

QUARTERLY REPORT OF INVESTIGATIONS OF SUSPECTED EXOTIC DISEASES

Vesicular disease ruled out

A veterinarian in private practice contacted MPI after being called to examine nine Jersey x Friesian cows out of a herd of 140 that over the preceding seven days had developed nasal ulcers, eye discharges and 0.5 cm ulcers on the commissures of their mouths. The cows were salivating but not pyrexia, they were not lame and they had no lesions on their udders or feet. Despite their discomfort, the cows were still eating well. Exotic vesicular disease was ruled out on history, clinical signs and photographic evidence supplied by the notifying veterinarian and the investigation was stood down. The main differential diagnosis considered was nasal catarrh secondary to infectious bovine rhinotracheitis (IBR). Four blood samples tested antibody-positive by ELISA for IBR but were PCR-negative at IDC. Histopathology showed that the epithelium was ulcerated and there was a small amount of necrotic tissue on the surface. Beneath this there was granulation tissue infiltrated with large numbers of eosinophils and lesser numbers of lymphoid cells. The adjacent intact epithelium was infiltrated with low numbers of eosinophils and there were infiltrates of eosinophils and lymphoid cells in the stroma beneath this. A pathological diagnosis of oral ulceration with intense eosinophilic inflammation and granulation tissue formation was reached, possibly caused by a hypersensitivity reaction or a response to foreign material. The palm kernel expeller being fed to the affected herd at this time was observed by the notifying veterinarian to be very coarse.

This case has marked similarities to that reported by McFadden *et al.* (2007), with a similar presentation (although less severe) and a similar incidence (6.4 vs 7.6 percent). It was in the same area (Putaruru) and the notifying veterinarian was also the same person in both cases.

A veterinarian phoned the MPI exotic pest and disease hotline after examining a clinically ill nine-year-old Appaloosa mare for chronic weight loss. On first examination the horse was not pyrexia but was coughing and had harsh lung sounds. After a second examination the veterinarian observed oral ulcers that looked like ruptured vesicles. Blood samples were requested and tested by virus-neutralisation test at the IDC Animal Health Laboratory, Wallaceville, for vesicular stomatitis virus varieties New Jersey and Indiana. Both tests were negative and the investigation was stood down. The horse

Exotic disease investigations are managed and reported by MPI's Investigation and Diagnostic Centre (IDC) Wallaceville. The following is a summary of investigations and response to suspected exotic disease during the period from April to June 2013.

did not improve with treatment and the veterinarian was considering an immune-mediated condition as the primary aetiology.

BSE ruled out

A veterinarian rang the MPI exotic pest and disease hotline to report that at ante-mortem inspection a three-year-old cow in the lairage at an abattoir in Oamaru had neurological signs that included head bobbing, aggression and muscle wastage. The cow was not lactating so a metabolic cause was unlikely. After consultation with the MPI surveillance team it was decided to send the brain to a Christchurch veterinary pathology laboratory for bovine spongiform encephalopathy (BSE) surveillance and histopathology, while the carcass was held in the cold store until cleared for human consumption. In fact the carcass was condemned at slaughter owing to acute arthritis with signs of septicaemia and there were also multiple joints with chronic arthritis and signs of previous pericarditis. The histopathology at Gribbles was negative for BSE and the investigation was closed.

Anthrax ruled out

A veterinarian in Canterbury called the MPI exotic pest and disease hotline with concern about anthrax following the sudden death of two R1 Jersey x Friesian heifers over the course of a week. On examination the second heifer was bloated with subcutaneous gas and had blood oozing from the nose, vulva and anus. The rest of the mob were unremarkable and there was no history of recent excavation on the property (often associated with exposure of buried anthrax spores) or access to toxic plants such as tutu (*Coriaria* spp.) or ngaio (*Myoporum* spp.). The mob was being fed baleage and grass and had been vaccinated with Ultravac 7 in 1 vaccine. Testing of peripheral ear blood smears at MPI IDC showed no evidence of rod bacteria resembling *Bacillus anthracis* (anthrax). *Clostridium sordellii*, the

causal agent for sudden death syndrome in cattle, is not covered by Ultravac 7 in 1 vaccine and remains a likely endemic differential.

