Association of Avian Veterinarians Australasian Committee Ltd.
Annual Conference 2016 pp 23-27

Mass Mortality Event in Australian Magpies and Australian Ravens in NSW in 2015.

Cindy Jarratt BVSc (HonsI) MANZCVS (Avian Health)
Albion Park and Gerringong Veterinary Hospitals
122a Tongarra Rd
Albion Park, NSW 2527
cindy.jarratt@gmail.com

Karrie Rose DVM DVSc
Australian Registry of Wildlife Health
Taronga Conservation Society of Australia
Bradleys Head Road Mosman NSW 2088
krose@zoo.nsw.gov.au

Introduction

In May 2015, a geographically and temporally focused mass mortality event involving Australian magpies (*Cracticus tibicen*) and Australian ravens (*Corvus coronoides*) occurred on the New South Wales South Coast. Eleven birds were presented dead and six birds were presented alive with neurological, respiratory and gastrointestinal signs. Four birds were given supportive therapy; recovered and were released. Two birds were presented moribund; died shortly after presentation and were necropsied. Pharyngeal swabs were found to be PCR negative for Avian Influenza Virus (AIV) and Newcastle disease Virus (NDV). Tissues were collected for histopathology and toxicology. Fresh tissues were stored frozen for future viral culture. Histopathological findings were consistent in both birds examined and were largely inflammatory. Toxicological tests on samples of liver and gut contents revealed low concentrations of fenthion, an organophosphate primarily used to control insect pests. The pattern of clinical signs and histopathological lesions seen in these birds appears to be consistent with what has previously been seen in two other mass mortality events on the East Coast of Australia involving Australian magpies, Australian ravens, Pied currawongs (*Strepera graculina*) and Magpie larks (*Grallina cyanoleuca*). A viral aetiology is strongly suspected, despite no viral growth in tissue cultures to date. This syndrome is described as Black and White Bird Disease.

Clinical Report

A syndrome of neurological, respiratory and gastrointestinal disease occurring in Australian magpies, Australian ravens, Pied currawongs (*Strepera graculina*) and Magpie larks (*Grallina cyanoleuca*) has emerged on the East Coast of Australia. Three epizootic events involving these Passeriformes have been identified. In 2006, 29 birds that seemed to represent the epizootic were examined through the Australian Registry of Wildlife Health (the Registry), Gribbles Pathology and CSIRO's Australian Animal Health Laboratory (AAHL). Another event occurring in July and August 2003, was identified through retrospective examination of the Registry's database. This report describes the third identified epizootic, involving 17 birds, which occurred in May 2015.

Over the course of two days, 11 dead birds (10 Australian magpies and one Australian raven) were presented by local wildlife rescuers, in a moderate state of decomposition. Six birds (five Australian magpies and one Australian raven) were presented alive. Each bird was found in or around one tree on the verge of a suburban street in Oak Flats on the New South Wales South Coast. Ten Australian magpies were described as male and four were described as female based on morphological appearance. Sex was not assigned to two Australian ravens and one juvenile Australian magpie. The majority of birds presented were adults (15 birds).

Live birds displayed a combination of neurological, respiratory and gastrointestinal signs. Live birds displayed a combination of neurological, respiratory and gastrointestinal signs. Neurological signs ranged from generalised paresis with birds in ventral recumbency (Figure 1) and lateral recumbency (Figure 2) to birds that would rest back on their hocks and stand when approached. Birds that were in ventral recumbency were alert when approached and would attempt to ambulate by scooting along with their wings. Some birds held their legs in extension, showed an exaggerated perch reflex and a delayed withdrawal reflex. Gastrointestinal signs ranged from blood in the oral cavity, haematochezia and haemorrhagic diarrhoea to reduced faecal output and diarrhoea. Respiratory signs included tail bob-

bing, tachypnoea and open -beak breathing.

Avian Influenza, Newcastle Disease, flaviviruses (West Nile virus, Kunjin virus, Murray Valley Encephalitis, Japanese Encephalitis), enteroviruses, chlamydiosis, systemic leucocytozoonosis and intoxications such as botulism, organophosphate and carbamate toxicity, mycotoxicosis and zinc and lead toxicity were included in a list of differential diagnoses for the event.

