Avian influenza surveillance programme

New Zealand's avian influenza surveillance programme is multi-faceted, incorporating active surveillance of resident wild birds, and enhanced passive surveillance. New Zealand has never had a case of highly pathogenic avian influenza virus (AIV) (infection with influenza A virus of high pathogenicity) in wild birds or poultry (World Organisation for Animal Health, 2016).

Wild bird surveillance

New Zealand is not on a migration pathway for waterfowl as observed in the northern hemisphere, although vagrant waterfowl from Australia are occasionally encountered. New Zealand lies at the southeastern extremity of the East Asian–Australasian Flyway which was (and still is) of particular relevance for the introduction of novel AI especially given the spread of H5N1 across Asia from 2004. Therefore, since 2004 the Ministry for Primary Industries (MPI), in conjunction with the New Zealand Fish and Game Councils, the Department of Conservation and other stakeholders, has annually carried out surveillance for avian influenza viruses in targeted migratory and resident birds. The first 6 years of surveillance focused primarily on migratory birds, in particular the bar-tailed godwit (*Limosa lapponica*), and red (lesser) knot (*Calidris canutus*), on their arrival each year from late September to November, at Miranda, their main North Island arrival site. These birds were targeted for surveillance because of their migration pathway, along which avian influenza viruses may be present: directly from the Arctic regions of Asia and North America in the case of the godwit, and from Arctic regions via the Pacific coast of Asia in the case of the knot. However, surveillance over this period indicated that migratory birds posed a very low risk for the introduction of high-pathogenicity avian influenza viruses into New Zealand, as no avian influenza virus was ever isolated. Therefore, since 2010, surveillance has focused on resident birds, mainly waterfowl.

Since 2004, non-migratory waterfowl, predominantly mallard ducks (*Anas platyrhynchos*) have been sampled in the summer months throughout New Zealand, with a particular focus on coastal areas where they might have had contact with migratory shorebirds.

In 2018, cloacal and oropharyngeal swabs were collected from 950 healthy resident mallard ducks (**Table 1**). A Fish & Game banding programme provided a convenient opportunity for MPI to collect samples from ducks for avian influenza surveillance at the same time. Individual bird samples were tested by the influenza A real-time RT-qPCR TaqMan (Spackman et al., 2003, Heine et al., 2015). Positive or suspect samples were then tested using real-time H5 and H7 RT-qPCR TaqMan (Slomka et al., 2007; Sidoti et al., 2010) and conventional H5, H7 RT-PCRs to obtain genomic information.

Influenza A RNA was detected in cloacal or oropharyngeal samples (or both) in 60.5 percent of the 950 ducks sampled. This is lower than in the previous year (73.2 percent). However, if we analyse AIV prevalence in ducks from individual collection sites, the percentage of AIV-positive ducks varies: 62 percent at Pipiroa; 16.7 percent at Lake Te Rotokare; 90.3 percent at the Kaituna River mouth; and 66.7 percent in the Gisborne area. AIV prevalence is rather dynamic and can change from year to year.

Influenza subtype H7 RNA was confirmed by PCR in 22 samples from one location in the North Island and one H7 virus was isolated. This virus was confirmed to be non-pathogenic, based on analysis of the cleavage site of the HA gene. RNA from three H5 viruses was detected but no H5 virus was isolated. To obtain information on AIV subtypes other than H5 and H7 circulating in mallard ducks in New Zealand, virus isolation was also carried out on a random selection of the remaining samples that tested positive for influenza A by RT-qPCR, and a number of influenza virus subtypes – H3, H4, H6 and H11 – were isolated.

Enhanced passive surveillance

MPI operates a 24/7 toll-free exotic pest and disease emergency hotline and receives calls relating to sick and dead wild and domestic birds from members of the public, veterinarians, regional laboratory pathologists and others. Where reports relate to native birds, they are handled collaboratively with the Department of Conservation.

