixed-age ewes died and another six became unwell over a five-day period on a West Otago sheep farm. The ewes became thin and tucked-up prior to death. Clinical examination of one ewe confirmed very poor body condition. The ewe was blind, stargazing and developed seizures during examination. The only gross abnormality noted at postmortem was a very pale liver with rounded margins. Microscopic examination confirmed severe hepatic lipidosis and accumulation of pale tan pigment in hepatocytes and macrophages. There were scattered clear vacuoles in the white matter tracts of the forebrain, particularly close to junctions of grey and white matter. Enlarged astrocytes with vesicular nuclei (Alzheimer type II cells) were seen in the cortical grey matter. The final diagnosis was **hepatic encephalopathy** secondary to severe hepatic lipidosis. Possible causes of hepatic lipidosis include negative energy balance, vitamin B12 deficiency and intoxication.

A two-month-old lamb on a large sheep and beef property running 2500 ewes in the eastern hills of the Rangitikei district was examined because of ill-thrift. The lamb was a Suffolk/Romney cross and was thought to have been mismothered at docking two weeks earlier. It was in poor condition with a tucked appearance to the abdomen, suggesting abdominal pain. At postmortem the lamb had few fat reserves, weighed an estimated 9 kg and had no milk in the abomasum. Gross postmortem findings were restricted to the abomasum where there was prominent oedema, crepitus and gas bubble formation throughout the abomasal wall. On histopathological examination, gas and oedema expanded the mucosa and submucosa of the abomasum. In most areas there was distortion and distension of the tissues, while in the submucosa there was haemorrhage and light infiltrates of inflammatory cells. On the surface of the abomasum were clusters of bacteria characteristic of ***Sarcinia*** sp., a common soil-borne and environmental bacterium associated with abomasal oedema and emphysema in pre-ruminant lambs, goats and calves. *Sarcinia* produces large volumes of carbon dioxide, resulting in **abomasal bloat** and often leading to abomasal rupture and death.

Ten one-year-old Romney stud hoggets from a flock of 300 on a Manawatu farm died suddenly over several days. The rams had been grazing white clover and plantain and were fully vaccinated against clostridial infections. Post-mortem examination showed diffuse marked reddening and congestion of the small intestine. Microscopically, there was marked congestion and extensive haemorrhage all through the intestinal wall, with sloughing of the superficial mucosa. The dietary history and pathological findings supported a diagnosis of **ovine redgut**. This is thought to be caused by arrested intestinal blood flow, which is due to mesenteric torsion and intestinal dilation. Torsions are often difficult to detect at necropsy as the rumen is relatively small on lush diets and the intestines may move after death.

Cervine

A mob of 600 yearling hybrid weaner deer had been grazing lucerne for six weeks on a Southland deer farm. They were then placed in holding paddocks and yarded the next day for anthelmintic treatment before being split into three mobs and placed on grass paddocks. The next day, more than 50 across the three mobs showed nervous signs of blindness, head-pressing and dullness. One deer found dead was necropsied but no gross lesions were seen. Histopathological examination of the fixed brain confirmed microscopic lesions of **polioencephalomalacia.**

Scabby, oedematous lesions were found all over the velvet antlers of numerous three-year-old stags on a Southland deer farm. This was confirmed as a **cervine parapox** infection by histopathological examination of fixed sections of affected antler from one stag. The stags had been grazing a paddock containing a large number of thistles and it was thought that skin trauma had permitted entry of the virus.

A Hawke's Bay deer farmer reported the death of five out of 135 one-year-old red stags. The deaths occurred over five days and were not preceded by noticeable illness. Post-mortem examination revealed fibrinopurulent pleural exudate in two of the stags. Histologically there were thick mats of fibrin, necrotic debris and degenerate neutrophils, and small bacterial rods were adhering to the pleural surface. Interlobular septae were expanded by fibrin and oedema. Culture of the pleural exudate yielded a heavy growth of ***Pasteurella multocida***. **Pasteurellosis** is occasionally implicated as a cause of sudden death in farmed New Zealand red deer (Wilson, 2002).

A Central Hawke's Bay deer farmer reported crusting lesions on the velvet of rising yearling spikers. Similar lesions had been observed in the same age-group in previous years. Older stags were not affected. Microscopic examination of a section of affected velvet showed severe necrosuppurative dermatitis with ballooning degeneration of keratinocytes, ulceration and crusting. The presumptive diagnosis of **parapoxviral dermatitis** was confirmed by testing scabs using PCR. Two different assays were performed, both resulting in strong positive detection of parapoxvirus.

Caprine

An adult Saanen doe was examined for weakness and diarrhoea on a goat farm in the Waikato where two other goats had died. The goat had pallor of the gums and conjunctiva so a whole blood sample was collected and a complete blood count performed. This confirmed **anaemia** as the packed cell volume was 0.14 (reference range 0.27–0.42), red blood cell count 6.65 x 10^{12}/L (reference range 13.5–22.8 x 10^{12}) and haemoglobin 50 g/L (reference range 89–138). A faecal egg count identified 1000 strongyle eggs per gram of faeces, confirming a significant **gastrointestinal parasite burden**, which was a possible reason for anaemia via blood loss.

A male Saanen goat of unknown age from the Auckland region presented for veterinary examination with swollen joints. A serum ELISA test was positive for antibodies to **caprine arthritis and encephalitis (CAE)** virus infection.

Porcine

Eleven litters of free-range piglets of various ages in Whangarei were coughing and dying. The only environment change that had occurred was that the pigs were being housed in old calf sheds with sawdust floors where previous cases of calf pneumonia had occurred. The piglets were immediately de-wormed and removed from the pens. Histopathology of lung, kidney, lymph node and spleen from two euthanased piglets revealed severe pleuropneumonia with intralesional bacterial organisms, mild interstitial nephritis, reactive lymphoid hyperplasia and splenic lymphoid depletion, respectively. Sputum culture from one of the piglets yielded a heavy growth of ***Streptococcus dysgalactiae*** and ***Escherichia coli*** and a moderate growth of ***Pseudomonas*** spp., confirming mixed **bacterial pneumonia**.

Canine

Enterococcus faecium was isolated from a persistent ear infection in an Otago dog. The isolate was resistant to all the antibiotic panels run. This bacterium can also infect humans, causing nosocomial bacteraemia, surgical wound infection, endocarditis and urinary tract infections.

Feline

A three-year-old cat had an acute onset of hindlimb ataxia. After eight days it was presented to a veterinarian for examination. A latex agglutination test for toxoplasma found a titre of 1:2048, confirming a protozoal myelitis caused by ***Toxoplasma gondii***.

A seven-month-old neutered male Skookum cat in the Auckland region had a three-month history of large-bowel diarrhoea and a pot-bellied appearance. It was up to date with deworming treatments. Faecal parasitology was negative for nematode eggs and coccidial oocysts. Faecal antigen ELISA was negative for *Cryptosporidium* but positive for ***Giardia*** spp., confirming a diagnosis of **giardiasis**.

Equine

An outbreak of abortions occurred in mares on a large Southland stud with 180 pregnant mares. Ten of 18 mares in the same paddock aborted over a 10-day period. All the mares were two to three weeks from term and the aborted foals were minimally decomposed. Necropsy of the foals revealed pulmonary oedema in the lungs. Typical **herpesvirus** lesions were found on histopathological examination of fixed liver and lung of one aborted foal examined.

Lagomorph

A two-and-a-half-year-old male neutered Lop rabbit in Auckland had a history of tachypnoea and radiographic evidence of lower respiratory tract disease. It had been treated with a prolonged course of oxytetracycline and some improvement had been noted but signs returned when the treatment was discontinued. Culture from a bronchoalveolar lavage sample revealed scant growth of ***Berkholderia capacia*** (previously *Pseudomonas capacia*), confirming **bacterial pneumonia**.

Avian

An 18-month-old pigeon (*Columba livia*) from Hamilton developed diarrhoea and a faecal screen for pathogens was undertaken. A coccidial count revealed extremely high numbers of coccidia and confirmed that **coccidiosis** was the aetiology of the diarrhoea.

A seven-month–old Araucana chicken from the Waikato was presented with intermittent diarrhoea and a history of decreased egg production. A faecal sample was examined for evidence of parasite eggs. None were found but there was an extremely heavy presence of coccidia, confirming **coccidiosis** was the aetiology of the diarrhoea.