Successful stabilisation treatment was given to 4 birds (3 Australian magpies and 1 Australian raven). Each bird received subcutaneous fluid therapy (33mL/kg; Hartmann's solution; Baxter healthcare Pty Ltd, Toongabbie, NSW, Australia) and oral rehydration therapy (15mL/kg; Glucose 5%; Baxter healthcare Pty Ltd, Toongabbie, NSW, Australia). Each of these birds was standing within twelve hours; was support fed a mixed insectivore diet by crop tube (15mL/kg; a/d Canine Feline Prescription Diet, Hill's Pet Nutrition Inc., Topeka, KS, USA; Insectapro, Vetafarm, Wagga Wagga, NSW, Australia) and was released to wildlife carers 24 hours after presentation. These birds were successfully released seven days after presentation.

Two Australian magpies died shortly after presentation and were submitted for necropsy. On external examination birds were in good body and feather condition, with good pectoral muscle mass. There was soiling of feathers around the vent with brown diarrhoea in one bird and haemorrhagic diarrhoea in the other (Figure 3). Gross internal lesions were found in the gastrointestinal organs and spleens of both birds. In the first bird examined, intraluminal haemorrhage was seen at all levels of the gastrointestinal tract. There was purple mottling of the serosal surfaces of the small intestines and pancreas. In the second bird examined, there was yellow, mucoid fluid filling the small intestines and copious light brown fluid present in the cloaca, but no evidence of haemorrhage. Both birds had large dark purple, mottled spleens, and moderately large livers with the hepatic border extending well beyond the sternal edge. There was epicardial haemorrhage in the first bird. The frist bird was a female, the second a male. Tissues were collected in 10% buffered formalin and processed routinely for histological examination at the Registry. Liver, brain, intestine and gut contents were stored at -8C pending histology.

Histopathological findings in both birds consisted of lymphoplasmacytic inflammation and acute vascular damage. Each bird had perivascular cuffs of nonsuppurative inflammation along the serosal surfaces of the viscera, nonsuppurative inflammation multifocally within the myocardium, pulmonary congestion and haemorrhage, and multifocal acute fibrinoid vasculopathy. The birds also had either fibrinonecrotising granulocytic air sacculitis or coelomitis. These histological changes were nonspecific. Histology images will be presented at the conference for discussion.

Both magpies had similar lesions in the gastrointestinal tract and liver, which varied in severity, distribution and chronicity. One bird had multifocal, acute hepatocellular and biliary necrosis and severe, acute necrotising, haemorrhagic enteritis. This animal also had fibrinonecrotising and granulocytic air sacculitis. The other bird had a more inflammatory and less necrotising intestinal lesion with moderate nonsuppurative enteritis. Similar to the first bird, this animal had multifocal hepatic necrosis, but it also had nonsuppurative cholangitis. The presence of intestinal mucosal coccidia and intravascular nematodes within the liver of this bird might confound these findings. The second bird also had fibrinonecrotising and granulocytic inflammation extensively along the coelomic surfaces.

Oesophageal nematodiasis and a protozoal infection, presumed leucocytozoonosis, based on histological appearance, were present in the first magpie examined and considered common incidental findings in wild magpies.

Pharyngeal swabs were collected from three birds; two Australian magpies during necropsy and one Australian raven given supportive therapy. The swabs were submitted to Elizabeth Macarthur Agriculture Institute to test for NDV via real time PCR and AIV via Influenza A matrix PCR. Each of these samples was negative for NDV and AIV.

Eleven frozen dead birds were collected by officers from the NSW Environment Protection Authority for toxicological testing. Liver samples were removed from four birds and gas chromatography was used to test for a wide range of acaricides, fungicides, organophosphates, carbamates, synthetic pyrethroids and organochlorine pesticides. Three samples tested positive for fenthion. One liver tested was found to contain 1.1mg/kg. The other two liver samples were found to contain traces of fenthion, however the concentrations were lower than the quantification limit of 0.15mg/kg.

Discussion

There have been three reported outbreaks of mortality in Australian magpies, Australian ravens, Pied currawongs and Magpie larks along the eastern coast of NSW demonstrating a consistent set of clinical signs, gross pathological and histopathological lesions. All birds presented during the 2015 event, came from Kilpa Place in Oak Flats on the NSW South Coast. Histologically consistent cases identified during the 2006 event, came from the Central Coast of NSW, Sydney's Northern Beaches, the Southern Suburbs of Sydney and Sydney's Western Suburbs. Other reported bird deaths during the 2006 event, fitting the case definition, came from Healesville (Australian Ravens, n=3) and Central Queensland (Common Koels, n=2).

The main species affected in the 2003 and 2006 events were Australian Magpies (70%) and Pied Currawongs (15%). There were anecdotal reports of affected birds including Crested pigeons (*Ocyphaps lophotes*), Common koels (*Eudynamys scolopacea*) and Silver gulls (*Larus novaehollandiae*) during the 2006 event. None of these cases were confirmed using histopathology.

