

Virology Projects

PCR for IBR (Wang, J., O'Keefe, J., **Cork, S.C.**, Orr (2007) International Recognition for new NZ- developed molecular test to determine infectious bovine rhinotracheitis (IBR) status of bovine semen for international trade. Biosecurity Magazine. Issue 77.
<http://www.biosecurity.govt.nz/publications/biosecurity-magazine/issue-77>

J. Wang^{1*}, J. O'Keefe, D. Orr, L. Loth, **S. Cork**, M. Banks, P. Wakeley, D. West, R. Card, G. Ibata, C. Van Maanen, P. Thoren, M. Isaksson, P. Kerkhofs (2007) Inter-laboratory Evaluation of a Real-Time PCR Assay for Detection of Bovine Herpesvirus 1 in Bovine Semen. 13 th International Symposium for the World Association of Veterinary Laboratory Diagnosticians. Melbourne, Australia. November. 2007

PCR for CSF (Kok-mun Tham)

Classical Swine Fever (CSF) is a severe and economically important disease of pigs which is caused by a pestivirus. CSF is exotic to New Zealand although pestiviruses of ruminant origin are common in the New Zealand cattle and sheep population. The objective of this project is to develop a rapid molecular test method (real-time RT-PCR) that will be suitable for the rapid detection (or rule out) of CSFV and the differentiation of CSFV from other swine pathogens that produce similar clinical signs.

A real-time RT-PCR assay using the SYBR Green chemistry has been developed and partially validated for the detection of CSFV. Test specificity has been determined using diagnostic samples from investigation cases of suspect CSFV in pigs undertaken at IDC Wallaceville. An avirulent strain of CSFV, obtained from the Netherlands, has been used as a positive control.

The real-time RT-PCR assay for CSFV has recently been validated at VLA Weybridge using six reference strains of CSFV which represented the three genotypes of CSFV. Validation of the real-time RT-PCR at VLA Weybridge also showed that the PCR assay developed and optimised at IDC Wallaceville is highly sensitive and specific, and can be used to detect CSFV in tissue samples during an outbreak of CSF

Pestivirus genotyping (Kok-mun Tham)

Pestiviruses of economic importance include Bovine Viral Diarrhoea virus, (BVDV), Border Disease virus (BDV) and Classical Swine Fever Virus (CSFV). In addition to these three types of pestiviruses affecting livestock animals; there is a wide range of other pestiviruses reported in the literature. This project currently focuses on BVDV isolates although other pestiviruses isolated from cattle and other ruminants are also examined.

Two genotypes of BVDV, BVDV type I and BVDV type II, can infect sheep and pigs in addition to cattle and are distributed world-wide. Previous limited work has demonstrated a somewhat greater diversity in New Zealand isolated strains of BVD type 1, as compared to Australia (Horner *et al.* 2000). BVDV type 2 is not currently thought to be present in the New Zealand cattle population.

This project aims to monitor the genotypes of BVDV (and potentially other pestiviruses) present in the New Zealand ruminant population. Samples are obtained from BVD cases identified by private veterinary diagnostic laboratories and from IDC incursion investigation cases.

Although an isolate of BVD type 2 has been isolated from a batch of commercial serum imported from overseas, results from this project has hitherto demonstrated that New Zealand cattle, sheep and pig populations are free of BVD type2

PCV-2 characterisation (Kok-mun Tham)

Porcine Circovirus type 2 (PCV2) has generally been considered to be a primary aetiological agent in Postweaning Multisystemic Wasting Syndrome (PMWS). In October 2003, PMWS was identified in a pig herd at Waikato and has subsequently been found on other pig farms in New Zealand. The role of PCV2 in PMWS, and other disease syndromes such as porcine dermatopathy and nephritis syndrome (PDNS) and congenital tremor, remains controversial.

The aim of this project is to obtain the complete nucleotide sequence of PCV2 viruses isolated from healthy pigs and from pigs with PMWS. The primary genomic nucleotide sequence of New Zealand isolates of PCV2 will be analysed and compared with isolates from overseas to examine the extent of genetic variation among New Zealand PCV2 isolates

In New Zealand PCV2 virus has now been isolated from healthy pigs, and pigs with PMWS. DNA extracted from these PCV2 isolates has been amplified using seven pairs of PCR primers that amplify gene sequences spanning the entire PCV2 genome. Amplicons generated from these PCV2 isolates have been sequenced so that the sequence data can be subjected to phylogenetic analysis.

Sequence data of NZ isolates of PCV2 were compared with Genbank data from other isolates obtained from various geographic regions i.e. Asia-Pacific, Europe and America (North and South) + South Africa.

PCR for influenza A and APMV-1 (Wlodek Stanislawek)

Avian influenza and Newcastle disease are the two most economically important avian diseases internationally. This is because of the great financial losses associated with outbreaks caused by pathogenic strains of these viruses. Pathogenic strains of avian influenza are usually of subtype H5 or H7. Avian paramyxovirus type 1 (APMV-1) is the cause of Newcastle disease. Other avian influenza viruses and paramyxoviruses generally have less clinical significance.