Three adult canaries in an aviary of 15 birds died suddenly over a period of several weeks. One bird was noted to be lethargic and fluffed-up prior to death. Necropsy was carried out on this bird. The spleen was enlarged and had a mottled appearance and finely granular texture. An impression smear of the spleen revealed clusters of bacterial rods and numerous heterophils. Multiple tissues were processed and examined histologically. There were randomly scattered large colonies of fine bacterial rods associated with necrosis and inflammation in the liver and spleen. Similar bacteria occluded blood vessels in the lung and kidney. The bacteria were Gram-negative. These findings were compatible with disseminated **yersiniosis**.

Amphibian

An African clawed frog from a small zoo collection stopped eating and became moribund over a period of two weeks. It had a history of unspecified infection seven years previously and had appeared slightly bloated since then. Generalised anasarca and ascites were noted at post-

mortem examination. There was an ill-defined mottled mass lesion about 12 mm in diameter in the coelomic adipose body. Histologically the mass was composed of granulomatous inflammation and fibrosis with scattered multinucleated giant cells. A Ziehl-Neelsen-stained section revealed occasional acid-fast bacilli within macrophages and multinucleated giant cells, confirming a diagnosis of **mycobacteriosis**. Mycobacterial species reportedly associated with disease in frogs include *M. ulcerans, M. marinum, M. liflandii, M. chelonae* and *M. xenopi*. Significant outbreaks may occur in research colonies (Fremont-Rahl *et al.*, 2011). As well as being contagious among frogs, mycobacteria have zoonotic potential.

Rodent

Two four-year-old female agoutis in a North Island zoo died acutely, one month apart. Both developed diarrhoea prior to death. Post-mortem examination revealed subcutaneous petechiae and ecchymoses and adrenomegaly in both cases. One animal also had hundreds of 1–2-mm diameter white foci scattered through the intestines, liver, spleen and lungs. Histologically, both agoutis had numerous foci of inflammation and necrosis centred on large colonies of bacterial rods in the liver. The one with grossly visible foci had numerous similar lesions scattered through the spleen, intestines and lungs. The bacteria were Gram-negative. Culture of the liver resulted in a growth of ***Yersinia pseudotuberculosis***, confirming a diagnosis of **yersiniosis**. Identification of the organism also used matrix-assisted laser desorption ionisation time-of-flight mass spectrometry.

Reptile

An 11-year-old male entire red-eared slider turtle from Auckland presented with anorexia and a thick white discharge from the mouth. Culture revealed a heavy growth of ***Aeromonas hydrophila***, confirming a diagnosis of **aeromoniasis**.

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Quarterly report of investigations of suspected exotic diseases

Exotic disease investigations are managed and reported by MPI Investigation and Diagnostic Centre (IDC) and Response, Wallaceville. The following is a summary of investigations of suspected exotic disease during the period from October to December 2015.

Exotic vesicular diseases ruled out

A veterinarian reported to the IDC a suspect vesicular disease in several three-month-old dairy calves. An Initial Investigating Veterinarian (IIV) from AsureQuality visited the property and examined the calves. Nine out of 17 calves in the mob (53 percent) were affected with mild oral erosive lesions. None were pyrexic or showed signs of systemic disease. Erosive lesions were present on the dorsal and ventral tongue. No lesions were present on the feet, nor was there any evidence of lameness in any of the calves.

A biopsy was taken from one lesion and a portion of the tissue collected was fixed in 10 percent buffered formalin. There were parakeratotic epithelial cells associated with mixed bacterial colonisation but nothing to indicate a specific aetiology. There were no significant findings either from blood biochemistry or a complete blood count carried out on blood from six affected calves. All of these calves tested negative by PCR for bovine viral diarrhoea and malignant catarrhal fever. In addition, virus culture of tissue did not show a cytopathic effect from two passes. Vesicular disease was excluded on clinical and epidemiological grounds but no specific aetiology was determined for the lesions observed.

A veterinarian called the MPI exotic pest and disease hotline after examining two ewes with mild pyrexia (40.1°C and 40.4°C), erythema, crusting and oedema affecting the muzzle, lips, eyelids and vulva. Although the findings were consistent with photosensitivity, the veterinarian was concerned because this condition had not previously been seen in the area and the presentation potentially aligned with exotic vesicular or bluetongue disease. Under the direction of an Incursion Investigator, the veterinarian (who was also an IIV contracted to AsureQuality) inspected the remainder of the flock (two separate mobs of about 250 each, all with 4–6-week-old lambs at foot) and found no other clinically affected animals. The worst-affected ewe was euthanased and a postmortem carried out. Gross findings were generally unremarkable apart from the liver appearing somewhat pale. No feet, mouth, gum, oesophageal or rumen pillar lesions were identified. Exotic vesicular disease was excluded on clinical and epidemiological grounds. Tissues were collected for histology and blood samples were collected from both ewes to help establish an endemic diagnosis. Haematology indicated an inflammatory leucogram, and serum biochemistry identified raised bilirubin, serum GLDH, AST and GGT, indicating mild-to-moderate hepatocellular damage with cholestasis. A presumptive diagnosis of hepatogenous (secondary) photosensitivity was made and the investigation was stood down.

Enzootic bovine leukosis excluded

A veterinarian notified MPI of two 18-month-old bulls that were positive by ELISA for enzootic bovine leukosis during pre-export testing. The bulls were from a shipment of 4000 animals destined for China. Samples were sent for confirmatory testing at the Livestock Improvement Corporation and both were negative on repeat testing. This meant the initial tests were false positives rather than indications of true exposure.

Calf deaths investigated

A farmer called the MPI exotic pest and disease hotline to report the death of seven 5-month-old Jersey crossbred calves bought from a local market about four months earlier. The calves had died over the previous 4–6 weeks, the final two only in the last 48 hours. The farmer was concerned that the calves might have developed an atypical disease, as routine treatments such as deworming had been ineffective. Under the direction of an Incursion Investigator, a local veterinarian visited the property and carried out post-mortem examinations of the two most recently dead calves. Gross findings were unremarkable except for poor fat reserves and loose faeces in both animals. Faecal examination identified coccidial oocysts in one calf and moderate strongyle egg counts in both. Histology identified moderately severe enteritis, consistent with coccidiosis. Findings were attributed to malnutrition associated with untreated enteric disease. Exotic disease was excluded and the investigation was stood down.

A veterinary pathologist called the MPI exotic pest and disease hotline to report unusual lung lesions in a calf that had died within 24 hours of birth. The calf had severe generalised pneumonia, with many alveoli containing proteinaceous material, neutrophils, macrophages and quite frequent multinucleated syncytial cells. Many small colonies of coccobacilli were seen. It is unusual for an animal to develop such lesions within 24 hours of birth and the calf might have suffered an *in utero* viral infection followed by secondary bacterial infection. The farm manager was interviewed by an Incursion Investigator. With calving 85 percent completed, the 250-cow herd had recorded 4 percent stillbirths or early neonatal deaths across all cow ages. Primiparous cows had 8 percent stillborn calves or early neonatal deaths. No respiratory or other diseases were noted among the calves. Immunohistochemistry testing on lung tissue at an overseas laboratory did not detect bovine herpesvirus type 1 (subtype 1.1 or abortifacient strain) – an exotic differential for weak calves at birth – so an atypical *in utero* manifestation of bovine respiratory syncytial virus infection was ruled out. Calving was

completed without any further losses, so no further samples were available for study and the investigation was closed.

Sporadic bovine encephalomyelitis excluded

A veterinary pathologist called the MPI exotic pest and disease hotline to report a case of encephalitis in a one-week-old calf from Hawke's Bay. The calf had died suddenly and histology indicated inflammation of the brain. The encephalitis resembled sporadic bovine encephalomyelitis (SBE), caused by infection with *Chlamydia pecorum*, a disease that seems to have increased in prevalence over recent years. Frozen tissues were sent to the AHL (Wallaceville) for *Chlamydia* PCR but unfortunately samples of fresh brain and lung (the two most commonly affected organs) had not been collected. Frozen kidney, liver, spleen, small intestine, blood, urine and lymph node all tested negative by PCR for *Chlamydia* spp. Besides SBE, possible causes of encephalitis in young calves include environmental bacteria such as *Escherichia coli*, *Staphylococcus* spp. and *Streptococcus* spp., but infection with any of these agents would typically present with a slightly different histological pattern (more suppuration) than the present case. The cause of death in this calf remains unknown. SBE was considered unlikely given the large range of tissues tested, though it could not be completely excluded. Other bacterial causes were not investigated. The investigation was stood down.

