

Molecular epidemiology of Newcastle disease and avian influenza in South
Africa

by

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Highly pathogenic avian influenza (HPAI) and velogenic Newcastle disease (ND) are devastating diseases of poultry that are notifiable to the World Animal Health Organization (Office International des Epizooties). RT-PCR, DNA sequencing, molecular characterisation and phylogenetic analyses were conducted on South African ND and AI virus strains isolated since the 1990s, to investigate the epidemiology of both diseases in the country. The first recorded outbreak of AI in chickens that started in 2002 was caused by two genetically distinct low pathogenicity avian influenza virus (LPAI) H6N2 genotypes that arose from a common ancestor. The ancestral virus appears to have been produced by reassortment between two ostrich viruses, A/Ostrich/South Africa/KK98/98 (H6N8), and A/Ostrich/South Africa/9508103/95 (H9N2). This highlighted the potential role that ostriches may play as mixing vessels for strains that may spill over into chickens when biosecurity breaks down. LPAI H3N8, H4N8 and H5N1 viruses isolated in 2004 from wild ducks in Gauteng were determined to be Eurasian in origin, but the LPAI H5N1 virus was not closely-related to the highly pathogenic avian influenza (HPAI) Asian (or Genotype Z) H5N1 strain. Pelagic shorebirds are implicated in the introduction of these viruses into South African wetlands that infect sympatric wild ducks and geese that in turn move extensively throughout the country. Interactions between wild ducks and ostriches are well-documented, and the transmission of AIV between these species was confirmed by the genesis of the HPAI

H5N2 ostrich outbreak strain of 2004 from the common ancestor of an LPAI H5N2 virus, isolated from an Egyptian goose (*Alopochen aegypticus*) in the same year.

Since the 1990s, outbreaks of velogenic Newcastle disease virus (NDV) in South Africa were caused by three distinct genotypes. The historic genotype VIII (lineage 3), enzootic since the 1970s, was replaced by genotype VIIb (lineage 5b) in the early 1990s during a panzootic, and VIIb was replaced by genotype VIId (lineage 5d) in 1999. Lineage 5d reemerged in 2003, causing an outbreak in 2004 in commercial and backyard flocks. Phylogenetic evidence indicated that pigeon paramyxoviruses (ND), similar to the aforementioned genotypes, were introduced into South Africa from Eurasian sources on several occasions. The consecutive replacement of Eurasian NDV genotypes, the lack of a unique South African lineage and the lack of conclusive evidence of a true cyclic reservoir challenges the mindset that NDV is enzootic in South Africa. This is the first time that molecular techniques were used to elucidate the status of these two important diseases in South Africa. It is evident that the region is at risk to the introduction of HPAI strains carried by migratory birds, but that with improved control, the enzootic status of NDV could be reversed, with important economic implications for the poultry industry. Improved biosecurity is therefore key to the prevention of future outbreaks of AI and ND in South Africa.

PAPERS AND CONFERENCE PROCEEDINGS

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
SUMMARY	ii
PAPERS AND CONFERENCE PROCEEDINGS	iv
LIST OF ABBREVIATIONS	xii
LIST OF FIGURES	xv
LIST OF TABLES	xxiii
CHAPTER ONE: LITERATURE REVIEW	1
1.1 AVIAN INFLUENZA	2
1.1.1 Introduction	2
1.1.2 Aetiology	2
1.1.3 Morphology and genome organization of influenza A viruses	2
1.1.4 Host range	4
1.1.5 Disease	5
1.1.6 Diagnosis	6
1.1.6.1 Virus isolation and identification	6
1.1.6.2 Assessment of pathogenicity	7
1.1.6.3 Nomenclature	7
1.1.7 The infection cycle	7
1.1.7.1 The surface glycoproteins	7
1.1.7.2 Receptor specificity and attachment	9
1.1.7.3 Endocytosis and escape into the host cell	10
1.1.7.4 Transcription, translation and particle assembly	10
1.1.8 HA, the primary molecular determinant of virulence	11
1.1.9 Other virulence and host range determinants	13
1.1.9.1 Neuraminidase	13
1.1.9.2 Host basal body temperatures and pH	14
1.1.9.3 Glycosylation and sialylation	15
1.1.9.4 Polypeptides of the replication complex	15

