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**Avian influenza  
and avian paramyxoviruses  
in the New Zealand bird population**

*A thesis presented in partial fulfilment  
of the requirements for the degree of  
Doctor of Philosophy in Veterinary Virology  
at Massey University  
Palmerston North, New Zealand*

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## Abstract

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A comprehensive study using virological and serological approaches was carried out to determine the occurrence of avian paramyxoviruses (APMV) and avian influenza viruses (AIV) in live healthy mallard ducks (*Anas platyrhynchos*) in addition to caged birds, wild birds (other than waterfowl), and poultry.

Thirty-three viruses were isolated from 321 tracheal and cloacal swabs from mallard ducks and were characterised as: 6 AIV (two H5N2 and four H4N6), 10 APMV-1, and 17 APMV-4. Of 335 serum samples tested for AIV antibodies, 109 (32.5%) sera were positive by nucleoprotein-blocking ELISA (NP-B-ELISA). Serum samples (315) were examined for antibody to APMV-1, -2, -3, -4, -6, -7, -8, and -9 by the haemagglutination inhibition (HI) test. The largest number of reactions, with titres up to  $\geq 1/64$ , was to APMV-1 (93.1%), followed by APMV-6 (85.1%), APMV-8 (56%), APMV-4 (51.7%), APMV-7 (47%), APMV-9 (15.9%), APMV-2 (13.3%), and APMV-3 (6.0%). All of the H5N2 isolates of AIV and the APMV-1 isolates from this and earlier New Zealand studies had low pathogenicity indices when assessed by the Intravenous Pathogenicity Index (IVPI) with the result 0.00 and Intracerebral Pathogenicity Index (ICPI) with results 0.00–0.16. Partial genomic and antigenic analyses were also consistent with the isolates being non-pathogenic. Phylogenetic analysis of the 10 APMV-1 isolates showed nine to be most similar to the reference APMV-1 strain D26/76 originally isolated in Japan and also to the Que/66 strain, which was isolated in Australia. The other isolate was very similar to a virus (MC 110/77) obtained from a shelduck in France.

Antibodies to APMV-1, -2, and -3 were detected in 4.8, 1.7, and 2.6%, respectively, of caged bird samples. The majority of these caged birds were "exotic" or "fancy" poultry breeds. Amongst wild birds, 4.2% had titres to APMV-2 and over half of these were passerine birds; 1.7% of the samples had titres to APMV-1 and 0.8% to APMV-3 antigen. No APMVs or AIVs were isolated from any of the cloacal swabs collected

from these birds. Of the 1778 poultry serum samples tested only five reacted with APMV-3 antigen and these were later found to be cross-reactions to APMV-1. No reactions were detected with APMV-2 antigen. Although, we can be confident that APMV-1 is present in caged birds, wild birds, and poultry of New Zealand, there is no conclusive evidence of the presence of APMV-2 and APMV-3 in poultry or APMV-3 in wild birds. The results also do not provide conclusive evidence for the presence of APMV-2 in wild birds.

Despite New Zealand being free from ND and highly pathogenic avian influenza (HPAI) in commercial poultry and the lack of evidence of pathogenic APMV-1 and AIVs in other birds, a number of possibilities were suggested by which virulent strains of APMV-1 and HPAI viruses could emerge in New Zealand including: (1) introduction by migratory birds; (2) importation of live birds and avian products; and (3) mutation in endemic viruses of low virulence.

The findings from this study and elsewhere emphasise the importance of good biosecurity measures on poultry farms, to prevent the introduction of viruses of low virulence, as well as monitoring for the presence and type of APMV-1 and AIV in wild and domestic birds. The situation is likely to be dynamic with new strains emerging and the occurrence of clinically important introductions is a real possibility.

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## Abbreviations

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|       |                                    |
|-------|------------------------------------|
| AGID  | agar gel immunodifusion test       |
| AIV   | avian influenza virus              |
| APMV  | avian paramyxovirus                |
| BLAST | basic local alignment search tool  |
| bp    | base pair                          |
| CPE   | cytopathic effect                  |
| ELISA | enzyme linked immuno sorbent assay |
| EM    | electron microscope/microscopy     |
| F     | fusion (glycoprotein)              |
| FA    | fluorescent antibody               |
| HA    | haemagglutinin                     |
| HI    | haemagglutination inhibition test  |
| HN    | haemagglutinin neuramidase         |
| HPAI  | highly pathogenic avian influenza  |
| ICPI  | intracerebral pathogenicity index  |
| IPX   | immunoperoxidase                   |
| IVPI  | intravenous pathogenicity index    |
| L     | large polymerase protein           |
| LPAI  | low pathogenic avian influenza     |
| M     | matrix protein                     |
| MAb   | monoclonal antibodies              |
| MDCK  | canine kidney cell                 |
| MDT   | mean death time                    |
| ML    | maximum likelihood (method)        |
| MP    | maximum parsimony (method)         |
| Mr    | relative molar mass                |
| mRNA  | messenger RNA                      |
| NA    | neuraminidase                      |
| ND    | Newcastle disease                  |
| NDV   | Newcastle disease virus            |

|            |                               |
|------------|-------------------------------|
| NIT        | neuraminidase inhibition test |
| NJ         | neighbour-joining (method)    |
| NP         | nucleocapsid protein          |
| NP-B-ELISA | nucleoprotein-blocking ELISA  |
| NS         | non-structural proteins       |
| OD         | optical density               |
| ORF        | open reading frame            |
| P          | phosphoprotein                |
| PA         | acidic protein                |
| PB         | basic proteins                |
| PBS        | phosphate buffered saline     |
| PCR        | polymerase chain reaction     |
| RBCs       | red blood cells               |
| RE         | restriction enzyme            |
| RNP        | ribonucleoprotein             |
| RT-PCR     | reverse transcriptase-PCR     |
| SDS        | sodium dodecyl sulphate       |
| SPF        | specific pathogen free        |
| ssRNA      | single stranded RNA           |
| UV         | ultraviolet                   |
| Vero       | African green monkey (cells)  |
| VNT        | virus neutralisation test     |
| vRNA       | viral RNA                     |
| VV         | viscerotropic velogenic       |

### **Amino acids**

|                             |        |
|-----------------------------|--------|
| Alanine                     | Ala, A |
| Arginine                    | Arg, R |
| Asparagine                  | Asn, N |
| Aspartic acid               | Asp, D |
| Asparagine or aspartic acid | Asx, B |
| Cysteine                    | Cys, C |

|                            |        |
|----------------------------|--------|
| Glutamine                  | Gln, Q |
| Glutamic acid              | Glu, E |
| Glutamine or glutamic acid | Glx, Z |
| Glycine                    | Gly, G |
| Histidine                  | His, H |
| Isoleucine                 | Ile, I |
| Leucine                    | Leu, L |
| Lysine                     | Lys, K |
| Methionine                 | Met, M |
| Phenylalanine              | Phe, F |
| Proline                    | Pro, P |
| Serine                     | Ser, S |
| Threonine                  | Thr, T |
| Tryptophan                 | Trp, W |
| Tyrosine                   | Tyr, Y |
| Valine                     | Val, V |