A risk assessment determines the need to investigate the report further. Key information used in the profile includes:

- history of the event: numbers affected and timeline of events;
- signs observed in dying birds;
- species of bird/s affected;

Table 1: Active surveillance for avian influenza viruses in wild birds, 2018

Location	Number of mallard ducks sampled	Number of samples tested (cloacal & oropharyngeal)	Number of RT/PCR positives		Confirmed
			H5	H7	H5 or H7 isolates
Pipiroa, Piako River	300	600	1	22	1 x H7*
Lake Te Rotokare, Hawke's Bay	240	480	3	0	0
Mouth of Kaituna River, Bay of Plenty	320	640	0	0	0
Gisborne area	90	180	0	0	0
Total	**950**	**1,900**	**4**	**22**	**1 x H7**

*The amino acid pattern of the HA cleavage site was consistent with low-pathogenic H7 viruses.

- availability of fresh samples (where unavailable, follow-up is instigated);
- location; and
- epidemiological trends over space and time.

Based on the risk assessment, the investigation is either stood down or expanded to look for a potential exotic or emerging disease aetiology.

A rapid field service is in place for sample collection and submission of unexplained bird deaths (Rawdon et al., 2007), using MPI-approved suppliers. A standardised investigation protocol coordinated by the IDC (Wallaceville) is applied to submissions. This includes necropsy and sample collection for histology, bacteriology and virology. The presence of avian influenza is assessed using influenza A real-time RT-PCR TaqMan (Spackman et al., 2003), with follow-up using real-time H5 and H7 RT/PCR TaqMan assays to exclude H5 and H7 subtypes (Slomka et al., 2007; Sidoti et al., 2010). Virus isolation is performed on samples that are positive in PCR assays (Stanislawek et al., 2002).

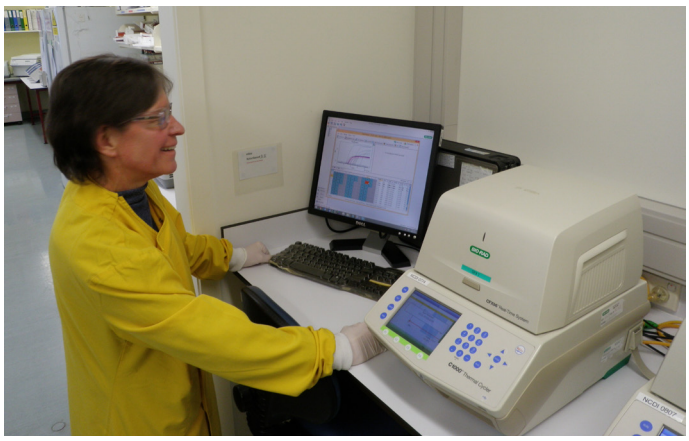
Reports on avian disease and mortality investigations are published quarterly in *Surveillance* as part of the DSS report of suspect exotic disease investigations. In 2018, 39 notifications were received and eight investigations were conducted (**Table 2**). Avian influenza was excluded by influenza A real-time RT-qPCR in all samples submitted for six of these investigations. In a further two avian disease investigations, endemic diseases were diagnosed and although influenza testing was not carried out, the investigation findings concluded that an endemic agent was the cause of the health event (see case histories below).

Table 2: Avian mortality notifications and investigations, 2018

Month	Notifications	Investigations
January	2	1
February	1	1
March	3	0
April	11	2
May	0	1
June	0	0
July	2	0
August	2	1
September	1	0
October	3	0
November	3	1
December	3	1

AHL scientist Harriett Sowman collecting samples from ducks at Lake Te Rotokare

AHL scientist Della Orr collecting samples from ducks at the mouth of the Kaituna River



AHL senior technician Maree Joyce testing field samples for presence of avian influenza

Respiratory disease investigated in backyard chicken flock

A Waikato veterinarian contacted MPI via the exotic pest and disease emergency hotline to discuss two deaths in a backyard chicken flock consisting of 15 five-year-old Red Shavers. The chickens were noticed to be unwell one morning after having been fine the previous evening. One presented with respiratory distress and was described as making a rattling noise as it breathed. Another was hunched over with its head lowered and eyes closed, and had a darkened comb. Both died within 4 hours. Unfortunately the chickens were disposed of and

unavailable for necropsy. While aged modern brown layers are prone to a variety of tumours that can present as these did, two deaths out of 15 birds on one day is unusual, so the exotic differentials avian influenza and Newcastle disease had to be eliminated. While the absence of clinical signs among the 13 in-contact birds suggested exotic disease was unlikely, nevertheless cloacal and oropharyngeal swabs from them, in avian transport medium, were requested by the duty Incursion Investigator. These were submitted to the AHL, where avian influenza and Newcastle disease were ruled out by PCR testing. The investigation was closed.