Bovine splenic enlargement investigated

An MPI meat plant veterinarian on the West Coast called the exotic pest and disease hotline to report two possible cases of theileriosis in slaughtered animals. One steer had an enlarged, reddened spleen and lymph nodes, and acute pleuritis and pericarditis. The other had an enlarged spleen but no other abnormalities. Frozen and fixed samples of spleen were collected and sent to the AHL (Wallaceville). Histology of the first spleen showed a monomorphic population of large, round cells, consistent with splenic lymphoma. The other spleen had an unusual lesion consisting of hyperplasia of the fibrohistiocytic and mesothelial tissue; this unusual and idiopathic change was considered most likely secondary to trauma or hypoxia. Both spleens were negative for *Theileria orientalis* sspp. Buffeli, Ikeda and Chitose by PCR. Since the first finding of *T. orientalis* Ikeda in New Zealand in 2013, this agent has been found in many places in both the North and South Islands, associated either with cattle movements from infected regions or with seasonal changes in tick populations. *T. orientalis* Ikeda has recently been found in a herd of cattle on the West Coast but is not believed to be widespread there at present.
This case presented two interesting differential diagnoses for vets to consider when attempting gross diagnosis of clinical theileriosis.

Scrapie ruled out

A member of the public called the MPI exotic pest and disease hotline regarding a seven-year-old Angora buck. It had been imported from Australia 18 months previously and since arriving in New Zealand it had had skin melanomas removed and confirmed on histopathological examination. The goat was now losing weight and the owner wanted the advice of an Incursion Investigator before having it euthanased. The goat did not display pruritus or nervous signs. Melanoma is the most common skin tumour in New Zealand goats (especially Angoras and Saanens), usually originating on the ears, muzzle or perineum and rapidly metastasising to regional lymph nodes and/or lungs. By the time melanomas are detected they have often metastasised, so the prognosis should always be guarded, even if the primary lesion appears to have been completely removed (Anonymous, 2012). The Incursion Investigator concluded that the weight loss was consistent with metastasised melanomas. Exotic disease was ruled out because firstly, the goat came from Australia, where the risk of transmissible spongiform encephalopathies is negligible; secondly, there were no nervous signs; and thirdly, the history of melanomas provided a complete explanation. Accordingly, the investigation was stood down.

Akabane virus excluded

A Massey University veterinary pathologist called the exotic pest and disease hotline after carrying out postmortems on three lambs with fetal abnormalities including absence of feet, joint fusion, kyphosis, scoliosis, cleft palate and undershot jaw. Multiple tissues from the lambs were submitted to the AHL (Wallaceville) for exclusion of exotic disease. Molecular assays were all negative for Schmallenberg, bluetongue and Akabane viruses at an Australian reference laboratory. A molecular assay for bovine viral diarrhoea virus was also negative. Histological abnormalities were limited to the spinal tract and included myelodysplasia, presence of multiple spinal canals and asymmetry of the ventral and dorsal horns. No changes consistent with a viral aetiology were identified. These findings were considered the result of either a genetic or toxic aetiology. DNA fingerprinting will be carried out to assess whether one or more sires produced the affected lambs. During the remainder of the lambing season four more deformed lambs were identified in the flock of about 1500 crossbred (Texel, Romney and Poll Dorset cross) ewes. Exotic disease was excluded and the investigation was stood down.

EHV-1 myeloencephalitis excluded

A veterinarian called the exotic pest and disease hotline to report an 11-month-old Thoroughbred yearling with urinary incontinence. In-contact horses remained clinically normal. Urinary incontinence can be associated with the neurological manifestation of equine herpesvirus-1 (EHV-1) infection (equine herpesvirus myeloencephalopathy).
While equine herpesvirus myeloencephalopathy is not an exotic disease, there has been only one recorded outbreak in New Zealand. Further investigation was carried out as it is a highly transmissible disease requiring prompt biosecurity measures to control spread. However, nasal swabs and bloods submitted to the AHL (Wallaceville) tested negative for EHV-1 by PCR. It was concluded that the urinary incontinence was a legacy of a pelvic injury that the yearling had suffered in its paddock four months earlier. The investigation was closed.

Contagious equine metritis ruled out

An equine reproduction veterinarian called the exotic pest and disease hotline to report an 11-year-old Standardbred mare with a recurrent grey vaginal discharge consistent with endometritis.

The mare was New Zealand-bred and had not been served naturally, but had been inseminated with imported (as well as New Zealand-sourced) semen. Uterine and clitoral swabs were submitted to the AHL (Wallaceville), where molecular techniques excluded *Taylorella equigenitalis* (the causative agent of contagious equine metritis). Routine bacterial culture of the uterine swab identified a heavy growth of *Streptococcus equi* ssp. *zooepidemicus*, which is commonly associated with equine endometritis. The infection resolved uneventfully after routine antibacterial and antifungal therapy. Exotic disease was excluded and the investigation stood down.

Canine histoplasmosis ruled out

A veterinary pathologist called the exotic pest and disease hotline to report possible exotic fungal disease in a five-year-old Bernese Mountain Dog seen for autopsy. The dog had no travel history and had been euthanased after presenting with persistent neutrophilia, infarcts in multiple organs and no response to supportive therapy. Histopathology showed granulomatous (macrophage-rich) gastritis, enteritis and lymphadenitis, with the mural wall often affected. Macrophages frequently contained small, round structures that stained positively with PAS and Young's fungal stains and resembled *Histoplasma capsulatum*, a fungus that is widely distributed but considered exotic to New Zealand (Hill, 1999). Fresh tissues and formalin-fixed paraffin-embedded (FFPE) tissues were sent to the AHL (Wallaceville), and the following tests were performed: secondary referral for histology to the state of Oklahoma (where histoplasmosis is endemic); PCR of FFPE tissues for *Histoplasma* spp.; and generic PCR of fresh and FFPE tissues. All tests were negative for fungi including *Histoplasma*. The identity of the intrahistiocytic structures was not determined but they could have been cellular debris or another non-infectious agent. The cause of the histiocytic inflammation in this dog remains undetermined. This case was investigated as thoroughly as possible given the post-mortem nature of sample collection and lack of additional cases. The exotic differential histoplasmosis was ruled out and the investigation was stood down.

Canine distemper excluded

A veterinarian called the exotic pest and disease hotline to report possible distemper in a five-month-old unvaccinated Mastiff cross dog with a three-week history of bilateral nasal discharge and progressive illness. There was no history of travel or exposure to imported animals. The dog initially presented with fever and bilateral discharge from the nose. It was treated with antibiotics but when seen again three weeks later the discharge had become severe, bilateral and mucopurulent. The dog was also polydipsic, retaining fluid (oedema) and was coughing. Distemper serology arranged by the AHL (Wallaceville) was negative for distemper antibodies. Bloodwork including CBC and blood chemistry was within normal limits except for mild leukocytosis, indicative of inflammation. Follow-up indicated that the dog was improving. Distemper was ruled out as the cause of disease and the investigation was stood down.

Brucella canis excluded

A veterinary practitioner called the exotic pest and disease hotline to report an imported eight-year-old Irish Setter with enlarged testicles. The dog had not been used for breeding. A serum sample was submitted to the AHL (Wallaceville) and *B. canis* ruled out by a *B. canis* card test. Exotic disease was ruled out and the investigation stood down. Further diagnostics attributed the signs seen to testicular neoplasia.

A veterinary pathologist called the exotic pest and disease hotline to report severe subacute focally extensive necrosuppurative and fibrosing orchitis and epididymitis in a swollen testicle submitted from a 10-year-old Huntaway x Pit Bull pig-hunting dog. The dog was observed to have "slowed down" in the previous two months. While this inflammation was most likely due to local trauma, brucellosis caused by *Brucella suis* or *B. canis* was a differential diagnosis as both of these agents are exotic to New Zealand. Unfortunately the dog died before a serum sample could be collected for serological testing. However fresh spleen and lymph node were collected and submitted along with the fixed testicle slides to the AHL (Wallaceville) for DNA extraction and generic *Brucella* PCR testing. Results were negative, so these exotic species were ruled out and the investigation was stood down.