1.1.9.5 Matrix proteins	16
1.1.9.6 N-structural proteins	17
1.1.10 Epidemiology	17
1.1.10.1 The prevalence of AIV in feral waterfowl	17
1.1.11 Control of AI	20
1.1.11.1 Vaccination	20
1.1.11.2 Chemotherapy	21
1.1.12 The zoonotic potential of avian influenza viruses	22
1.1.12.1 Historic human influenza epidemics and avian influenza	22
1.1.12.2 The emergence and spread of Asian HPAI H5N1 (genotype Z)	23
1.1.13 History of avian influenza in South Africa	26
1.2 NEWCASTLE DISEASE	27
1.2.1 Introduction	27
1.2.2 Aetiology	27
1.2.3 Disease	28
1.2.4 Host range	29
1.2.5 Morphology and genome structure	29
1.2.6 The infection cycle	32
1.2.6.1 Adsorption and penetration	32
1.2.6.2 Transcription and replication	34
1.2.6.3 Virus assembly and release	35
1.2.7 F ₀ , the primary molecular determinant of virulence	35
1.2.8 Other virulence determinants	36
1.2.9 Zoonotic potential of NDV and the use in cancer treatment for humans	37
1.2.10 Genotypes and global epidemiology	38
1.2.10.1 Lineage 1	39
1.2.10.2 Lineage 2	40

1.2.10.3 Lineage 3	40
1.2.10.4 Lineage 4	40
1.2.10.5 Lineage 5	41
1.2.10.6 Lineage 6	41
1.2.11 Vaccination and control	41
1.2.11.1 Live attenuated vaccines: Lentogenic strains	42
1.2.11.2 Live attenuated vaccines: Mesogenic strains	43
1.2.11.3 Inactivated vaccines	44
1.2.11.4 Limitations of vaccination	44
1.2.12 The role of wild birds in the perpetuation and spread of Newcastle disease	45
1.2.13 Transmission and spread	48
1.2.14 The history of Newcastle disease in South Africa	49
1.2.15 The threat of Newcastle disease to sustainable livelihoods	52
1.3 OBJECTIVES OF THE INVESTIGATION	55
CHAPTER TWO: PHYLOGENETIC ANALYSIS OF LPAI H6N2 VIRUSES ISOLATED FROM CHICKEN OUTBEAKS (2001-2005)	57
2.1 INTRODUCTION	58
2.2 MATERIALS AND METHODS	59
2.2.1 Viruses	59
2.2.2 RNA extraction	61
2.2.3 First strand cDNA synthesis	61
2.2.4 PCR	61
2.2.5 DNA sequencing and phylogenetic analysis	64
2.3 RESULTS	65
2.3.1 H6 Hemagglutinin genes	65
2.3.2 Neuraminidase genes	74
2.3.2.1 N8 Neuraminidase genes	74
2.3.2.2 N2 Neuraminidase genes	76
2.3.3 Matrix protein (M) genes	82
2.3.4 Nonstructural protein (NS1) genes	84