Contagious bovine pleuropneumonia ruled out

An unusual presentation of chronic interstitial pneumonia in dairy cattle was notified to MPI by a pathologist. Infection with *Mycoplasma* spp., including *M. mycoides*, which causes contagious bovine pleuropneumonia, could not be ruled out clinically. The herd had been experiencing respiratory problems for several months: cows would sporadically start coughing, progressing to abdominal breathing and respiratory distress. All had been treated for lungworm and antibiotic therapy of affected animals did not resolve the problem. Out of the herd of 285 cows, 20 had been affected, with three deaths. Post-mortem histology of one 10-year-old cow revealed severe chronic interstitial pneumonia, lymph node hyperplasia and chronic membranoproliferative glomerulonephritis. Bronchial swabs and fresh lung tissue were submitted from this cow to the Animal Health Laboratory, IDC Wallaceville. PCR and culture for *Mycoplasma* spp. was negative. The type of pneumonia present in the post-mortemed cow arises from diffuse alveolar damage, which can be from a variety of causes, the most common being lungworm or 3-methylindole, while the glomerulonephritis was also non-specific and of immune-complex origin.

Bovine digital dermatitis investigated

A vet in the Manawatu contacted the MPI exotic pest and disease hotline to report a case of bovine digital dermatitis in dairy cattle. The lesions were observed on two occasions on one farm. Lameness was not a clinical sign in these cases and spread between animals was not noted. The vet was aware of the BDD monitoring work being performed by Massey University and a working group that includes MPI, and had viewed the website giving information regarding the condition and the working group at <https://sites.google.com/site/digitaldermatitisnz>. Histopathology from the two cases exhibited characteristics consistent with BDD and silver staining of sections of the lesions showed organisms consistent with spirochaetes invading the deeper layers of the foot. These findings are consistent with previous cases

of BDD identified in New Zealand (van Andel *et al.*, 2012; Vermunt, 2004).

A site visit was arranged to examine the herd and to assess the prevalence of the condition on the farm. Three animals with lesions were identified and all cattle (about 300) on the property were examined. All lesions seen were consistent with those identified in previous investigations, having mixed chronic and active elements. No lameness problem has been identified in this herd by farm managers or attending veterinarians. The low prevalence observed (about 1 percent) is consistent with the previous reports of BDD-like lesions in New Zealand.

This site visit enabled the investigators to confirm that the animals were affected with the syndrome as observed previously in NZ (van Andel *et al.*, 2012) and to trial the examination process and data recording sheets prepared by the working group for the Massey BDD project.

EIA/EVA ruled out

A Gribbles veterinary pathologist reported a six-month-old New Zealand-born Thoroughbred colt with mild anaemia (HCT= 0.27; reference range 0.31–0.41) to the MPI exotic pest and disease hotline. All other horses on the property were in good health. Paired serum samples were submitted to the Animal Health Laboratory and tested by serology for equine viral arteritis (EVA) and equine infectious anaemia (EIA). Both tests yielded negative results. The colt had an uneventful recovery.

In another case, a colt in the quarantine facility awaiting export to China tested positive for EVA at a titre of 1:6. The colt had recently been vaccinated and this was considered to have caused a non-specific reaction leading to a false positive result. A titre of 1:2 was found on re-testing 14 days later. The colt was considered to be uninfected and the investigation was stood down.

A veterinary pathologist notified the MPI exotic pest and disease hotline after examining a blood sample from a five-year-old Quarterhorse gelding with ventral oedema. The blood sample showed a severe neutropenia and leucopenia. The samples were forwarded to MPI and tested negative for EVA and EIA.

Anaemia was observed in a three-year-old Miniature horse with accompanying neutropenia and a history of lethargy. Three weeks earlier the affected horse had been in contact with a pregnant mare that had arrived from stud. A blood sample was requested and tested for EVA at

IDC Wallaceville. The sample was negative at a titre of < 1:2 and the investigation was closed.