Meliodosis excluded

A Gribbles veterinary pathologist called the exotic pest and disease hotline to report the culture of a bacterium that on preliminary assessment was consistent with *Burkholderia pseudomallei* (previously *Pseudomonas pseudomallei*). The culture was from a swab taken from the soft palate of a 14-year-old Munsterlander dog in which a skin biopsy had confirmed a diagnosis of erythema multiforme (immune-mediated skin disease). As well as a generalised purulent stomatitis/gingivitis, the dog had dermatitis affecting the ears, eyelids and anal area. The culture was referred to the AHL (Wallaceville) for evaluation and tested negative for *B. pseudomallei* and *B. mallei* by PCR; also it did not conform with the expected growth patterns for these organisms. Sequencing of the 16S PCR product from the culture showed highest similarity to *Serratia marcescens* (Enterobacteriaceae). This organism is associated with soil, considered widespread and has been associated with opportunistic and often hospital-acquired infections in humans who are immunocompromised or have other chronic debilitating diseases. In dogs it has occasionally been associated with disease including abscessation after dental extraction and endocarditis. *S. marcescens* can induce myelostimulation and activate macrophages with release of proinflammatory cytokines, which raises the possibility that it was the inciting aetiology of the infection seen in this dog (Maddison *et al.*, 2008). The dog had eaten a smoked beef bone a few days before developing the stomatitis, raising the possibility that gum or mouth trauma associated with gnawing on the bone may have incited this episode. Exotic disease was excluded and the investigation was stood down.

Dirofilaria immitis ruled out

A veterinary practitioner called the exotic pest and disease hotline regarding a 12-year-old spayed Domestic Shorthaired cat with a cough of four months' duration. The cat had been imported from Rarotonga shortly before the cough began. Furthermore, the cat's owner had recently died from complications of pulmonary tuberculosis

caused by *Mycobacterium tuberculosis*. Serology, PCR testing and culture of a bronchoalveolar lavage were completed and tuberculosis was ruled out. The exotic differential feline heartworm (*Dirofilaria immitis*) was ruled out at an overseas laboratory by antibody ELISA testing (the preferred diagnostic test for heartworm in cats). Parallel investigation by the referring veterinarian attributed the cough to bronchopneumonia caused by *Pasteurella* spp. and complicated by feline immunodeficiency virus. The cat responded to antibiotic treatment. Exotic disease was ruled out and the investigation was stood down.

Exotic feline haemoparasites excluded

A veterinary pathologist called the exotic pest and disease hotline to report possible rickettsia-like disease in an imported cat. The elderly cat had been imported from the US and presented to a veterinarian with a complex array of clinical and laboratory signs including weight loss, increased appetite and a CBC featuring a regenerative anaemia with 30–35 percent Heinz bodies. *Ehrlichia* spp. and other blood-borne bacterial parasites can be a cause of anaemia with Heinz bodies, and are exotic to New Zealand. DNA was extracted from blood samples at the AHL (Wallaceville) and battery testing for haemoparasites was subcontracted to Vector Borne Diagnostic Laboratory, North Carolina. Immunofluorescent antibody testing was negative for *Bartonella vinsonii, B. hensalae* and *B. koehlerae*; PCR was negative for *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Ehrlichia* spp., *Mycoplasma* spp. and *Rickettsia* spp. Extensive further diagnostic testing was performed by the veterinarian at the owner's expense. Based on elimination of other likely causes, inflammatory bowel disease was tentatively diagnosed as the cause of the weight loss and systemic illness. The cause of the Heinz bodies was unclear, though some systemic illnesses can reportedly cause Heinz bodies in cats. Other causes (e.g., onions, acetominophen, propylene glycol) were ruled out based on owner history. Exotic disease was ruled out as a cause of illness and the investigation was stood down.

Rabbit syphilis ruled out

A veterinarian called the exotic pest and disease hotline to report a suspected case of rabbit syphilis (*Treponema paraluiscuniculi,* aka *T. cuniculi*) in a male neutered domestic rabbit with severe perineal ulcerative dermatitis. *T. paraluiscuniculi* is thought to be a worldwide pathogen of rabbits, but has not previously been confirmed in New Zealand, despite the suspicion that it is likely to be present (Midwinter & Fairley, 1999) and a single case of spirochaetes in rabbits (Townsend, 1994). The rabbit was undergoing antibiotic therapy with penicillin prior to notification, and when it did not respond as favourably as expected, bacteriology and cytology were also performed. Causes of perineal dermatitis in rabbits include husbandry and urinary tract issues, as well as *T. paraluiscuniculi* infection (which can also cause lip and nasal lesions).

In this case, serological testing was employed as samples had been obtained after antibiotic treatment. Immunofluorescent antibody testing returned a negative serology result for *T. paraluiscuniculi.* General bacterial culture of the healing perineum showed growth of *Staphylococcus xylosus* and a minor growth of *S. capitis. S. xylosus* is usually considered a component of normal skin flora of mammals, though it has been reported as an opportunistic pathogen, for example in immunocompromised laboratory mice (Won *et al.*, 2002). Cytological examination yielded normal results, with populations of inflammatory cells present as would be expected in a healing lesion. *Treponema* infection was ruled out in this case, the rabbit continued to improve with antibiotic treatment and a change in husbandry, and the investigation was stood down.

Infectious bursal disease ruled out

As part of routine infectious bursal disease (IBD) surveillance carried out by the Poultry Industry Association of New Zealand, serum reactors in the screening ELISA are tested by virus-neutralisation test (VNT) at the AHL (Wallaceville). During February and March 2015, three broiler barns from different farms were identified with serum reactors in the IDEXX IBD ELISA test. Samples were sent to the AHL (Wallaceville), where low-positive titres in the VNT were confirmed in all three barns. MPI's Incursion Investigation team then carried out a comprehensive investigation, with testing focused on the next batch of birds placed in each barn. Mortality monitoring and bursal evaluation of daily mortalities in each barn was carried out by each farm's veterinarian. No abnormalities were detected. At processing, samples of serum (20), and bursae (70 fresh and 10 fixed) were collected from each barn. Serological assessment using the IDEXX IBD ELISA was followed by IBD VNT testing. Testing of all the fresh bursae from VNT-positive barns was carried out using two molecular assays, for IBD serotypes 1 and 2. One farm had no ELISA reactors, while the other two had ELISA reactors at a prevalence that varied from 5 to 58 percent. VNT titres from the ELISA positives were low (predominantly 1:16). Both molecular assays were negative for IBD virus in all bursa tested. Of the three farms investigated here, serum reactivity at the follow-up sampling only persisted in the two free-range operations. In common with most broiler farms in New Zealand, no birds on these farms were vaccinated against any viral or bacterial agents, so any non-specific serum reactivity cannot be attributed to antigen or adjuvant received at vaccination. Persistent serum IBD reactivity is uncommon and rarely seen in broiler birds. It is interesting to note that a previous comprehensive investigation into ongoing serum reactors also involved a free-range management system with layer birds (Bingham *et al.*, 2006). Repeated longitudinal sampling of birds at processing from each of the broiler farms investigated here is being undertaken to help assess seasonal patterns, in an attempt to gain a better understanding of the possible factors contributing to non-specific reactivity. A comprehensive assessment of multiple indicators of IBDV infection including clinical signs, mortality, bursal pathology, serology and presence of antigen, has excluded infection with IBD virus on the three farms investigated here. Exotic disease was excluded and the investigation was stood down.

In another, unrelated investigation, during August 2015 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 (Wallaceville), where low-

positive titres in the VNT (1:6, 1:12) were identified. 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. Mortality monitoring and bursal evaluation of daily mortalities was carried out by the farm's veterinarian. No abnormalities were detected and, at processing, samples of serum (25) and bursae (80 fresh, 10 fixed) were collected from each barn. Serological screening using the IDEXX IBD ELISA identified no reactors, so there was no need to follow up serological or molecular assessments. Exotic disease was excluded and the investigation was stood down.

Granulomatous disease in a kea

A veterinarian from Wellington Zoo called the exotic pest and disease hotline to report a kea (*Nestor notabilis*) with recurrent subcutaneous fungal granulomas. The regional veterinary laboratory was having difficulties in confirming the identity of the fungus. The kea originally had three granulomas around the face and jaw area, which were surgically removed. A few months later nodules were identified over the keel and legs. Molecular testing was negative for chlamydophila (tissue swab), beak-and-feather disease virus (feathers) and polyoma virus (blood). In each case, histology confirmed chronic granulomas with intralesional fungal hyphae and there was no evidence of bacteria or mycobacteria on Gram or acid-fast staining. Fungal cultures were submitted to the AHL (Wallaceville), where *Candida albicans* was confirmed using biochemical and germ-tube testing. A CT scan of the parrot indicated a likely nidus of fungal infection in the respiratory or gastrointestinal tracts, and long-term antifungal therapy was instituted. Exotic disease was excluded and the investigation was stood down.