2.3.5 Nucleocapsidprotein (NP) genes	87
2.3.6 Polymerase A (PA) genes	89
2.3.7 Polymerase PB1 (PB1) genes	91
2.3.8 Polymerase B2 (PB2) genes	93
2.4 DISCUSSION	98
CHAPTER THREE: PHYLOGENETIC ANALYSIS OF AVIAN INFLUENZA VIRUSES ISOLATED FROM OSTRICHES AND WILD WATERFOWL IN SOUTH AFRICA IN 2004	102
3.1 INTRODUCTION	103
3.2 MATERIALS AND METHODS	106
3.2.1 Viruses	106
3.2.1.1 Ostrich virus	106
3.2.1.2 Wild duck viruses	106
3.2.2 RNA extraction	107
3.2.3 Real-time RT-PCR	107
3.2.4 First strand cDNA synthesis and PCR	109
3.2.5 DNA sequencing and phylogenetic analysis	109
3.3 RESULTS	110
3.3.1 The hemagglutinin genes	110
3.3.1.1 Hemagglutinin (H5) genes	110
3.3.1.1.1 Real time RT-PCR detection of the HPAI H5N2 ostrich H5 gene	110
3.3.1.1.2 Phylogenetic comparison of H5 genes	111
3.3.1.1.3 RT-PCR Detection of an H5 virus co-infection of A/Duck/South Africa/1108/04 (H3N8) and phylogenetic analysis of the partial sequence	119
3.3.1.2 Hemagglutinin (H3) genes	122
3.3.1.3 Hemagglutinin (H4) genes	124
3.3.2 The Neuraminidase genes	126
3.3.2.1 Neuraminidase (N2) genes	126
3.3.2.2 Neuraminidase (N8) genes	131

3.3.2.3 Neuraminidase (N1) genes	133
3.3.3 Matrix protein (M) genes	138
3.3.4 Nonstructural protein (NS1) genes	140
3.3.5 Nucleocapsidprotein (NP) genes	144
3.3.6 Polymerase A (PA) genes	146
3.3.7 Polymerase B1 (PB1) genes	148
3.3.8 Polymerase B2 (PB2) genes	150
3.4 DISCUSSION	152
CHAPTER FOUR: PHYLOGENETIC ANALYSIS OF SOUTH AFRICAN VELOGENIC NEWCASTLE DISEASE OUTBREAK STRAINS OF THE 1990s (GENOTYPES VIII AND VIIIb)	159
4.1 INTRODUCTION	160
4.2 MATERIALS AND METHODS	162
4.2.1 Viruses	162
4.2.2 RNA extraction	162
4.2.3 RT-PCR	162
4.2.4 DNA sequencing and phylogenetic analysis	163
4.3 RESULTS	166
4.3.1 Genotype VIII (Lineage 3d)	166
4.3.2 Genotype VIIIb (Lineage 5b)	169
4.4 DISCUSSION	179
CHAPTER FIVE: PHYLOGENETIC ANALYSIS OF PIGEON PARAMYXOVIRUSES ISOLATED IN SOUTH AFRICA	181
5.1 INTRODUCTION	182
5.2 MATERIALS AND METHODS	185
5.2.1 Viruses	185
5.2.2 RNA extraction	185
5.2.3 RT-PCR	185
5.2.4 DNA Sequencing and phylogenetic analysis	185

5.3 RESULTS	187
5.4 DISCUSSION	194
CHAPTER SIX: THE MOLECULAR EPIDEMIOLOGY OF NDV GENOTYPE 5d/VIIId (“GOOSE PARAMYXOVIRUS”) IN SOUTH AFRICA FROM 1999-2006	196
6.1 INTRODUCTION	197
6.2 MATERIALS AND METHODS	199
6.2.1 Viruses	199
6.2.2 RNA extraction	204
6.2.3 RT-PCR	204
6.2.4 DNA Sequencing and phylogenetic analysis	205
6.2.5 Geographic Information System maps	205
6.3 RESULTS	206
6.3.1 South African lineage 5d strains are closely-related to strains from the Far East	214
6.3.2 The ND outbreak in KwaZulu/Natal from 1999-2000	214
6.3.3 The ND outbreak in KwaZulu/Natal in 2003	214
6.3.4 The rise of the epidemic strain in KwaZulu/Natal	215
6.3.5 The spread of the outbreak throughout South Africa	216
6.3.6 Mapping of specific genetic variants to gain insight into how ND is spread in South Africa	217
6.4 DISCUSSION	220
CHAPTER SEVEN: CONCLUDING REMARKS	223
REFERENCES	235
APPENDICES	276
Appendix 1: Map of the main areas of poultry production in South Africa	276
Appendix 2: GIS maps of the distribution of Lineage 5d in South Africa	277
Appendix 3: Migratory flyways	285
Appendix 4: List of Palaearctic migrant waterfowl that over winter in South Africa	286