Accurate and speedy diagnosis is critical particularly when an exotic disease such as avian influenza or Newcastle disease has to be excluded. Rapid molecular tests such as real time reverse transcriptase polymerase chain reaction (RRT-PCR) offer the high sensitivity provided by conventional RT-PCR with the advantage that it is quicker. A

preliminary result can be achieved within 3-4 hours of receipt samples at the laboratory. However, conventional RT-PCRs will still play important role in the diagnosis of avian influenza and other diseases because they can assist with sub-typing and provide genomic information when a virus is sequenced.

Assays such as real time RT-PCR (RRT-PCR) have the potential to enhance the current diagnostic capability at IDC and also have application as a screening test for surveillance studies for AIV and AMPVs in New Zealand domestic and wild bird populations. With further evaluation the generic influenza A RRT-PCR also has the potential to be used for surveillance in other species such as the horse.

Surveillance of migratory birds for influenza A/AMPV-1 (Wlodek Stanislawek)

The spread of a number of strains of highly pathogenic avian influenza subtype H5N1 around the world has prompted MAF to determine the current influenza A status (subtype H5N1 in particular) of migratory birds arriving in New Zealand. No strains of AIV, including H5N1 viruses, have been isolated in previous studies of migratory birds arriving in New Zealand

One of the most important pathways for entry of new strains of AIV and APMV is the movement of migratory birds. Due to New Zealand's relative geographical isolation, it is not on the main migratory flyways. However, there are a number of species that migrate to New Zealand regularly. Shorebirds, in particular the bar-tailed godwit, red (lesser) knot, ruddy turnstone, and Pacific golden plover make up the highest numbers of the up to 200,000 birds of 45 species that visit New Zealand each year. Most of them breed in the Arctic regions of Europe, Asia, and North America and migrate south for the boreal winter. New Zealand does not have migratory waterfowl although there are a few species of ducks and geese that occasionally reach New Zealand from Australia.

For migratory birds to introduce new avian viruses to New Zealand, they would have to be infected before migration or during migration. From our limited knowledge regarding their migratory routes, godwits are believed to fly directly to New Zealand. Lesser knots have stopovers in East Asia and ruddy turnstones have a number of stops in Hong Kong, Taiwan, and China before they reach New Zealand.

On arrival to New Zealand migratory birds infected with avian influenza viruses have the potential to shed viruses and introduce new strains of AIV and AMPV to the environment where they co-habit with endemic shorebirds, gulls, waterfowl, and other species including humans, livestock, and wildlife.

The epidemiology of AIV and AMPV in shorebirds is still not well defined, although from studies carried out in the Northern Hemisphere the peak of prevalence of avian influenza viruses occurs in spring and is also associated with autumnal migration. AMPV have a similar epidemiology to AIV.

Migratory shorebirds start to arrive in New Zealand in late September through to November. With the assistance of wader study groups, attempts are made to trap the birds for banding and sampling as soon as possible after arrival as possible. Cloacal swabs are collected and used for virus isolation and PCR assays. Samples from this, and the waterfowl surveillance project, are currently being used to evaluate a real time influenza A PCR assay.

Stanislawek, W.L., **Cork, S.C**, Rawdon, T.G., Thornton, R.N., Melville, D (2007) Surveillance for avian influenza in wild birds in New Zealand. Option for the control of influenza VI, Conference, Toronto, Canada. June 2007.

Surveillance of wild ducks for influenza A/AMPV-1 (Wlodek Stanislawek)

The recent outbreaks of avian influenza in Europe and Asia, caused by strains of avian influenza virus (AIV) subtype H5N1 has prompted enhanced monitoring of influenza viruses in New Zealand birds. To date, no H5N1 subtype viruses have been identified in samples collected from waterfowl in New Zealand. However, previous work identified a number of other subtypes of influenza A virus in New Zealand waterfowl and also the presence of a number of paramyxoviruses.

Wild birds, particularly waterfowl (mallards and other ducks, teal, geese, swans) are known to be the natural reservoir for avian influenza viruses. Different populations of wild waterfowl can maintain a wide range of, predominantly harmless, avian influenza viruses. A range of subtypes have been identified in birds and these are characterised by combinations of H (16) and N (9) types as determined by laboratory testing. Ducks are also recognised as a reservoir for some types of paramyxoviruses, including avian paramyxovirus type 1 (APMV1, \virulent forms cause Newcastle disease), and information on these viruses may also be obtained in this survey.

The interaction of ducks with other birds, including migratory birds, makes it particularly appropriate to survey duck populations for the prevalence of influenza viruses.

The opportunity for waterfowl to become infected by avian viruses introduced by migratory shorebirds theoretically exists where species mix at estuaries and coastal lakes. Sampling sites have been selected to assess this risk. Every year ducks are trapped by Fish and Game personnel, who also provide other relevant information including species, sex, and age of birds. Cloacal swabs and blood samples are collected by animal health laboratory (AHL) staff. Ducks can shed AIV and APMV for several weeks in faeces which increase the chance of detecting the virus. Cloacal swabs are tested using virus isolation and PCR assays. Serum from blood samples is tested using the Haemagglutination inhibition (HI) test for antibodies to H5 and H7 subtype viruses. Samples from this project are currently being used to evaluate the diagnostic sensitivity of a real time PCR assay for influenza A.