Exotic honey bee diseases excluded

A Marlborough beekeeper called the exotic pest and disease hotline to report dysentery in one of 50 queen breeder hives. Dysentery indicates a digestive system disturbance that causes bees to defecate on the combs or near the hive entrance. Causes include fermented honey stores, excessive moisture in the hive or prolonged confinement to the hive caused by bad weather. Nevertheless, an investigation was carried out to ensure that no exotic or endemic bee pathogens were involved. A site inspection was conducted by an Apiculture Auditor (AP2) under instruction from an AsureQuality Apiculture Technical Adviser, and bees were collected for testing. The AP2 observed that the one affected colony had no food stores, that until recently the hive entrance had been occluded by long grass, the hive had a damp interior and it was sited in a shaded area. The exotic Israeli acute paralysis virus was ruled out in testing at the AHL (Wallaceville). Bees tested at dnature Gisborne were negative for *Nosema ceranae, N. apis* and chronic bee paralysis virus but positive for black queen cell virus, deformed wing virus, Kashmir bee virus and *Lotmaria passim*. It was determined that the endemic agents identified were not unusual background findings and that the dysentery was the result of poor nutrition. The dysentery resolved with the onset of spring pollen flows.

Honey bee mortalities investigated

A backyard beekeeper called the exotic pest and disease hotline to report the sudden death of 100–200 bees a day or two previously, in one of her two hives. An apiary specialist carried out a site investigation and found that the colony was strong, with no current evidence of dieoff. The bees that had died were unsuitable for disease testing. The apiary specialist suspected accidental poisoning, as occasionally occurs with the use of pesticides. The beekeeper was asked to report the incident to the Environmental Protection Authority, the government department responsible for maintaining a register of pollinator incidents. Exotic disease was excluded on clinical and epidemiological grounds, and the investigation was closed.

Exotic bees excluded

A member of the public in Hastings called the exotic pest and disease hotline to report an aggressive type of bee that she had not seen before. She saw several of the distinctive insects attacking honey bees in her garden. An Apicultural Officer, contracted by MPI through AsureQuality, discussed the behaviour and appearance of the insects and concluded that they were most likely to be wool carder bees, *Anthidium manicatum*, which are an established species in New Zealand. They were first discovered here in Nelson and Napier in 2006, and the males can be extremely territorial towards honeybees when defending flower resources.

A member of the public called the exotic pest and disease hotline to report that a swarm of what he described as "unusual small bees" had entered his house. He was concerned that they might have come from a neighbouring property that had recently received household goods from Asia. The notifier killed the insects with a household pesticide spray but was unable to provide photos to help identify them. An MPI Plant and Environment Incursion Investigator visited the site and collected a sample of the insects, which were sent to the PHEL (Tamaki) for identification. They were identified as two types of beetle native to New Zealand; *Phyllotocus macleayi*, a species that on warm summer nights can flock to windows, with some finding their way indoors; and *Parisopalpus nigronotatus*, a species that is often attracted to light. The exotic bee species of concern (*Apis cerana*) was ruled out and the investigation stood down.

A freight company manager called the exotic pest and disease hotline to report a nest of aggressive bees found on the corner casting of a recently-imported container at a Transitional Facility in Auckland. The bees were treated as possibly exotic (e.g. African or Asian honeybees) and killed by a contractor arranged by the Incursion Investigator. The contractor noted that the bees were small and made an unusual humming rather than buzzing sound. Samples were sent to the PHEL (Tamaki) and identified as European honey bees (*Apis mellifera*). Follow-up DNA testing confirmed the species identification. Screening for exotic mites was negative. Although it is unlikely that bees would survive on the outside of a container for three weeks, the biosecurity risk that exotic bees and their diseases could pose warrants rapid knock-down and diagnosis of suspect cases such as this. European honeybees swarm during the spring, and this is thought to have been a local swarm that had alighted on the container after it arrived. This investigation was stood down.

Nosema disease in bumblebees excluded

A member of the public reported the death of bumblebees outside a hive. The informant said the bees showed signs of dysentery. Signs such as these have a wide range of potential causes such as nutritional problems (e.g., through lack of pollen or from a viral agent). *Nosema* was considered to be the prime differential but dead bees tested negative for this agent by PCR so it was excluded. No further follow-up was considered necessary.

Exotic ticks intercepted

Two investigations of suspect ticks took place during this quarter, one from a medical laboratory in Wellington and the other in Dunedin. In both cases, the reports were of a single tick found on a patient who had recently returned from Australia. Both ticks were identified as adult female *Ixodes holocyclus* (Australian paralysis tick) at PHEL (Tamaki). The removal and destruction of these ticks negated the risk from these interceptions. The paralysis tick has strict temperature and humidity requirements that may prevent it from establishing in all but the most northern parts of New Zealand (Heath & Hardwick, 2011). In humans, this tick can transmit *Rickettsia australis*, the agent of Queensland tick typhus. It can also cause reactions ranging from localised swellings to anaphylactic reactions and tick paralysis. The patients were reported to have remained in good health.

Rhipicephalus sanguineous excluded

A lifestyle block owner near Kaukapakapa called the exotic pest and disease hotline to report finding a tick that he suspected was *Rhipicephalus sanguineus,* the brown dog tick. The tick was an engorged female, found three days after family members returned from travelling widely in the US, including time in national parks in contact with wildlife. A second, smaller tick was found a day after the initial notification. Both ticks were identified as *Haemophysalis longicornis,* the endemic cattle tick, at PHEL (Tamaki) and the investigation was stood down. The brown dog tick is exotic to New Zealand but is distributed widely in the US and many other countries.

Parananniziopsis australasiensis excluded

A wildlife veterinarian called the exotic pest and disease hotline to report a tuatara (*Sphenodon* sp.) with skin lesions resembling those of *Parananniziopsis australasiensis* (PA, formerly known as CANV, which stands for "*Chrysosporium* anamorph of *Nannizziopsis vriesii"*). The tuatara originated from Queenstown. At postmortem, skin lesions and liver granulomas were found. Generic fungal culture of the lesions was performed at AHL (Wallaceville). Fungal isolates were negative for *P. australasiensis* and no significant veterinary fungal pathogens were isolated. The investigation was stood down.

To date, *P. australasiensis* has been detected at two captive tuatara facilities in the North Island, but its range is not thought to extend to captive populations in the South Island. Formerly grouped with the now-defunct *Chrysosporium* anamorph of *Nannizziopsis vriesii*, *P. australiasiensis* is now thought to be a distinct, possibly less pathogenic species (Sigler *et al*., 2013). However, more work needs to be done to determine whether it is an endemic pathogen of tuatara and whether it causes significant primary disease.

A wildlife veterinarian called the exotic pest and disease hotline to report a possible fungal dermatitis in a native gold-striped gecko (*Hoplodactylus chrysosireticus*). This species is endemic to New Zealand, being limited in range to the Taranaki coast and to Mana Island reserve. The sample was cultured for *Parananniziopsis australasiensis* (formerly CANV: see previous item). Although fungal colonies were isolated, all were saprophytic fungi and no species of veterinary significance (including *P. australasiensis*) was isolated. The case was stood down. To date, *Parananniziopsis* infections have been restricted to captive tuatara and non-native Eastern water dragons. However, more work remains to determine the range of this genus among New Zealand reptiles, and its significance.
A veterinarian at Massey Wildbase called the exotic pest and disease hotline to report dermatitis consistent with fungal infection in a native Otago skink (*Oligosoma otagense*). In the past, MPI has investigated an emerging condition of fungal dermatitis in tuataras, associated with *Parananniziopsis australasiensis*, but this agent has not yet been found in other native reptiles. A skin sample was submitted to the AHL (Wallaceville), where fungal culture yielded a light growth of mixed fungi that were not considered to be of veterinary significance. No PA was isolated and the investigation was stood down.

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Quarterly report of investigations of suspected exotic marine and freshwater pests and diseases

Exotic marine pest and aquatic disease investigations are managed and reported by MPI Investigation and Diagnostic Centre and Response, Wallaceville. The following is a summary of investigations of suspected exotic marine diseases and pests during the period from October to December 2015.