LIST OF ABBREVIATIONS

°C	:	degrees Celcius
%	:	percentage
A	:	adenine
AGID	:	agar gel immunodiffusion test
AI	:	avian influenza
AIV	:	avian influenza virus
APMV-1	:	avian paramyxovirus type 1
bp	:	base pair
C	:	cytosine
cRNA	:	complimentary RNA
DIVA	:	differentiating infected from vaccinated animals
e.g.	:	for example
ELISA	:	enzyme-linked immunosorbent assay
EM	:	electron microscope
ER	:	endoplasmic reticulum
EU	:	European Union
F ₀	:	fusion protein precursor
G	:	guanidine
GIS	:	geographic Information System
GPMV	:	goose paramyxovirus
H ₀	:	hemagglutinin protein precursor
HA	:	hemagglutinin protein
HA	:	hemagglutination
HAU	:	hemagglutinating units
HI	:	hemagglutinin inhibition
HN	:	hemagglutinin-neuraminidase protein
HPAI	:	high pathogenic avian influenza
HPNAI	:	high pathogenic notifiable avian influenza
ICPI	:	intracerebral pathogenicity index
IVPI	:	intravenous pathogenicity index
kDA	:	kilodaltons
KZN	:	KwaZulu-Natal province
L	:	large polymerase protein
LPAI	:	low pathogenic avian influenza
LPNAI	:	low pathogenic notifiable avian influenza

M	:	matrix protein
M1	:	matrix protein-1
M2	:	matrix protein-2
MAb	:	monoclonal antibody
MDT	:	mean death time
min	:	minutes
M-MLV	:	mouse Moloney murine leukemia virus
mRNA	:	messenger RNA
NA	:	neuraminidase protein
NASBA	:	nucleic acid sequence-based assay
NDA	:	National Department of Agriculture
NeuAc	:	N-acetylneuraminic acid
NeuGc	:	N-glycolylneuraminic acid
NI	:	neuraminidase inhibition
ND	:	Newcastle disease
NDV	:	Newcastle disease virus
NP	:	nucleoprotein
NS	:	non-structural
nt	:	nucleotide
NWP	:	North-West province
OIE	:	Office International des Epizooties (World animal health organization)
ORF	:	open reading frame
OVI	:	Onderstepoort Veterinary Institute
P	:	phosphoprotein
PA	:	Polymerase A
PB1	:	Polymerase B1
PB2	:	Polymerase B2
PCR	:	polymerase chain reaction
PPMV-1	:	pigeon paramyxovirus type 1
RBS	:	receptor binding site
RNA	:	ribonucleic acid
RNP	:	ribonucleoprotein
RT-PCR	:	reverse transcription polymerase chain reaction
rRT-PCR	:	real-time reverse transcription polymerase chain reaction
SA	:	sialic acid
SAN	:	specific antibody negative

SAPA	:	South African Poultry Association
sec	:	seconds
SPF	:	specific pathogen free
T	:	thymidine
TNF α	:	tumor necrosis factor alpha
UAE	:	United Arab Emirates
UTR	:	un-translated region
VLA	:	Veterinary Laboratory Agency
vRNA	:	viral ribonucleic acid
RNAP	:	viral RNA-dependent RNA polymerase
vRNP	:	viral ribonucleoprotein
WHO	:	World Health Organization