Equine multinodular pulmonary fibrosis confirmed

A veterinary pathologist called the MPI exotic pest and disease hotline to report a case of suspected equine multinodular pulmonary fibrosis (EMPF) thought to be caused by equine herpes virus 5 (EHV-5) in a 10-year-old Polo pony from the Auckland region. Clinical signs included weight loss over the winter and no notable response to antibiotic therapy. Post-mortem examination revealed multiple coalescing and poorly demarcated cream-coloured firm areas with little normal lung tissue remaining. No other horses on the property were affected. Histopathology revealed evidence of interstitial lung damage and intra-nuclear inclusion bodies were found in macrophages in the lung tissue. These inclusion bodies are consistent with EMPF. Virus isolation was attempted both at the Animal Health Laboratory, Wallaceville and at Massey University, without success. PCR testing at Massey University confirmed the presence of EHV-5 in a sample of lung tissue from the horse and the result was confirmed by sequencing of the PCR product. The first case of EMPF in the world was diagnosed in 2007 and although the role of EHV-5 is not fully proven, there is strong circumstantial evidence that the virus is associated with the lung lesions. EHV-5 is common in New Zealand horses and has been isolated from healthy foals and adult animals. No specific strain type of EHV-5 has been identified as being associated with EMPF and the disease seems to be a rare outcome for horses associated with EHV-5 infection. This is the first report of EMPF in New Zealand but no further action was deemed necessary because EHV-5 is an endemic virus in New Zealand.

Brucella canis ruled out

A veterinarian phoned MPI to report purulent epididymitis in a dog that had been surgically neutered. The dog had no history of travel and was New Zealand-born and bred but the veterinarian was concerned about the possibility of *Brucella canis* infection. Histopathology on the excised tissue did not find evidence of inflammation and the dog also had a negative serum agglutination test that ruled out *B. canis*. The investigation was closed.

A veterinarian reported to the MPI exotic pest and disease hotline a six-year-old Boxer dog that had developed unilateral orchitis. The New Zealand-bred dog had not travelled overseas or been used for mating. A blood sample was submitted to the IDC Wallaceville, where *B. canis* was excluded after serum tested negative in the *B. canis* card agglutination test.

A veterinary pathologist called the MPI exotic pest and disease hotline to report epididymitis resembling canine brucellosis (*B. canis*) in a four-year-old male Labrador dog with a single enlarged testis. Histologically there was chronic active epididymitis, vasculitis of adjacent vessels and inflammation of the spermatic cord. The dog had no travel history and was otherwise clinically well. A serum agglutination test was negative for antibodies to *B. canis*. Endemic causes of epididymitis in dogs include gram-negative rods such as *E. coli* and others. The case was stood down.

Mycoplasma haemocanis excluded

A veterinary pathologist called the MPI exotic pest and disease hotline to report suspect *Mycoplasma haemocanis* in a geriatric dog with a mild regenerative anaemia and erythrocytes containing basophilic punctate structures. *M. haemocanis* (formerly *Haemobartonella canis*) has been rarely reported in New Zealand, but has never been confirmed by PCR (Anon., 1997, 2008; Thompson, 1998). Whole-blood samples tested negative by PCR for both *M. haemocanis* and the more recently described *M. haematoparvum*. The identity of the punctate structures was not determined, but in the face of the negative PCR result they are likely to be non-infectious (e.g., ribosomal RNA aggregates). The investigation was stood down.

A veterinary pathologist contacted MPI after observing small structures on the red blood cells of an 11-year-old Miniature Schnauzer dog with severe anaemia. The pathologist suspected *Mycoplasma haemocanis*. Upon receipt by IDC Wallaceville the samples were forwarded to Langford Veterinary Services, Bristol, UK for PCR testing. Results were negative and the investigation was closed down.

Aspergillus terreus confirmed

A veterinary pathologist rang the MPI exotic pest and disease hotline to report a case of disseminated mycosis

in a two-year-old German Shepherd bitch. The initial fungal identification by the referring veterinary laboratory was confirmed by IDC Wallaceville as *Aspergillus terreus*. Disseminated mycosis caused by *A. terreus* has not previously been recorded in New Zealand, although it has been reported in Australia. The affected dog was imported from Australia when only three months old, with no observable health issues. The dog has since been euthanased on humane grounds and cremated.