Yersinia ruckeri ruled out

On 26 December, New Zealand Veterinary Pathology (NZVP) contacted the MPI Animal Health Laboratory (AHL) regarding a suspected case of *Yersinia ruckeri* in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from a South Island freshwater hatchery. Exotic strains of this bacterium, including the Hagerman strain, are unwanted organisms under the Biosecurity Act 1993, so an investigation was initiated to rule out exotic disease. The serotype 01b (Biotype 1) of *Y. ruckeri* is considered endemic in New Zealand (Keeling *et al.*, 2012) and has previously been isolated from salmon hatcheries on the east coast of the South Island (Diggles *et al.*, 2002). 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 (Diggles *et al.*, 2002). Endemic *Y. ruckeri* is generally confined to salmon cultured in fresh water and is not considered a threat to marine systems. Cultures sent to AHL were confirmed as *Y. ruckeri* by PCR. From clinical and epidemiological evidence, the endemic rather than the exotic strain of *Y. ruckeri* was suspected. This evidence included clinical presentation (ocular haemorrhage, circling and death), a low mortality rate (peaking at about 0.4 percent per day in the affected batch), the identification of environmental/husbandry factors exposing this batch of fish to stress and opportunistic disease before the mortality event, and the lack of mortality in other batches of fish on the farm. To definitively rule out exotic strains, isolates were sent to the Australian Animal Health Laboratory for serotyping. The isolate was examined directly by macroscopic agglutination against cross-absorbed rabbit antisera for *Y. uckeri* serotypes 01a and 01b. No significant agglutination was observed with the test sample in 01a antiserum, whereas strong agglutination was observed with the test sample in 01b antiserum. This confirmed that the *Y. ruckeri* strain isolated from the fish was the endemic one. Results were communicated to NZVP, the veterinarian and the farmer and the investigation was stood down.

Exotic copepods confirmed

A freshwater adviser from the Department of Conservation called the exotic pest and disease hotline to report the appearance of a non-native calanoid copepod, *Skistodiaptomus pallidus* (Herrick, 1879), in Lake Kereta, Northland. This first detection of *S. pallidus* at this location coincided with the first release of grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), into the lake, and the introduction of this copepod may have been associated with this release.

S. pallidus has been present in Auckland, Taranaki and Canterbury since at least 2000 and has been found among zooplankton being sold as fish food in the aquarium trade. Three other exotic freshwater crustaceans were simultaneously brought to the attention of MPI as recent invaders: another calanoid copepod, *Sinodiaptomus valkanovi* (Kiefer, 1938), and two cladocerans: *Daphnia galeata* (Sars, 1864) and *D. pulex* (Leydig, 1860). *S. valkanovi* has a restricted distribution, being found in three constructed waterways in Auckland and Waihi, where it has been established for at least 10 years. D. *galeata* is established and widespread in Auckland, Waikato, Rotorua and Tauranga, while *D. pulex* is found in Southland, Otago and Canterbury and is purportedly spreading further in the South Island.

These non-native zooplankton species are well established over a wide geographic range and are suggested to have negligible impacts. There are limited management tools available and eradication is not feasible. However, freshwater species transfers provide an opportunity for the accidental transfer of zooplankton such as these, so biosecurity procedures to reduce the spread are being investigated by MPI in conjunction with the relevant aquaculture industry representatives. There is also a parasite of carp, the Asian tapeworm, *Bothriocephalus acheilognathi* (Yamaguti, 1934) that has an intermediate copepod host phase. This has also been raised as a potential concern but there are no records of it in New Zealand, nor is there any evidence to suggest it is currently present. *B. acheilognathi* has a wide range of both intermediate and definitive hosts, and if currently present it would likely have already been detected in a number of suitable hosts in aquaculture facilities, aquaria or in native or sports-fish populations. As no further biosecurity risk was identified, the investigation was stood down.

Sabella spallanzanii confirmed

A diver called the MPI pest and disease hotline after seeing a suspect specimen while diving by Taranaki Wharf in Wellington Harbour. Photos and video footage suggested it was likely to be the Mediterranean fanworm, *S. spallanzanii*, so the investigator asked for the specimen to be collected (and thus destroyed). It was confirmed by the Marine Invasive Taxonomic Service (MITS) as an immature specimen. Since this was a range expansion of an unwanted organism and the first record of *S. spallanzanii* in Wellington, it was referred to the Biosecurity Response Group.

In another report, the supervisor of the Waikawa Marina, Picton, called the exotic pest and disease hotline to report a suspected incursion of *S. spallanzanii*

on a jetty float. A Marlborough District Council Biosecurity Officer examined the float and reported that the organism was not *S. spallanzanii.* An unfamiliar anemone species was collected and submitted to the Marine Invasives Taxonomic Service (MITS) for identification, but it proved to be the native species *Haliplanella lineata*.

A member of the public notified MPI of a suspected *S. spallanzanii* incursion in the Bay of Islands. The notifier reported that it was fouling the hull of a boat moored in Matauwhi Bay, Russell, and provided photos of specimens he had removed from the hull. The photos were sent to taxonomic experts at NIWA but the image quality was inadequate to identify the species. Northland Regional Council Biosecurity Officers were notified and they examined the vessel. The inspection did not reveal any *S. spallanzanii* or other unwanted organisms so the investigation was closed.

New sea slug confirmed

MPI received notification of a new to New Zealand nudibranch, *Ercolania boodleae*, commonly known as the boodlea sap-sucker. Until the 1970s, *E. boodleae* occurred naturally in the northwestern Pacific Ocean from Russia to Hong Kong, including Japan. However, over the past 50 years this species has become established in northern California, the Gulf of California and Victoria, Australia. Eight individuals were found at Ti Point, on the northern side of the entrance to Whangateau Harbour, Northland.This is the first report of this species in New Zealand. NIWA was asked to provide a Marine Exotic Species Note (Willan *et al*., 2014), which includes an assessment of any risk this species may pose to the New Zealand environment.

E. boodleae has black, finger-like processes (cerata) covering its notum, and can grow to 15 mm extended crawling length, although 6–9 mm is usual for adults. The specimens found were large and therefore assumed to be adults and reproductive; furthermore, two of them were mating at the time of collection. *E. boodleae* is a sacoglossan sea slug. Sacoglossans are specialised nudibranchs that feed solely by piercing the cell walls of siphonaceous green algae and sucking out the contents. Because of this host specificity, their distribution matches that of their food species and they are most often found on or close to their hosts. Water temperatures in northern New Zealand are considered high enough to support this species, and siphonaceous green algae found on our coastline may provide a suitable diet. If so, an established population could affect certain seaweed populations and other organisms for which those algae form a major part of the diet. *E. boodleae* may also compete with the endemic *E. felina*, an intertidal species. It is likely that *E. boodleae* has become spread to New Zealand by ships. It has been slowly spreading for the past 50 years but there are no indications that it displays invasive characteristics. While it is likely to eventually become established in New Zealand, the biosecurity risk it poses is considered to be low. Accordingly, the investigation was closed.

Macrobrachium rosenbergii confirmed

A member of the public notified MPI of a pet shop selling giant Malaysian river prawns (*Macrobrachium rosenbergii*), and asked whether it was legal to sell or keep them. Although it is legal to keep these prawns in New Zealand without restriction, an investigator went to the pet shop to take photos to confirm identification and rule out the possibility that it was a different species of exotic prawn. The photos were not high quality but the taxonomist was able to confirm that of the prawns were a species of *Macrobrachium* and that they looked very like juvenile *M. rosenbergii*. Since it was very likely to be a tropical species known to be established in captivity but unlikely to survive in the wild in New Zealand, the biosecurity risk was considered negligible and the investigation was closed.

Exotic crabs excluded

A member of the public brought some crabs from Marsden Cove Marina, Whangarei, to MPI for identification. The notifier was concerned that they might be an invasive species. Photographs were sent to the Marine Invasives Taxonomic Service at NIWA and identified as belonging to the genus *Notomithrax*, commonly known as camouflage crabs and indigenous to New Zealand. As there was no biosecurity risk, the investigation was closed.

Risk from imported crayfish excluded

A member of the public called the exotic pest and disease hotline, concerned that freshwater crayfish imported from China could act as a vector for an aquatic mould, *Aphanomyces astaci*, the cause of a disease commonly known as crayfish plague. The notifier understood that the crayfish were susceptible to this disease and that the fungal zoospores might remain viable even in frozen crustaceans. The notifier was concerned that this might pose a risk to the endemic freshwater crayfish, *Paranephrops zealandicus* (southern koura) and *P. planifrons* (northern koura).

Crayfish plague is a serious disease of freshwater crayfish in Europe, where it has caused large-scale mass mortality of several wild populations (OIE, 2012). All European species of freshwater crayfish are thought to be susceptible, while those in North America are considered resistant but act as vectors. The disease may be transmitted by introduction of a suitable host, but also the zoospores may remain viable in water contaminated with the disease and on any equipment used in contaminated water. Hence, outbreaks of crayfish plaque in the 20th century were largely linked to movements of North American crayfish introduced for farming.

Australia and New Zealand have never had an outbreak of crayfish plague and are considered free of the disease (OIE, 2012).

Chinese crayfish are imported into New Zealand for food and are very popular in Chinese restaurants. All imported consignments of crustaceans must meet the requirements of the import health standard for the importation of marine fisheries products for human consumption from all countries. Essentially, this means all imported crustaceans must be dead and therefore frozen.