LIST OF FIGURES

Fig. 1.1	Structure of the influenza A virus.	3
Fig. 1.2	Relative sizes of AIV RNA segments.	4
Fig. 1.3	Genomic organization of Newcastle disease virus, with relative gene sizes indicated below.	29
Fig. 1.4	Classification of NDV lineages.	39
Fig. 2.1(a)	Dendogram of H6 type hemagglutinating gene sequences (1315 nt). The tree is rooted with A/duck/Hong Kong/202/77. South African isolates are indicated in boldface, and sub-lineages corresponding to Fig 2.1(a) are indicated (I and II; (a) to (g)).	65
Fig. 2.1(b)	Dendogram of partial H6 type hemagglutinating gene sequences, corresponding to the region between nucleotides 836 and 951 (116 nt) of the viruses presented in Fig 2.1(a). The tree is rooted with A/duck/Hong Kong/202/77. South African isolates are indicated in boldface, and sub-lineages corresponding to Fig 2.1(c) are indicated (I and II; (a) to (g)).	65
Fig. 2.1(c)	Dendogram of partial H6 type hemagglutinating gene sequences, corresponding to the region between nucleotides 836 and 951 (116 nt). The tree is rooted with A/duck/Hong Kong/202/77. South African isolates are indicated in boldface, and sub-lineages are indicated (I and II; (a) to (g)).	66
Fig. 2.2	Multiple amino acid alignment of partial H6-type hemagglutinin genes (residues 273 to 310).	67
Fig. 2.3	Phylogenetic tree inferred from a 1367-nt multiple sequence alignment of the hemagglutinin (H6) genes of South African (in boldface) and other viruses. Sub-lineages A to H are indicated.	69

- Fig. 2.4 Amino acid alignment of full-length H6 genes. The hemagglutinin peptide cleavage site (H₀) at position 339 to 357 is underlined. South African viruses are indicated in boldface. Sub-lineages are indicated in square brackets. 72
- Fig. 2.5 Phylogenetic tree inferred from a 1314-nt multiple sequence alignment of the neuraminidase (N8) genes of *A/Ostrich/South Africa/KK98/98* (H6N8) (in boldface) and other viruses. Sub-lineages A to C are indicated. 75
- Fig. 2.6 Phylogenetic trees inferred from a 1104-nt multiple sequence alignment of the neuraminidase (N2) genes of South African H6N2 (in boldface) and other viruses. Deletions or insertions were excised to simplify the phylogenetic analysis. Sub-lineages A to I are indicated. 76
- Fig. 2.7 Multiple alignment of full-length N2 peptide sequences. Lineages are indicated in square brackets, and deleted amino acids are represented by (-). 78
- Fig. 2.8 Multiple amino acid alignment of the N-stalk region of selected South African H6N2 (in boldface) and reference virus N2 genes. Sub-lineages I and II are indicated in brackets. Deleted amino acids are represented by (-). 80
- Fig. 2.9 Phylogenetic tree inferred from a 797-nt multiple sequence alignment of the matrix (M) protein genes of South African H6N2, H6N8 (in boldface) and other viruses. Sub-lineages A to I are indicated. 82
- Fig. 2.10(a) Phylogenetic tree inferred from a 763-nt multiple sequence alignment of the nonstructural (NS1) protein genes of South African H6N2, H6N8 (in boldface) and other viruses. Sub-lineages A to M are indicated. 84
- Fig. 2.10(b) Radial version of Fig. 2.10(a). South African viruses are indicated by “*”. 85
- Fig. 2.11 Phylogenetic tree inferred from an 863-nt multiple sequence alignment of the nucleoprotein (NP) genes of South African H6N2, H6N8 (in boldface) and other viruses. Sub-lineages A to I are indicated alongside. 87