Diarrhoea investigated in pigeon flock

A pigeon breeder contacted MPI via the exotic pest and disease emergency hotline to report pigeon deaths over a period of 6–8 weeks, which he believed might be due to salmonellosis. Clinical signs included inactivity, loss of appetite, weight loss, loss of flight and watery droppings, leading to death. The breeder had been treating the birds with metronidazole (dose unknown), to no effect. An avian specialist veterinarian was engaged to assist with diagnosis and rule-out of exotic agents including pigeon rotavirus (an emerging disease in Australia) and pigeon paramyxovirus. The avian veterinarian interviewed the notifier and arranged for faecal samples to be submitted to the laboratory. Samples were found to contain very high levels of *Capillaria* spp. (18,300–28,000 eggs per gram). Also known as hairworm, capillariasis is a well-known cause of diarrhoea and wasting in pigeons. The flock responded to anthelminthic dosing and no further deaths were reported by the breeder. Exotic agents were ruled out by diagnosis of exclusion, and the investigation was closed.

Suspect avian paramyxovirus type 1 in commercial layer flock

A specialist poultry veterinarian contacted MPI via the exotic pest and disease emergency hotline to discuss a disease outbreak in a layer flock with up to 20 percent mortality in an 8-week period. Post-mortem investigation had identified peritonitis and lesions in the reproductive tract that were consistent with pasteurellosis in mature layer hens, and culture yielded a heavy growth of *Pasteurella multocida*. Strains of *P. multocida* have been differentiated into Heddleston serotypes based on surface lipopolysaccharide (LPS) molecules, and bacterins based on these types are used for protective vaccination. The vaccine available in New Zealand captures Heddleston types 1, 3 and 4. The isolated strains were sent to an overseas laboratory and typed to LPS group 3, corresponding to Heddleston types 3 and 4. In an attempt to look for a predisposing cause of the infection, the veterinarian had serological testing (Bio Check ELISA) carried out for avian paramyxovirus type 1 (APMV-1) at a poultry industry laboratory. Two of three affected layer birds were seropositive, while two of five unaffected birds were also positive.

Several different pathotypes of APMV-1 are recognised. Pathogenic APMV-1 is exotic to New Zealand, while non-pathogenic APMV-1 is present in New Zealand (Dunowska et al., 2013). There were no lesions consistent with pathogenic APMV-1, and there was no intestinal pathology that can sometimes be found with very low pathogenic APMV-1 viruses. For further investigation of this seropositivity, serum samples from sick and healthy birds were submitted to the AHL, where a range of low positive titres (1:8 to 1:16) were identified in the APMV-1 hemagglutination inhibition test. These titres supported exposure of the flock to a non-pathogenic APMV-1 at some point. In addition, oropharyngeal and cloacal swabs, cecal tonsil, liver and spleen from up to 30 birds were submitted to the AHL and were found to be negative for APMV-1 by real-time PCR. The presence of an exotic APM-1 pathotype or a new emerging *P. multocida* strain was ruled out and the investigation closed.

In addition to investigating sick and dead bird reports, MPI collects data from approved veterinary diagnostic laboratories on avian submissions from veterinary practitioners. **Table 3** summarises submission data from the MPI passive surveillance system (Watts et al., 2016).

Table 3: Avian submissions to MPI's passive surveillance system 2004–2018

Year	Approved veterinary diagnostic laboratory submissions	MPI notifications	MPI investigations
2004	116	30	8
2005	340	85	8
2006	360	154*	24
2007	33	60	14
2008	120	37	10
2009	163	151**	7
2010	174	25	7
2011	142	19	7
2012	290	19	8
2013	664	19	6
2014	385	30	13
2015	503	45	14
2016	824	28	11
2017	723	18	12
2018	785	39	8

* The aberration in the number of bird mortality reports for 2006 was generated as a result of public interest following excessive media reporting – see McFadden et al. (2007).

** The aberration in the number of bird mortality reports for 2009 was due to a toxicity event in August of that year relating to grey side-gilled sea slugs (*Pleurobranchaea maculata*) in the Auckland region.

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