Leptospira canicola excluded

Serum from two dairy bulls was submitted to the IDC Wallaceville for routine export testing. Both animals were intended semen donors. Both animals produced low positive titres to MAT testing for *Leptospira canicola*, a strain of leptospirosis not known to be present in New Zealand, and gave the same result on resampling. They were in good health and had been fully vaccinated for strains of leptospirosis known to be present in New Zealand. They had also received prophylactic antibiotics that would have cleared any infection on arrival at the exporting property. The reasons for low-level test results of this nature include true infection in the past, with localisation of the agent in the kidneys and persistent low-level shedding in the urine, cross-reaction with another strain of leptospirosis, or non-specific reaction to the MAT. Given that both animals were in good health, had been fully vaccinated and received prophylactic antibiotics that would later have cleared any infection, the presence of true infection with an exotic strain was regarded as unlikely.

In this case, the AHL was in contact with the OIE reference laboratory in the US that supplies leptospires for the *L. canicola* MAT testing. It was ascertained that the particular batch of *canicola* leptospires had been identified as causing low-level reactivity of unknown cause – just as was identified in these two bulls. The batch was replaced by the supply laboratory. Serum from both animals was sent to another reference laboratory in the UK and both animals returned negative results on MAT testing. The investigation was stood down after all parties were informed of the results.

Exotic ticks ruled out

A member of the public reported to the exotic pest and disease hotline a fully engorged tick on her six-month-old infant after returning from Australia. The child had

been presented to a physician with non-specific flu-like symptoms. The tick was identified by an entomologist at IDC PHEL Tamaki as *Ixodes holocyclus* (Australian paralysis tick). The tick tested negative by PCR for *Borrelia* (Lyme disease). The child responded to treatment with antibiotics but had mild lymphadenopathy for some weeks after the tick had been removed.

Mycoplasma in kiwis investigated

Two sets of tissues were submitted for mycoplasma culture by veterinary pathologists. One set was from a two-to-three-week-old Haast tokoeka kiwi chick (*Apteryx australis*), which died following sinusitis and pneumonia and was sent to the New Zealand Wildlife Health Centre (Wildbase) for post mortem. The other tissue was from a juvenile female brown kiwi (*A. australis*) with an unusual pneumonia pattern. Both kiwis were being hand-raised in captive-rearing facilities. Mycoplasma disease has not been seen in kiwis previously but has been widely diagnosed in other species including poultry and wild birds and is associated with sinusitis and chronic respiratory disease. In the Haast bird, lesions seen consisted of acute (heterophilic) bronchitis and sinusitis. No other birds were reportedly affected. No bacteria were visible on H&E sections in either bird, meaning that the more common bacterial disease were less likely. Mycoplasma culture was inconclusive owing to mixed bacterial overgrowth but mycoplasma PCR was negative. Based on the negative PCR test and lack of available tissues for follow-up culture, the investigation was stood down.

Mycoplasma lipofaciens infection confirmed in Fiordland crested penguin

A veterinary pathologist submitted lung tissue from an adult male Fiordland crested penguin (*Eudyptes pachyrhynchus*) with pneumonia. The penguin had been found on a Wellington beach and was undergoing rehabilitation in Otago when it died suddenly following moult and was submitted for post-mortem examination at Wildbase Pathology. On histology both lungs had severe extensive heterophilic and lymphoplasmacytic pneumonia, somewhat similar to lesions seen in the two juvenile kiwis reported above. No aetiological agent could be identified histologically. Similar lesions have been described in birds infected with *Mycoplasma* spp. and *Bordetella avium*. *B. avium* is exotic to New Zealand.