The majority of birds necropsied during the 2003 and 2006 events were found to be male (63%). Sex was not confirmed in 16% of birds. In the 2015 event, the sex of 15 birds was not confirmed by necropsy. In order to identify a sex bias, necropsy examination should be performed on as many possible dead birds during future mortality events.

The majority of birds presented during the 2003 and 2006 events were adults (68%). Twenty-nine percent were described as subadult based on plumage and histological appearance of the gonads. Three percent were juveniles. These findings suggest that adult male birds may be over-represented in these events.

Four out of 17 birds presented during the 2015 event were given supportive treatment and released. During the 2006 event, most clinical cases resulted in death within 12 to 24 hours. However, there was reported survival to 10 days or more. Some birds appeared to recover after intensive treatment including the use of thiamine, activated charcoal and corticosteroids. These findings demonstrate a high mortality rate during these events.

The predominant gross necropsy findings in all three events include birds in good body condition, with diarrhoea and haemorrhage into the gastrointestinal tract. Epicardial and myocardial haemorrhage and hydropericardium were seen grossly in birds during the 2003 and 2006 events.

Histopathological lesions in all three events have focused in and around blood vessels, cardiac and skeletal muscles, coelomic membranes, intestines, liver and lung. Histopathological diagnoses in each event include: vasculopathy with mild to severe fibrinonecrotising vasculopathy of coronary blood vessels, skeletal muscle and intestinal serosal vessels; nonsuppurative inflammation and myodegeneration of cardiac and skeletal muscle, acute foci of hepatic necrosis, cholangiolar cell proliferation, enteritis, acute enteric necrosis and haemorrhage, perivascular pulmonary haemorrhage and acute heterophilic bronchitis.

No lesions were evident in the brain of the two birds examined histologically in 2015. Peripheral nerves and spinal cord were not collected at necropsy. Only two birds examined during outbreaks in 2003 and 2006 had lesions involving the central nervous system with foci of microgliosis and nonsuppurative inflammation. Nonsuppurative inflammation was present in soft tissues surrounding peripheral nerves in some cases. Two birds had foci of acute haemorrhage within the spinal cord that was not accompanied by any evidence of trauma or haemorrhage in the surrounding tissues. These findings highlight the importance of collecting a full set of tissues, including brain, spinal cord and peripheral nerves, for histopathological examination during epizootics. The aseptic collection of additional tissues for storage frozen allows a full investigation into a multitude of differential diagnoses.

The findings of oeseophageal and hepatic nematodiasis and protozoal infection in the two magpies examined in 2015 were presumed to be incidental. Presumed incidental findings in other confirmed cases include microfilaraemia, systemic leucocytozoon species infection, intestinal coccidiosis, pancreatic and hepatic trematodiasis, small intestinal cestodiasis, proventricular nematodiasis and intracellular sarcocyst-like organisms in skeletal or cardiac muscle unrelated to any inflammation. The leucocytozoon infections may be an indicator of high exposure to biting insects, which could serve as vectors for other viruses and parasites.

Samples from birds presented during the 2006 event

tested negative for the notifiable diseases AI, NDV, West Nile Virus, and *Chlamydia psittaci* via PCR. Attempts at viral isolation via culture in chick embryos and mammalian cells were unsuccessful.

Due to the mass mortality of birds found to be in good body condition at a single point location, toxicity was initially suspected during the 2015 Oak Flats mortality event. Toxicological examination for a wide range of pesticides and herbicides revealed concentrations of fenthion in liver tissues in three out of four birds tested. These levels were interpreted to be below concentrations sufficient to have caused clinical effects. The Australian magpie is the most common species of wild bird involved in confirmed poisoning events reported to Wildlife Health Australia. This species represented almost a third of all reported events between April 2009 and March 2014 (Grillo et al. 2014). Poisoning of Australian ravens has also been commonly reported (Grillo et al. 2014).

Collectively, Black and White Bird disease is a syndrome characterised by acute mass mortality of Australian magpies, Pied currawongs and Australian ravens. When birds present alive they are most often in good body condition, and may exhibit a range of clinical signs related to the respiratory, gastrointestinal and neurological systems. The most common presentation includes dyspnoea and ventral recumbency. Birds appear paralysed, but will stand and walk if stimulated. Consistent post mortem findings include: vasculopathy; nonsuppurative perivascular inflammation along coelomic surfaces, myocardium and skeletal muscle; hepatic necrosis; and enteritis or enteric necrosis.