An MPI import risk analysis for crustacean products (currently being reviewed by external experts) examined the likelihood of entry of *A. astaci* through imported frozen freshwater crayfish (frozen for > 72 hours). The risk was assessed to be negligible. Further, the viability of *A. astaci in vivo* and *in vitro* has been studied and it is killed by short exposure to temperatures of 60+°C or -20°C for 48+ hours (Alderman, 2000).

As the product meets the import health standard and has received biosecurity clearance, the investigation was closed.

Fish mortality investigated

A DOC staff member called the pest and disease hotline to pass on information from a third party regarding a fish kill at Coromandel, where many hundreds of fish had died in the tidal Te Mata creek. The MPI investigator spoke to the original notifier and established that the dead fish had been there for a few days and he had become alerted to them by increased bird feeding activity in the area. The notifier was unsure whether fish were still dying, but thought they might be. Samples were requested and sent to the Animal Health Laboratory, Wallaceville, but were too decomposed for diagnostic testing. Information from people in the field suggested that this had been a one-off event and that no further fish had died. A request was made for DOC and Fisheries Officers in the area to remain alert for any further mass mortalities and to provide fresh samples if this occurred. In the absence of samples suitable for diagnostic testing, this investigation was closed.

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Plants and environment investigation report

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

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Ghost ants intercepted

A member of the public who had recently returned from holiday in Bali found exotic ants (both alates and brood) in a box of children's toys and immediately placed them in a plastic bag and sprayed them with insecticide. He was told to place the bag in the freezer for 48 hours, then send a sample to MPI for identification. The sample was identified as *Tapinoma melanocephalum* (ghost ant). Ant specialists were contracted to carry out follow-up surveillance in early December to ensure no further ants were present. None were found but toxic baits were laid as a precaution.

Coptotermes termites intercepted

A member of the public reported strange white insects in a box of bath and beauty products they had recently purchased. The product was returned and the retail store notified its distribution centre, who contacted MPI. More insects were found in a full carton of the products, which had originated from China. The goods were sprayed with fly spray and then couriered to the distribution centre. MPI entomologists identified the insects as *Coptotermes formosanus*, a highly invasive termite species and Unwanted Organism under the Biosecurity Act 1993. All products from this consignment and any wooden pallets they had been in contact with were shrink-wrapped and withdrawn from all 56 retail stores and sent back to the distribution centre. Risk of establishment from infested products that had already been sold to the public was considered to be low.

Ten of the 56 retail stores that sent the remaining products from this consignment back to the Auckland distribution centre reported signs of product infestation. All items were fumigated according to MPI standards, in 40-ft shipping containers that were only half-full to allow the fumigant to circulate. Care was taken to ensure the plastic wrapping did not impede penetration of the fumigant into the products. After venting, 10 percent of the items were inspected and dead termites and one live spider were found. The spider was identified by MPI as *Badumna* sp., a local species likely to have entered the container while venting. An information sheet was developed to describe signs of *C. formosanus* nest establishment in a structure and this was distributed to all retail stores. Store managers were advised to use the information to check their storerooms and retail buildings for signs of nest establishment. No evidence of nests was found. The infested products had entered New Zealand in two shipping containers. The first had not been used since, and was inspected by MPI and found to be free from termite infestation. The second container had been steam-cleaned and left New Zealand containing a shipment of kiwifruit. Staff packing the kiwifruit had checked the container for signs of dirt and unusual smells and had not reported any problems. No evidence of insects was noted while steam-cleaning. However, an alert was placed on this container so that it will be inspected for termites when it next enters New Zealand.

It seems unlikely that either of the containers contained any termite nests but an alert was placed on products shipped by the China-based supplier to two other New Zealand businesses around the same time. Both businesses were asked to inspect their remaining stock for signs of insect infestation, and photos of the termite-damaged products were provided as an identification aid. No evidence of insect infestation was found, nor were there any customer complaints. Nevertheless, a further alert was placed on the supplier, requiring future shipments to be inspected. Five consecutive clean shipments will be required before the alert will be removed.

African powder-post beetle intercepted

An importer contacted MPI after finding three exit holes, fresh frass and a borer-type insect that had emerged from a set of wooden boxes imported from India. PHEL Entomology identified the specimen as *Lyctus africanus*, the African powder-post beetle, which is an Unwanted Organism under the Biosecurity Act 1993. The notifier agreed to put the box in the freezer for seven days to eliminate any residual risk. A total of 40 units (each a set of four boxes that stacked into each other) had been imported and all had been fumigated before export. Of the 21 customers who had bought this product, four were contacted to see if they had noticed any insects. No one reported any such signs.

Wasps found in coriander seeds

Small flying insects were found in a sealed bag of coriander seeds bought from a Wellington shop in December 2015, about two weeks before the buyer reported the issue. The seeds originated

from India and the insect was identified as *Systole albipennis*, a chalcid wasp that is an Unwanted Organism under the Biosecurity Act 1993. The shop owner was contacted and the remaining 21 packets were immediately removed from sale. The owner noticed insects in one of the packets, indicating that wasp contamination was present throughout the consignment. All packets were put in a freezer for 48 hours and then disposed of. The total amount of coriander seed in this consignment was 280 kg and destruction of the entire stock was considered the only practicable option for reducing the risk. An alert was prepared, with a recommendation to target future importations of coriander seed from the importer for additional inspection to ensure compliance.

New rust species, *Uredinopsis pteridis*, on bracken in Auckland

Landcare Research reported a new to New Zealand organism on bracken fern (*Pteridium aquilinum*) collected from Muriwai, South Piha and Maraetai in Greater Auckland. This organism morphologically matched the fir-bracken rust, *Uredinopsis pteridis*, which may be established and widespread in New Zealand but was previously undetected. It was first reported from bracken fern in Tasmania in 2014 and may have recently become self-introduced.

U. pteridis is considered a cosmopolitan rust fungus, with *Abies* (fir) as an alternate host. The disease is more prevalent on fir seedlings and saplings. Infection is known to occur in the northern hemisphere during May and June, and in November and December in New Zealand. The most characteristic symptom is the white tube-like fruiting structures (aecia) on the lower needle surface. Aecia of *U. pteridis* can mature on current-year needles in autumn or winter but usually develop on the previous year's needles in spring. Aeciospores can infect bracken fern at any time during the growing season and there are several spore stages, some of which can re-infect other bracken plants. Fruiting bodies called telia develop on the fern and overwinter on dead fronds, after which the spores germinate in spring and re-infect other fir plants, completing the two-host cycle. Symptoms on fern include chlorotic markings on the upper surface and white pustules (*uredinia*) on the lower surface. The impact of this rust fungus is considered low.

Suspect brown marmorated stink bug ex Japan

When a traveller opened a wallhanging she had bought in Japan as a gift two and a half weeks earlier, she found a live insect that she described as a shield-shaped beetle with long antennae. Emailed photographs showed a suspected brown marmorated stink bug (BMSB) (*Halyomorpha halys*) and an Incursion Investigator was dispatched to pick up the insect and provide the notifier with information on this species, which is an Unwanted Organism under the Biosecurity Act 1993. PHEL Tamaki confirmed it was a female BMSB with no egg development. The notifier was advised to thoroughly check her suitcases, clothing and the rooms in which she unpacked. No further BMSBs were found.

The notifier had visited Japan as a member of a party of 20 people and the tour company was contacted and advised of the find. An email was forwarded to all tour members, asking them to thoroughly check their baggage and houses. A BMSB information sheet was also sent. No suspect BMSBs were found and it is thought this find was a lone hitch-hiker, in which case the biosecurity risk was mitigated when it was caught.

Suspected disease on flax plants

The notifier saw a flax plant (*Phormium tenax*) with a distorted inflorescence in Mahoe Reserve, Christchurch, about six weeks before reporting it, and had since found another plant there with the same symptoms. The inflorescences had become shrivelled and the insides of the stalks were spongy and necrotic. The notifier consulted a flax specialist at Landcare Research, who found similar symptoms in mountain flax (*P. cookianum*) in the Landcare Research collection. Samples from affected *P. tenax* and *P. cookianum* plants were analysed by the virology and mycology teams at MPI's PHEL but no pathogenic viral or bacterial agents were detected. It was therefore concluded that the symptoms were due to abiotic factors, most likely a recent heavy frost.

