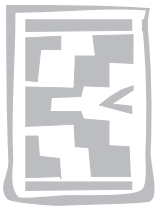
Onderstepoort Journal of Veterinary Research, 70:157–160 (2004)



RESEARCH COMMUNICATION

Characterization of a pigeon paramyxovirus (PPMV-1) isolated from chickens in South Africa

C. ABOLNIK[1], R.F. HORNER[2], R. MAHARAJ[2] and G.J. VILJOEN[1]

ABSTRACT

ABOLNIK, C., HORNER, R.F., MAHARAJ, R. & VILJOEN, G.J. 2004. Characterization of a pigeon paramyxovirus (PPMV-1) isolated from chickens in South Africa. *Onderstepoort Journal of Veterinary Research,* 71:157–160

A paramyxovirus with a thermostability of 60 min (typical of velogenic viruses) and a mean death time of > 90 h (typical of lentogenic viruses) was isolated from layers near Mooi River, South Africa. Our results, based on comparative nucleotide sequence data indicated that the virus is pigeon paramyxovirus 1 (PPMV-1), a variant of Newcastle disease virus. The F_0 cleavage site contains a ^{112}RRKKRF^{117} motif, and the virus had 98 % sequence identity with PPMV-1 strains from the Far East. PPMV-1 was last reported in South Africa during the 1980s, with this being the first report of PPMV-1 isolated from chickens in South Africa.

Keywords: Chickens, Newcastle disease virus, nucleotide sequence data, phylogenetics, pigeon paramyxovirus

INTRODUCTION

Newcastle disease virus (NDV), or avian paramyxovirus-1, is a member of the *Avulavirus* genus in the family Paramyxoviridae (Van Regenmortel, Fauquet, Bishop, Carsten & Maniloff 2000). It is classified as a list A disease by the Office Internationale des Epizooties (OIE) because it is highly contagious and causes severe disease and high mortalities in susceptible birds. A pandemic caused by the pigeon variant of avian paramyxovirus-1 (PPMV-1) arose in the late 1970s, and reached Europe by 1981 before spreading worldwide (Collins, Strong & Alexander 1994; Alexander 1998), including South Africa in 1986 (Pienaar & Cilliers 1987).

Despite vaccination, PPMV-1 is still enzootic in pigeons in some countries (Alexander 2001). In recent years, Europe has been experiencing an epidemic of Newcastle disease (ND) caused by PPMV-1 in feral doves and pigeons (Alexander, Manvell & Frost 1998, 1999; Capua & Cancelloti 1999; Terregino, Cattoli, Grossele, Bertoli, Tisato & Capua 2003). In Japan, PPMV-1 has been recorded regularly since 1984 (Mase, Imai, Sanada, Yuasa, Imada, Tsukamoto & Yamaguchi 2002). PPMV-1 represents a threat to poultry production, although PPMV-1 infection of poultry is not as severe as an infection with a velogenic virus strain (Werner, Römer-Oberdörfer, Köllner, Manvell & Alexander 1999). Several studies have demonstrated an increase in virulence after sequential passages of some PPMV-1 isolates in chickens (Alexander & Parsons 1984, 1986; Kissi 1988; Alexander 1997, 1998; Kommers, King, Seal & Brown 2001, 2003). Here we report on the first isolation and characterization of a PPMV-1 virus from chickens in South Africa.

[1] Biotechnology Division, Onderstepoort Veterinary Institute, Private Bag X05, Pretoria, 0110, South Africa

[2] Allerton Provincial Veterinary Laboratory, Pietermaritzburg

Accepted for publication 11 November 2003—Editor

MATERIALS AND METHODS

Virus characterization

South African isolate ZA469/PPMV1/02 was isolated from 28-week-old layers with symptoms of moderate mucoid tracheitis from the Mooi River area in KwaZulu-Natal Province, South Africa in December 2002. Pooled tissues of trachea and caecal tonsils were prepared and inoculated into the allantoic cavity of 9–11 day-old embryonated chicken eggs. Mean death time (MDT) tests were performed as described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (2000).

RNA extraction and RT-PCR

Viral RNA was extracted from allantoic fluid using TRIzol® reagent (Gibco, Invitrogen), according to the manufacturer's instructions. Random hexamers were used to generate first strand cDNA according to the method described by Sambrook, Fritsch & Maniatis (1989). The oligonucleotide primers mentioned below were used to amplify an 1 180 base pair fragment spanning the regions between nucleotides 581 of the fusion protein and nucleotides 610 of the matrix protein, which includes the F_0 cleavage site. Reaction mixtures were subjected to 35 cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min.