Mycoplasma culture of the lung isolated an unusual species, *M. lipofaciens*, which has previously been reported in healthy adult chickens and has been isolated from the infertile egg of a raptor in Europe (Lierz *et al.*, 2007). Experimentally it can cause disease in poultry embryos and hatchlings. Culture has been infrequently performed on samples from Fiordland crested penguins, and given the non-migratory nature of these birds, this pathogen is most likely a native bacterium of this species. It is unclear what role *M. lipofaciens* had in the development of pneumonia in this bird. Subsequently, aerobic culture isolated *Hafnia alvei*, a member of the Enterobacteriaceae family of facultative anaerobes that is widespread throughout the world as a commensal intestinal flora species.

This is the first known isolation of *M. lipofaciens* in New Zealand. Communication with industry, including PIANZ and DOC, has been undertaken, and a short article published (Buckle *et al.*, 2013). It is believed to pose a negligible risk to the NZ poultry industry and wildlife, given the high likelihood that this is a first detection of a historically present organism.

Infectious bursal disease ruled out

Routine surveillance for infectious bursal disease (IBD) on two adjacent sites in the Waikato found that five sheds of eight tested containing 39- and 57-week-old Ross broiler breeders were weakly positive for IBD on ELISA and VNT. The affected flocks were commercial broiler breeders housed with strict biosecurity (shower-on facilities) protocols. Vaccination was with non-adjuvanted (live) vaccines only, including Marek's disease, live coccidiosis vaccine (Immucox), live attenuated salmonella vaccine (Meganvac), combined avian encephalomyelitis, fowl pox and chicken anaemia vaccine, infectious laryngotracheitis vaccine and infectious bronchitis vaccine. The birds had previously tested IBD-negative at six to 11 weeks of age, when they were housed at different sites.

Initial positive prevalence ranged from 10 to 30 percent for each shed by ELISA, with all titres within a low positive range. A rebleed two weeks later showed increased ELISA values and increased prevalence of positive test results, but all ELISA positives were still within low ranges (highest was 1213, most were 500–600, cutoff was > 396 optical density). A virus-neutralisation test (VNT) was performed for the second

set of 10 samples at the IDC Wallaceville and returned similar results.

During follow-up testing at the MPI Animal Health Laboratory 100 samples were tested by VNT, with 20 birds tested per shed. Out of 83 samples that had sufficient sample volume, six were positive (7.2 percent) (using a cutoff of 1:8). The highest VNT results were 1:16 (n= 6). The low range of values is inconsistent with recent IBD infection: no subsequent flocks reared in the same sheds tested positive for IBD via ELISA and adjacent properties and sheds have likewise remained negative for IBD by routine ELISA screening. Owing to low titres, low numbers of test-positive birds and absence of apparent spread between initial and subsequent bleedings, this case is considered to be inconsistent with true IBD infection. True outbreaks of IBD, whether low- or high-virulence, are associated with seroprevalence verging on 100 percent, with much higher titres that do not drop for several months. Furthermore, there was no evidence of any true disease outbreak of any sort, as there was no associated decrease in egg production and mortality rates were low, with no change. Previous similar cases of IBD false-positive serology results have been blamed on adjuvant exposure through vaccination with killed vaccines. As these birds lacked a history of killed vaccine exposure the cause of the seropositivity is most likely to be a non-specific response to another immune stimulus.

European fowlbrood investigated

Two of four honey samples submitted by a Dunedin research organisation I were positive on PCR for European fowlbrood (EFB, *Melissococcus pluton*). Attempts to culture EFB were unsuccessful but *Paenibacillus alvei*, which can be a proxy for EFB infection, was produced. The honey from which the samples originated was held in a containment facility, which was inspected by a quarantine inspector at the request of IDC and found to be well managed and secure. A copy of the import permit was also examined and found to be in order. After consultation with the MPI risk analysis team the investigation was closed.

Tracheal mites ruled out

While conducting routine surveillance of beehives for MPI anASUREQuality apicultural inspector was shown a hive with dead bees. It was the only affected hive on the property and the fact that the dead bees were only outside

of the hive suggested poisoning, though no sources of potential poison could be identified. Samples of bees were submitted to IDC PHEL Tamaki for entomological examination to rule out tracheal mites (*Acarapis woodi*), the main differential diagnosis to be considered. No tracheal mites or any other exotic parasites were found and the investigation was closed.

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