Fig. 2.12	Phylogenetic tree inferred from a 735-nucleotide multiple sequence alignment of the polymerase A (PA) genes of South African H6N2, H6N8 (in boldface; *) and other viruses. Sub-lineages A to I are indicated.	89
Fig. 2.13(a)	Phylogenetic tree inferred from a 668-nt multiple sequence alignment of the polymerase B1 (PB1) genes of South African H6N2, H6N8 (in boldface) and other viruses. Sub-lineages A to I are indicated.	91
Fig. 2.13(b)	Radial version of Fig. 2.13(a). South African viruses are indicated by “*”.	92
Fig. 2.14	Phylogenetic tree inferred from a 738-nt multiple sequence alignment of the Polymerase B2 (PB2) genes of South African H6N2, H6N8 (in boldface) and other viruses. Sub-lineages A to I are indicated.	94
Fig. 2.15	Schematic representation of the origins of genes of South African H6N2 viruses	95
Fig. 3.1(a)	rRT-PCR amplification curve for A/Ostrich/South Africa/N227/04(H5N2) partial HA sequence (red). dH2O (blue) represents the negative water control. Black and pink indicate a failed positive control. Two oligonucleotide sets were tested: “My primers” represents the H5-specific oligonucleotides of Lee <i>et al</i> (2001) whereas “Amanda’s primers” are the H5HA1/HA2 oligonucleotide pair (Starick <i>et al</i> , 2002).	110
Fig. 3.1(b)	Melting curve for A/Ostrich/South Africa/N227/04(H5N2) partial HA sequence	111
Fig. 3.2	Phylogenetic tree inferred from a 1338-nt multiple sequence alignment of the HA (H5) genes of the outbreak strain A/Ostrich/South Africa/N227/05 (H5N2) and A/Wild duck/South Africa/811/04 (H5N1) (in boldface) and related sequences. Sub-lineages A to D are indicated	112
Fig. 3.3	Multiple H5 HA amino acid alignment. The South African viruses are indicated in boldface, and the H0 cleavage site is underlined. Sub-lineages are indicated in square brackets.	113

Fig. 3.4	Phylogenetic tree inferred from a 373-nt multiple sequence alignment of the H5 hemagglutinin genes including that of A/Egyptian Goose/AI23/04 (H5N2) (in boldface). Sub-lineages A to D are indicated, corresponding to Fig 3.2.	117
Fig. 3.5	Multiple amino acid sequence alignment of partial H5 gene sequences including the H ₀ cleavage site (underlined).	118
Fig. 3.6	Phylogenetic trees inferred from a 308-nt multiple sequence alignment of a partial H5-type virus detected by RT-PCR in A/Duck/South Africa/1108/04 (H3N8) (in boldface) and related sequences. Sub-lineages A to E are indicated.	120
Fig. 3.7	Partial peptide sequence alignment of North American lineage H5 genes and the South African sequence (boldface) including the H ₀ cleavage site (underlined).	121
Fig. 3.8	Phylogenetic tree inferred from a 1104-nt multiple sequence alignment of the H3 genes of A/Duck/South Africa/1108/04 (H3N8) (in boldface) and related sequences. Sub-lineages A to I are indicated.	122
Fig. 3.9	Phylogenetic trees inferred from a 459-nt multiple sequence alignment of the H4 genes of A/Duck/South Africa/1233/04 (H4N8) (in boldface) and related sequences. Sub-lineages A, B and C are indicated.	124
Fig. 3.10	Phylogenetic tree inferred from a 1073-nt multiple sequence alignment of the NA (N2) genes of the outbreak strain A/Ostrich/South Africa/N227/05 (H5N2), A/Egyptian Goose/AI23/04 (H5N2) (both in boldface) and related viruses. Sub-lineages A to K are indicated	126
Fig. 3.11	Multiple amino acid alignment of N2 genes. South African strains are sequenced in this study are indicated in boldface.	128
Fig. 3.12	Phylogenetic tree inferred from a 1305-nt multiple sequence alignment of the N8 genes of A/Duck/South Africa/1233/04 (H4N8) and A/Duck/South Africa/1108/04 (H3N8) (in boldface) and related sequences. Sub-lineages A to	131

D are indicated.