M610 5'- CTG TAC AAT CTT GCG CTC AAT GTC -3' (forward primer)

NDVF581 5'- CTG CCA CTG CTA GTT GTG ATA ATC C -3' (reverse primer)

Sequencing and phylogenetic analysis

DNA was sequenced using the ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer's instructions, in an ABI377™ automated sequencer.

A 374 nucleotide (nt) fragment of the fusion protein gene, including the F_0 cleavage site was aligned using GCG Seqlab (Wisconsin package version 10.1-UNIX, Genetics Computer Group, Inc). Phylogenetic trees were drawn with the DNAML program from the PHYLIP software package (version 3.4) (Feselstein 1991).

RESULTS AND DISCUSSION

The thermostability assay of the virus that was isolated gave a value of 60 min, which is typical for velogenic ND viruses. This was in contrast to the value obtained (> 90 h which is typical for lentogenic viruses). RT-PCR was performed on the viral

TABLE 1 Viruses used in the phylogentic analysis and comparison of F_0 cleavage site sequences

Isolate/ year of isolation	Country	Amino acid sequence at F_0 cleavage site[a] 111 120	Genotype	Accession number
Tochigi/95	Japan	V R R **K** K R F I G A	VIc	AB070419
Utsonomiya/95	Japan	V R R **K** K R F I G A	VIc	AB070420
Shiga/96	Japan	V R R **K** K R F I G A	VIc	AB070422
Fukushima/96	Japan	V R R **K** K R F I G A	VIc	AB070423
Saitama/97	Japan	V R R **K** K R F I G A	VIc	AB070426
ZA469/PPMV1/02	**South Africa**	V R R **K** K R F I G A	VIc	AY445669
2736/00	Italy	V R R **K** K R F I G A	VIc	AF520965
177/01	Italy	V R R **K** K R F I G A	VIc	AF520971
Gunma/2000	Japan	A R R **K** K R F I G A	VIc	AB070434
Ch/98-1	China	E K R Q K R F I G A	VIb	AF358785
Js/2/98/Go	China	E K R Q K R F I G A	VIc	AF456439
GB 1168/84	Britain	G G R Q K R F I G A	VIb	AF109885
1811/00	Italy	G G R Q K R F I G A	VIb	AF520969
907/00	Italy	G G R Q K R F I G A	VIb	AF520966
1444/00	Italy	G G R Q K R F I G A	VIb	AF520968
99299	France	G R R Q K R F I G A	VIc	AJ306304
99106	France	G R R Q K R F I G A	VIc	AJ306305
1166/00	Italy	G R R Q K R F I G A	VIc	AF520967
2874a/00	Italy	G R R Q K R F I G A	VIc	AF520972
4400/00	Italy	R G R Q K R F I G A	VIb	AF520970
ASTR/74	Russia	G R R Q K R F I G A	VIa	Y19012

[a] Residues 112–117 form the F_0 cleavage site; the unusual 114K for Q substitution is printed in bold

RNA using NDV-specific oligonucleotides, and the F_0 cleavage site was sequenced in order to determine the pathotype. The partial nucleotide sequence of the F gene and amino acid sequence at F_0 cleavage site was typical of PPMV-1. Phylogenetic analysis indicated that, although similar to recent European PPMV-1 isolates, the South African isolate is most closely related to Japanese strains of pigeon paramyxoviruses isolated in the late 1990s (at 98 % nucleotide sequence homology). The phylogenetic relationships furthermore suggest that currently circulating Italian strains 117/01 and 2736/00 could share a common origin with the strains from the Far East. In a recent epidemiological study of 155 NDV isolates isolated in South Africa over the past 12 years, no evidence of PPMV-1 was found. PPMV-1, however, was present in South Africa during the 1980s (Pienaar & Cilliers 1987). Prolonged absence of reported ND in local doves and pigeons, together with the close sequence similarity with Japanese isolates, suggests that ZA469/PPMV1/02 is a recent introduction into South Africa. This is in agreement with the results of the aforementioned epidemiological survey, in which outbreaks of ND in South Africa are linked to NDV strains circulating in the Far East (Abolnik, Horner, Bisschop, Parker, Romito & Viljoen 2004). The routes of transmission are speculative (e.g. via migratory water birds nesting in