Suspected new to NZ psyllid found

A possibly new to New Zealand psyllid was reported on Port Jackson fig (*Ficus rubiginosa*) in Albert Park, Auckland. The psyllid was *Mycopsylla obliqua*, which is native to New Caledonia. Both a male and female were found on the same plant and submitted to PHEL for identification. A few days later, scattered exuviae of the same species were found on another Port Jackson fig tree in a park in Parnell, Auckland. More *M. obliqua* were also found on fig trees on Princes Street (which adjoins Albert Park), and on Greenlane West, suggesting that this species may be widespread in Auckland. The PHEL lead entomologist concluded that the specimens seen should be assigned the name *Mycopsylla* sp. nr. *obliqua*, as they show a number of characters that are distinct from those described for *M. obliqua*. Genetic analysis to determine the species was inconclusive. As the only known host is an exotic *Ficus* species, it is unlikely that this psyllid will become a biosecurity threat. The distribution of specimens found also suggests a breeding population may have long been established in Auckland and this further suggests that its impacts are likely to be benign. The investigation was closed.

Quarterly report of biosecurity responses

The Biosecurity Response Group was managing 39 high-priority active responses and four low-priority active responses (i.e. full responses were not initiated) at the end of the October to December 2015 reporting period. During that period three new responses were initiated and six were closed or stood down. **Figure 1** shows the number of active biosecurity responses from December 2014 to December 2015.

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

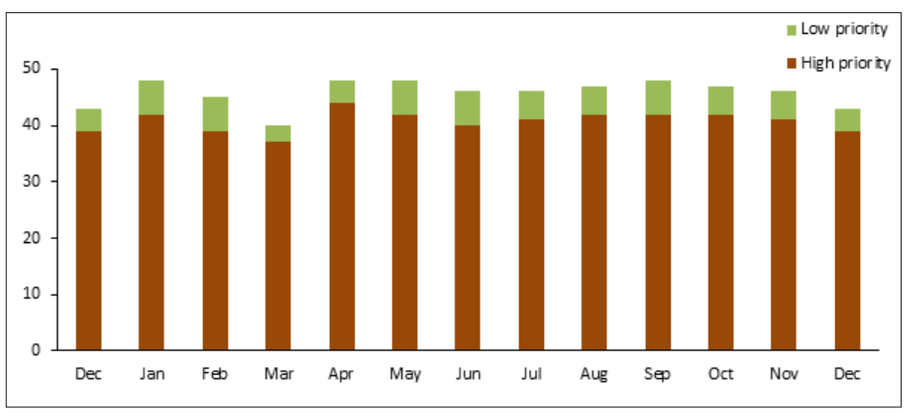

Figure 1: Active biosecurity responses from December 2014 to December 2015.

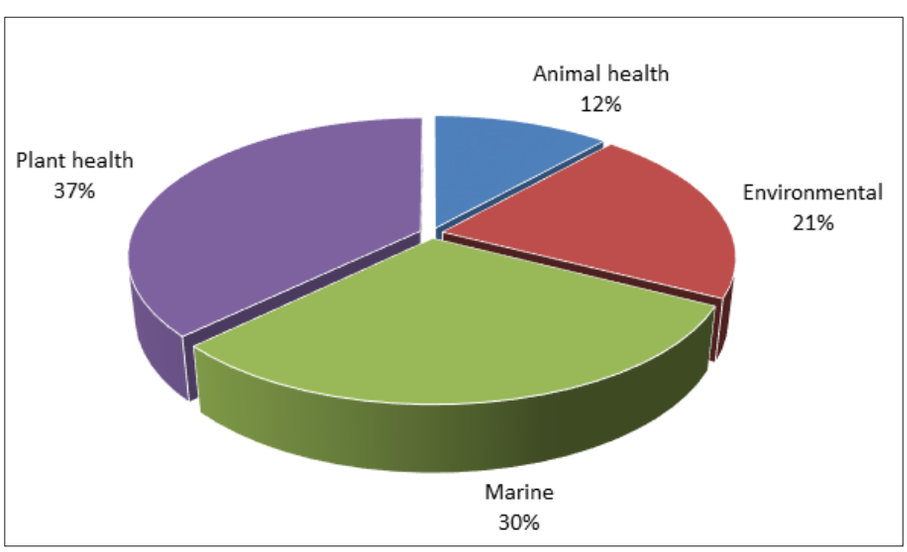

Figure 2: Sector breakdown for open responses at the end of the October–December 2015 reporting period.

The Group manages biosecurity responses affecting plant, environment, animal and marine sectors (**Figure 2**).

Queensland fruit fly eradicated

The detection of a single male Queensland fruit fly (*Bactrocera tyroni*) in a fruit fly surveillance trap in suburban Auckland in February 2015 prompted the immediate initiation of a biosecurity response by MPI and industry partners Kiwifruit Vine Health and Pipfruit New Zealand. There are no known established populations of Bactrocera fruit flies in New Zealand and the aim of the response was to eradicate any further Queensland fruit flies associated with this detection. This has now been achieved and eradication was declared in December 2015.

Eucalyptus leaf beetle eradicated

A single Eucalyptus leaf beetle (*Paropsisterna beata*) (ELB) was found at an Upper Hutt property in December 2012. A biosecurity response was initiated, with aerial and ground spraying of the detection site, and surveillance was undertaken out to 3 km. No further ELBs were found after 4 October 2013. Together with the advice from an MPI entomologist on the ELB life cycle, this led to eradication being declared in December 2015.

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PEST WATCH: 24 October – 15 February 2016

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

This information was collected from 24 October 2015 – 15 February 2016. 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
chromist	*Phytophthora niederhauserii* *no common name*	*Vitis vinifera* grape	Bay of Plenty	IDC & R (General Surveillance)	This pathogen can cause root and crown rot in various host plants. It was first described in 2014.
fungus	*Neonectria microconidia* no common name	*Actinidia chinensis* kiwifruit	Northland	IDC & R (General Surveillance)	This fungus was isolated from stem cankers, but the significance of its association with the cankers is unknown.
fungus	*Pestalotiopsis telopeae* no common name	*Actinidia chinensis* kiwifruit	Northland	IDC & R (General Surveillance)	This fungus was first described in 2014.
fungus	*Septoria lavandulae* no common name	*Lavandula angustifolia* English lavender	Auckland	IDC & R (General Surveillance)	This fungal pathogen causes leaf spots on lavender plants.
insect	*Parochmastis hilderi* no common name	*Lavandula angustifolia*	Auckland	Landcare Research (General Surveillance)	*P. hilderi* is a Norfolk Island endemic. Little has been reported regarding its biology or ecology.
mite	*Disella* sp. no common name	*Coprosma robusta* karamu	Auckland	IDC & R (High Risk Site Survey)	Possibly an undescribed native species. *Disella* currently contains 22 species worldwide, with one other previously recorded from New Zealand.
mite	*Lambella* sp. no common name	*Coprosma robusta* karamu	Auckland	IDC & R (High Risk Site Survey)	Likely an undescribed native species. Found on the leaf undersurface.
mite	*Rhizoglyphus howensis* no common name	*Persea americana* avocado	Northland	IDC & R (General Surveillance)	R. howensis was previously recorded from seeds of palm in Lord Howe Island and seeds of *Quercus patula* in Australia
mite	*Schwiebea elongata* no common name	*Persea americana* avocado	Northland	IDC & R (General Surveillance)	Known from the USA, Canada and Japan.
mite	*Tarsonemus* sp. no common name	*Coprosma robusta* karamu	Auckland	IDC & R (High Risk Site Survey)	An undescribed species. Members of this genus are generally considered to be primarily fungivores.
virus	*Southern tomato virus* *STV*	*Solanum lycopersicum* tomato	Mid Canterbury	IDC & R (General Surveillance)	STV was first described in 2009 from the USA.

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

Veterinary Diagnostic Laboratories

gribbles veterinary pathology

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  Courier: 37–41 Carbine Road, Mount Wellington, Auckland 1060
  Postal: PO Box 12049, Penrose, Auckland 1642
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- HAMILTON
  Courier: 57 Sunshine Ave, Hamilton 3240
  Postal: PO Box 195, Hamilton 3240
  Tel: 07 850 0777 Fax: 07 850 0770

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

- CHRISTCHURCH
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  Postal: PO Box 3866, Christchurch 8140
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- DUNEDIN
  Courier: Invermay Research Centre, Block A, Puddle Alley, Mosgiel, Dunedin 9053
  Postal: PO Box 371, Dunedin 9053
  Tel: 03 489 4600 Fax: 03 489 8576

NEW ZEALAND VETERINARY PATHOLOGY

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

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

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

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

0800 80 99 66

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

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

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

New Zealand Government

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Manatū Ahu Matua