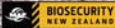
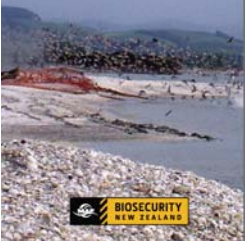
SURVEILLANCE FOR AVIAN INFLUENZA IN WILD BIRDS IN NEW ZEALAND

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background

The spread of a number of strains of highly pathogenic avian influenza (AI) virus subtype H5N1 around the world has prompted Hawkestry New Zealand to expand surveillance programmes to determine the current status of AI viruses in migratory shorebirds arriving in New Zealand as well as its resident waterfowl.

Shorebirds, in particular the bar-tailed godwit (*Limosyta hypoleucos*), red (dusky) knot (*Calidris canutus*), noddy turnstone (*Actonotus nigripes*), and Pacific golden plover (*Pluvialis fulva*) make up the majority of the ~200,000 birds of 43 species that visit New Zealand each year. Most of them breed in the Arctic regions of Europe, Asia, and North America and migrate south for the boreal winter.

For these birds to introduce AI viruses to New Zealand, they would have to be infected en route or close to departure and shed the virus in New Zealand on arrival. From our limited knowledge regarding their migratory routes, godwits are believed to fly directly to New Zealand. Lesser knots have stopovers in East Asia and noddy turnstones have a number of stops in Hong Kong, Taiwan, and China before they reach New Zealand.



On arrival infected migratory birds may shed AI virus into the environment which they share with endemic shorebirds, gulls, waterfowl, and other species including humans, livestock, and wildlife.

New Zealand's waterfowl do not migrate internationally as some species do in the northern hemisphere but they can maintain endemic AI viruses and introduce them to other species.



methodology

Since 2004 a total of 834 clinical swabs have been collected from newly arriving migratory birds in particular the bar-tailed godwit, red (dusky) knot, and noddy turnstone. Sampling was carried out at Miramira in the North Island, the main New Zealand arrival site, but was expanded to other sites in 2006.

A total of 2754 clinical swabs were collected from waterfowl (mainly mallard ducks) caught using wire mesh traps in coastal areas during the summer months (December-February). Blood samples (385) were also collected from some of these waterfowl.



results

Migratory birds

No AI viruses were isolated from clinical swabs collected from migratory shorebirds. However, AI viral RNA was confirmed in two samples using nucleic acid (Table 1). When the sample matrix genomes (780bp) sequences obtained from the above were compared to sequences deposited in GenBank, they had 96.5% identity to the matrix protein genome sequences of AI A/Chicken/Hubei/1997. AI virus subtypes H5 and H7 were excluded by specific RT-PCR.

Waterfowl

A total of 50 AI virus subtypes H5, H2, H4, H6, and H11 were isolated from clinical swabs collected from resident waterfowl (Table 2). These represents 1.7% overall prevalence of AI virus in resident waterfowl in New Zealand. A total of 105/944 were reacted with H5 H8 antigen with titres between 1.8 and 1.128 and 24/384 reacted with H7 H8 antigen with titres between 1.8 and 1.12.

conclusions

During the study a number of AI viruses were isolated from New Zealand waterfowl as has been found in other countries. To date, no pathogenic strains of AI virus have been isolated from New Zealand birds.



Surveillance results for H5 and H7 antibodies should be treated with caution as they could represent a cross-reaction with the mammalian antigens. Therefore, further work is required before the significance of these results can be determined.

Only two samples collected from migratory shorebirds contained viral RNA consistent with influenza A. However, no AI viruses were isolated indicating that, currently, these species are not a likely means of introducing AI viruses to New Zealand.



Although the risk of highly pathogenic AI viruses entering and becoming established in New Zealand via the movement of migratory birds is considered to be very low, on-going monitoring of shorebirds and waterfowl will continue.

Table 1
 Avian influenza surveillance results for migratory birds in New Zealand

Species	Sample	AI Virus	AI Virus Subtype
Bar-tailed godwit	1	+	H5N1
Red (dusky) knot	1	+	H7N1
Noddy turnstone	0	-	-
Pacific golden plover	0	-	-
Other	0	-	-
Total	2	2	-

* AI virus influenza surveillance RT-PCR was conducted using primers H5N1 and H7N1.

Table 2
 Avian influenza surveillance results for waterfowl in New Zealand

Species	Sample	AI Virus	AI Virus Subtype
Mallard duck	1	+	H5N1
Mallard duck	1	+	H2N1
Mallard duck	1	+	H4N1
Mallard duck	1	+	H6N1
Mallard duck	1	+	H11N1
Other	0	-	-
Total	5	5	-

* AI virus influenza surveillance RT-PCR was conducted using primers H5N1 and H7N1.