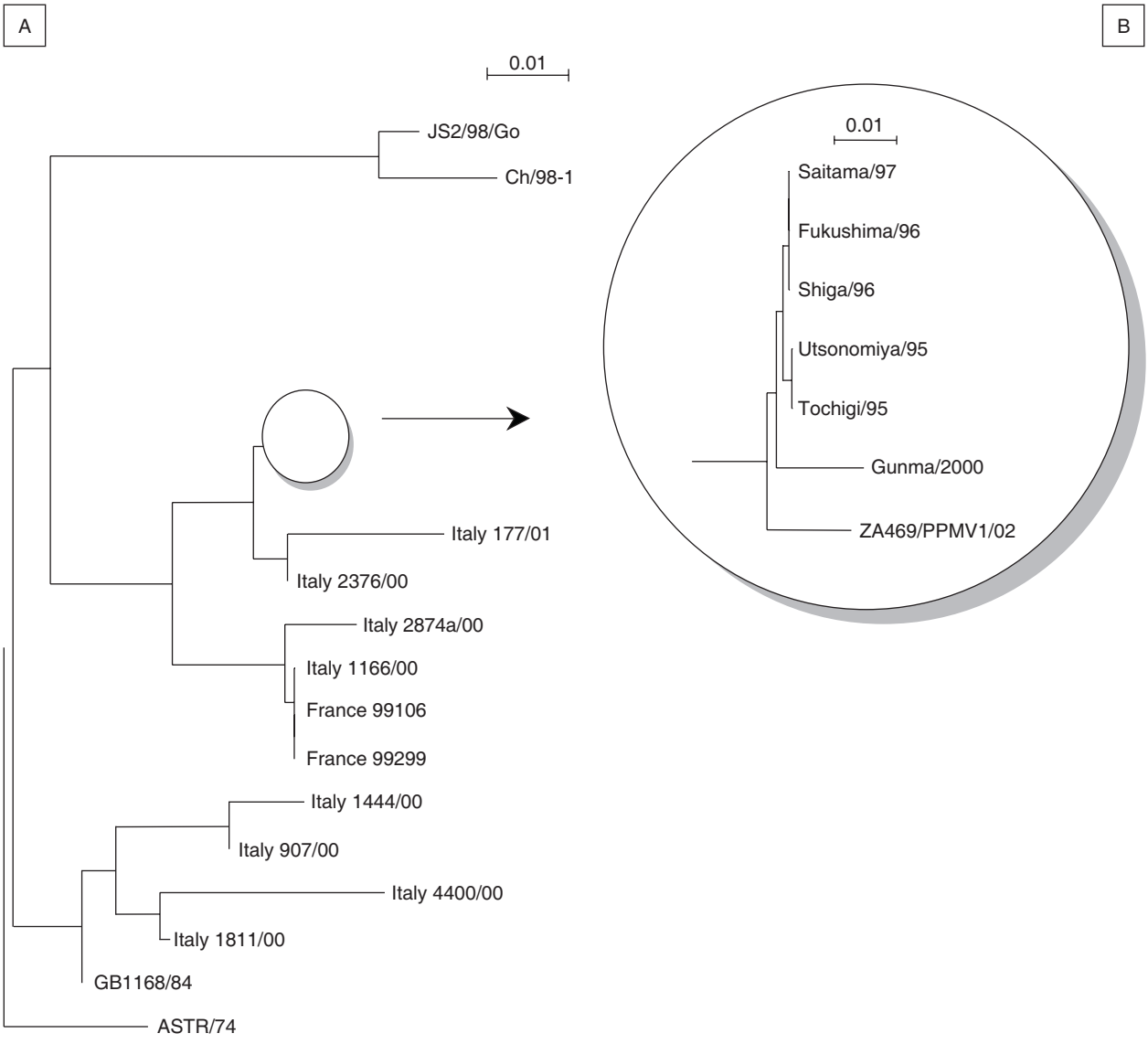


FIG. 1 Phylogenetic relationships of PPMV-1 isolates in Table 1 based on (A) nucleotides 269 to 374 (106 bp) of the F protein gene and (B) nucleotides 1 to 374 of the F protein gene, with insert showing higher resolution of the phylogenetic relationship of some of these viruses

Siberia, which mingle there with others migrating from the Far East). The isolation of a pigeon paramyxovirus from chickens with the potential of causing disease is nevertheless a further motivation for highlighting the importance of vaccinating poultry and domestic racing pigeons against ND.

ACKNOWLEDGEMENTS

We thank Dr M. Romito for his critical reading of the manuscript.

REFERENCES

ABOLNIK, C., HORNER, R.F., BISSCHOP, S.P.R., PARKER, M.E., ROMITO, R. & VILJOEN, G.J. 2004. A phylogenetic study of South African Newcastle disease virus strains isolated between 1990 to 2002 suggests epidemiological origins in the Far East. *Archives of Virology,* 149:603–619.

ALEXANDER, D. & PARSONS, G. 1984. Avian paramyxovirus type 1 infections of racing pigeons (2). Pathogenicity experiments in pigeons and chickens. *The Veterinary Record*, 114: 466–469.