- Fig. 3.13 Phylogenetic trees inferred from a 1025-nucleotide multiple sequence alignment of the N1 genes of A/Duck/South Africa/811/04 (H5N1) (in boldface) and related sequences. Sub-lineages A to F are indicated. 133
- Fig. 3.14 Multiple amino acid sequence alignment of neuraminidase (N1 genes). Sub-lineages are indicated in square brackets. 135
- Fig. 3.15 Phylogenetic tree inferred from a 674-nt multiple sequence alignment of the M genes of South African AI viruses isolated in 2004 (in boldface) and related sequences. Sub-lineages A to F are indicated. 138
- Fig. 3.16 Phylogenetic tree inferred from a 675-nt multiple sequence alignment of the NS1 genes of South African AI viruses isolated in 2004 (in boldface) and related sequences. Alleles A and B and sub-lineages A to I are indicated. 140
- Fig. 3.17 Multiple amino acid alignment of the NS1 protein genes of the South African H5N2 viruses and related genes. The position of the 2004 Thai HPAI H5N1 viruses' NS1 deletion is underlined. 142
- Fig. 3.18 Phylogenetic tree inferred from a 776-nt multiple sequence alignment of the NP genes of A/Ostrich/South Africa/N227/04 (H5N2), A/Duck/South Africa/811/04 (H5N1), A/Duck/South Africa/1108/04 (H3N8) and A/Duck/South Africa/1233/04 (H4N8) (in boldface) and related sequences. Sub-lineages A to F are indicated. 144
- Fig. 3.19 Phylogenetic tree inferred from a 695-nt multiple sequence alignment of the PA genes of A/Ostrich/South Africa/N227/04 (H5N2), A/Duck/South Africa/811/04 (H5N1), A/Duck/South Africa/1108/04 (H3N8) and A/Duck/South Africa/1233/04 (H4N8) (in boldface) and related sequences. Sub-lineages A to J are indicated. 146
- Fig. 3.20 Phylogenetic tree inferred from a 753-nt multiple sequence alignment of the 148

PB1 genes of A/Ostrich/South Africa/N227/04 (H5N2), A/Duck/South Africa/811/04 (H5N1), A/Duck/South Africa/1108/04 (H3N8) and A/Duck/South Africa/1233/04 (H4N8) (in boldface) and related sequences. Sub-lineages A to D are indicated.

- Fig. 3.21 Phylogenetic trees inferred from a 726-nt multiple sequence alignment of the PB2 genes of A/Ostrich/South Africa/N227/04 (H5N2), A/Duck/South Africa/811/04 (H5N1), A/Duck/South Africa/1108/04 (H3N8) and A/Duck/South Africa/1233/04 (H4N8) (in boldface) and related sequences. Sub-lineages A to J are indicated. 150
- Fig. 3.22 Pairwise amino acid alignment of a full-length PB2 gene with A/Ostrich/South Africa/N227/04 (H5N2) PB2. The amino acid at position 627 (boxed) is a glutamate (E) residue. 151
- Fig. 3.23 Reassortment between South African AIVs isolated in 2004. 155
- Fig. 4.1 Dendrogram of a 374 nt 3'-end region of the fusion proteins of South African genotype VIII (lineage 3d) viruses. Viruses whose fusion protein sequences were determined in this chapter are indicated in boldface. 166
- Fig. 4.2 Multiple amino acid alignment of partial fusion (F) protein genes of genotype VIII viruses, including the F₀ cleavage site (underlined). Viruses whose nucleic acid sequences were determined in this study are indicated in boldface. 157
- Fig. 4.3(a) Phylogenetic tree of genotype VIIIb isolates. South African viruses whose nucleic acid sequences were determined in this study are indicated in boldface. Ostrich isolates are indicated by arrows. Portuguese and Spanish viruses are boxed. Enlargements of views (a) and (b) are presented in Figs. 4.3(b) and 4.3(c) respectively. 169
- Fig. 4.3(b) Enlargement of view (a) 170