The cause of the syndrome has not been established, but a viral aetiology seems to best match the epidemiology and histological lesions. Samples from birds affected through each event have been submitted for next-generation sequencing in an attempt to look for the presence of viral pathogens.

Investigating these sorts of events reminds us of the importance of using personal protective equipment (PPE) in any veterinary hospital that regularly treats wildlife as we may be faced with zoonotic or emerging pathogens of uncertain zoonotic potential. When a potentially infectious disease is encountered, strict quarantine should be applied to minimise the risk of disease spread among captive birds. PPE should be worn to protect staff, volunteers, and carers from potential zoonoses.

These findings highlight the importance of appropriate disease investigation in cases of wildlife mortality. Having an efficient disease monitoring program for wildlife allows early detection of new and emerging diseases, some of which may have serious zoonotic and economic implications. Necropsy examination of fresh carcasses, histopathological examination of a full range of tissues, specific exotic disease testing, toxicological testing and more advanced culture and molecular pathogen detection capabilities are vital to rule out exotic diseases and elucidate the cause of mortalities. This data may then contribute towards event response, wildlife management, and public communication. Black and White Bird Disease should be considered as a differential diagnosis in Passeriformes, displaying neurological, respiratory and gastrointestinal signs, particularly in the face of mass mortality.

Acknowledgements

NSW Department of Primary Industries, CSIRO's Australian Animal Health Laboratory, Tiggy Grillo, Wildlife Health Australia.

Reference

Grillo T, Cox-Witton K and Post L 2014. Wildlife Health Australia report in Animal Health Surveillance Quarterly 19: 1, 7-9.

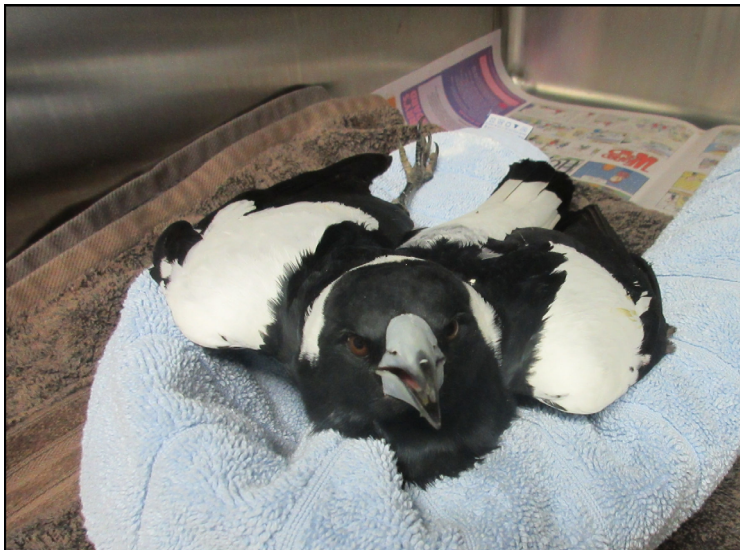

Figure 1: Bird in ventral recumbency

Figure 2: Bird in lateral recumbency

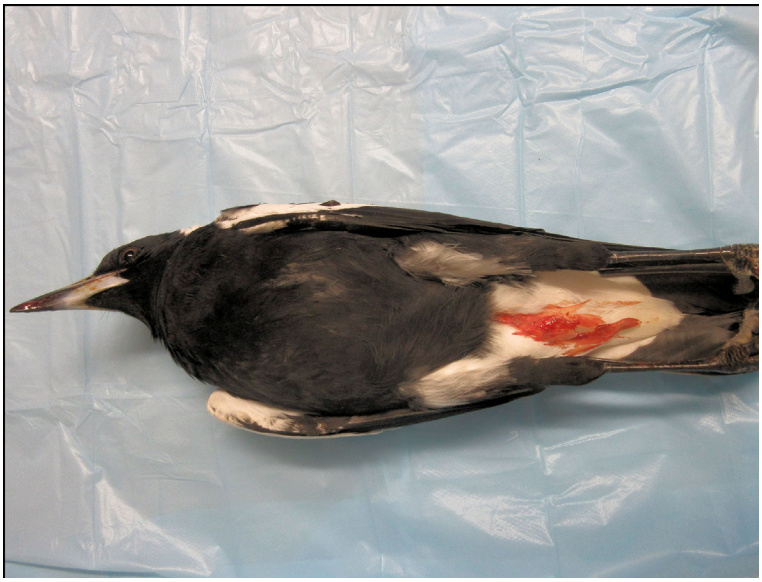

Figure 3: Blood-stained feathers around vent