ALEXANDER, D. & PARSONS, G. 1986. Pathogenicity for chickens of avian *Paramyxovirus* type 1 isolates obtained from pigeons in Great Britain during 1983–85. *Avian Pathology*, 15:487–493.

ALEXANDER, D.J. 1997. Newcastle disease and other avian paramyxoviridae infections, in *Diseases of poultry*, edited by B.W. Calneck, H.J. Barnes, L.R. McDougall, Y.M. Saif & C.W. Beard, 10th ed. Ames: Iowa State University Press.

ALEXANDER, D.J. 1998. Newcastle disease virus and other paramyxoviruses, in *A laboratory manual for the isolation and identification of avian pathogens*, edited by D.E. Swayne, J.R. Glisson, M.W. Jackwood, J.E. Pearson & M.W. Reed, 4th ed. Kennet Square, New York: American Association of Avian Pathologists.

ALEXANDER, D.J., MANVELL, R.J. & FROST, K.M. 1998. Report of the European Union Reference Laboratories for Avian Influenza and Newcastle Disease 1998. *Proceedings of the Joint Fifth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 9–10 November 1998, Vienna*: 61–66.

ALEXANDER, D.J, MANVELL, R.J. & FROST, K.M. 1999. Report of the European Union Reference Laboratories for Avian Influenza and Newcastle Disease 1999. *Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 29–30 November 1999, Brussels*: 72–77.

ALEXANDER, D.J. 2001. Gordon Memorial Lecture. Newcastle disease. *British Poultry Science*, 42:5–22.

CAPUA, I. & CANCELLOTI, E.M. 1999. Newcastle disease: situation in Italy during 1998 and 1999. *Proceedings of the Joint Sixth Annual Meetings of the National Newcastle Disease and Avian Influenza Laboratories of Countries of the European Union, 29–30 November 1999, Brussels*: 30–31.

COLLINS, M.S., STRONG, I. & ALEXANDER, D.J. 1994. Evaluation of the molecular basis of pathogenicity of the variant Newcastle disease viruses termed 'pigeon PMV-1 viruses'. *Archives of Virology*, 134:403–411.

FESELSTEIN, J. 1991. *PHYLIP manual*, version 3.4. University Herbarium. Berkeley: University of California.

KISSI, B. 1988. Studies on the virulence of pigeon paramyxovirus-1 (PMV-1). I. Changes in the virulence of pigeon PMV-I strains isolates in Hungary upon passage in chickens, embryonated hen's eggs and pigeons. *Acta Veterinaria Hungarica*, 36:238–292.

KOMMERS, G.D., KING, D.J., SEAL, B.S. & BROWN C.C. 2001. Virulence of pigeon-origin isolates of Newcastle disease for domestic chickens. *Avian Diseases*, 45:906–921.

KOMMERS, G.D., KING, D.J., SEAL, B.S. & BROWN, C.C. 2003. Virulence of six heterogeneous-origin Newcastle disease virus isolates before and after sequential passages in domestic chickens. *Avian Pathology*, 32:81–93.

MASE, M., IMAI, K., SANADA, Y., SANADA, N., YUASA, N., IMADA, T., TSUKAMOTO, K. & YAMAGUCHI, S. 2002. Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. *Journal of Clinical Microbiology*, 40:3826–3830.

OFFICE INTERNATIONAL DES EPIZOOTIES. 2000. *Manual of standards for diagnostic tests and vaccines*: 221–232.

PIENAAR, A.C.E. & CILLIERS, J.A. 1987. The isolation of a paramyxovirus from pigeons in South Africa. *Onderstepoort Journal of Veterinary Research*, 54:653–654.

SAMBROOK, J., FRITSCH, E.F. & MANIATIS, T. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

TERREGINO, C., CATTOLI, G., GROSSELE, B., BERTOLI, E., TISATO, E. & CAPUA, I. 2003. Characterization of Newcastle disease virus isolates obtained from Eurasian collared doves (*Streptopelia decaocto*) in Italy. *Avian Pathology*, 32: 63–68.

VAN REGENMORTEL, M.H., FAUQUET, C.M., BISHOP, D.H.L., CARSTEN, E.B. & MANILOFF, J. (Eds). 2000. Seventh report in virus taxonomy. *Reports of the international Committee on Taxonomy of Viruses*. New York: Academic Press.

WERNER, O., RÖMER-OBERDÖRFER, A., KÖLLNER, B., MANVELL, R.J. & ALEXANDER, D.J. 1999. Characterization of avian paramyxovirus type 1 strains isolated in Germany during 1992 to 1996. *Avian Pathology*, 28:79–88.