Fig. 4.3(c)	Figure 4.3(c). Enlargement of view (b)	171
Fig. 4.4	Multiple amino acid alignment of partial fusion (F) protein genes of genotype VIIb viruses, including the F ₀ cleavage site (underlined). Reference sequences retrieved from Genbank are indicated in boldface.	172
Fig. 4.5	Multiple amino acid alignment of partial fusion (F) protein genes of genotype VIIb viruses, including the F ₀ cleavage site (underlined). Viruses whose nucleic acid sequences were determined in this chapter are indicated in boldface.	174
Fig. 5.1(a)	Dendrogram inferred from the 374 bp region of the 3' end of the fusion protein (F) gene of PPMV-1 strains isolated in South Africa (boldface) and strains from Genbank. Enlargements of the regions representing subgroups 4bi and 4bii are presented separately in Figs 5.1(b) and 5.1(c) respectively.	187
Fig. 5.1(b)	Enlargement of Fig 5.1(a) depicting Subgroup 4bi phylogeny	188
Fig. 5.1(c)	Enlargement of Fig 5.1(a) depicting Subgroup 4bii phylogeny	189
Fig. 5.2	Multiple amino acid alignment of the translated 374-nt region of the fusion (F) protein of South African PPMV-1 strains (underlined) and other viruses, including the F ₀ cleavage site (underlined).	190
Figure 5.3	Nucleotide sequence alignment of variable sites within the 374 nt region of the 3' end of the fusion (F) protein. The South African isolates (in boldface) are compared with selected representatives of sub-lineages A to I	191
Fig. 6.1(a)	Phylogenetic tree based on nucleotide sequences of South African lineage 5d viruses (in boldface) and reference strains. The tree is rooted with LaSota/46 virus. Sub-lineages (a) to (o) are indicated. Enlargements of Subgroups I and II are presented in Figs. 6.1(c) and 6.1(d) respectively.	206
Fig. 6.1(b)	Radial (unrooted) version of Fig. 6.1(a)	207

Fig. 6.1(c)	Enlargement of Fig 6.1(a) depicting Subgroup I phylogeny	208
Fig. 6.1(d)	Enlargement of Fig 6.1(a) depicting Subgroup II phylogeny	209
Fig. 6.2	Multiple nucleotide sequence alignment of variable sites only within the fusion protein gene (nt 61-374) of selected South African (in boldface) and other lineage 5d strains. Sub-lineages (a) to (o) are indicated in square brackets.	210
Fig. 6.3	Multiple amino acid alignment (residues 21 to 124 of the fusion protein) of South African (in boldface) and other lineage 5d strains. The F ₀ cleavage site is underlined.	212
Fig. 6.4	Prevalence of NDV GPMV isolates collected from September 2004 to May 2006	213

LIST OF TABLES

Table 2.1	South African LPAI H6N2 and H6N8 viruses isolated from 1998 to 2005	60
Table 2.2	Thermal cycling conditions used to amplify AIV genes	62
Table 2.3	Oligonucleotide primers used to amplify and sequence South African AIV isolates	63
Table 2.4 (a-c)	Percent nucleotide identities between genes	96
Table 3.1	Viruses isolated/detected in South Africa during winter of 2004	107
Table 3.2	LightCycler experimental protocol to amplify the HPAI H5N2 HA gene	108
Table 4.1	Velogenic South African ND viruses characterised in this study	164
Table 5.1	South African Pigeon Paramyxovirus isolates analysed in this study	186
Table 6.1	Virulent ND viruses isolated in South Africa from 1999-2006 (n=257)	199