DOI: 10.7589/2015-07-199 *Journal of Wildlife Diseases*, 52(3), 2016, pp. 599–608
© Wildlife Disease Association 2016

COMPLETE GENOMIC SEQUENCE OF VIRULENT PIGEON PARAMYXOVIRUS IN LAUGHING DOVES (*STREPTOPELIA SENEGALENSIS*) IN KENYA

Vincent Obanda,[1,9] George Michuki,[2] Michael J. Jowers,[3,4] Cecilia Rumberia,[2] Mathew Mutinda,[1] Olivia Wesula Lwande,[5] Kihara Wangoru,[2] Jacquiline Kasiiti-Orengo,[6] Moses Yongo,[1] and Samer Angelone-Alasaad[7,8,9]

[1] Department of Veterinary Services, Kenya Wildlife Service, PO Box 40241-00100, Nairobi, Kenya
[2] International Livestock Research Institute, PO Box 30709-00100, Nairobi, Kenya
[3] CIBIO/InBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos), Universidade do Porto, Campus Agrario De Vairão, 4485-661 Vairão, Portugal
[4] National Institute of Ecology, 1210, Geumgang-ro, Maseo-myeon, Seocheon-gun, Chungcheongnam-do 33657, Korea
[5] Department of Clinical Microbiology, Division of Virology, Umeå University, SE-901 85 Umea, Sweden
[6] Department of Veterinary Services, Ministry of Livestock Development, Private Bag 00625, Kangemi, Nairobi, Kenya
[7] Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas (CSIC), C. Americo Vespucio s/n, 41092 Sevilla, Spain
[8] Institute of Evolutionary Biology and Environmental Studies (IEU), University of Zurich, Switzerland, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[9] Corresponding authors (email: vobanda@kws.go.ke; sameralasaad@hotmail.com)

ABSTRACT: Following mass deaths of Laughing Doves (*Streptopelia senegalensis*) in different localities throughout Kenya, internal organs obtained during necropsy of two moribund birds were sampled and analyzed by next generation sequencing. We isolated the virulent strain of pigeon paramyxovirus type-1 (PPMV-1), PPMV1/Laughing Dove/Kenya/Isiolo/B2/2012, which had a characteristic fusion gene motif ^{110}GGRRQKRF^{117}. We obtained a partial full genome of 15,114 nucleotides. The phylogenetic relationship based on the fusion gene and genomic sequence grouped our isolate as class II genotype VI, a group of viruses commonly isolated from wild birds but potentially lethal to Chickens (*Gallus gallus domesticus*). The fusion gene isolate clustered with PPMV-I strains from pigeons (Columbidae) in Nigeria. The complete genome showed a basal and highly divergent lineage to American, European, and Asian strains, indicating a divergent evolutionary pathway. The isolated strain is highly virulent and apparently species-specific to Laughing Doves in Kenya. Risk of transmission of such a strain to poultry is potentially high whereas the cyclic epizootic in doves is a threat to conservation of wild Columbidae in Kenya.

Key words: Epizootics, genotype, Newcastle disease, pigeon paramyxyovirus, poultry, vaccine, wild birds.

INTRODUCTION

The *Paramyxoviridae* is a composite family of multiple human and animal viruses that cause severe diseases including measles, mumps, and respiratory tract infections (Hines and Miller 2012). The genomic structure of these viruses is complex, consisting of single-stranded RNA of 13–19 kb and containing 6–10 genes that encode up to 12 proteins (Saif et al. 2008). Some of the proteins include the matrix (M), fusion (F), phosphoprotein (P), nucleocapsid (NP), hemagglutinin-neuraminidase (HN), and RNA-dependent RNA polymerase (L) (Hines and Miller 2012). Virulent strains of avian paramyxovirus serotype 1 (also called Newcastle disease virus [NDV]), a member of the *Paramyxoviridae*, is the causal organism of Newcastle disease (Mayo 2002). The full genome of NDV is about 15.2 kb and is classified into three sequence types, with lengths of 15,198 nucleotides belonging to class I (one genotype) while class II (18 genotypes) has nucleotide lengths of 15,192 and 15,186 (Courtney et al. 2013). Nearly all orders of avian species are susceptible to NDV (Lomniczi et al. 1998) infection and, depending on the strain virulence, it causes diverse clinical symptoms that lead to variable rates of morbidity and mortality. Virulent strains of NDV are enzootic in many countries and responsible for huge economic losses in the poultry industry, especially in growing economies (Xiao et al. 2012). Outbreaks of Newcastle

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

disease in African poultry are common, but genomic details of the causal viruses remain mostly unknown. Recently, novel NDV strains have been detected in West Africa (de Almeida et al. 2013) as well as other parts of Africa (Byarugaba et al. 2014), which suggests that Africa may have more genetically diverse strains within and among local NDV populations (Samuel et al. 2013). Since 1926, virulent strains of NDV have caused devastating panzootics, taking place in waves, with the third and fourth wave still ongoing and the associated strains rapidly evolving (Diel et al. 2012). Recently, Miller et al. (2015) suggested an emergence of the fifth panzootic wave, which resulted from coevolution of NDV strains of subgenotype VIIi and VIIh. The third panzootic, which began in the 1980s, was caused by a pigeon-adapted variant of NDV (Saif et al. 2008) that is commonly known as the pigeon paramyxovirus type-1 (PPMV-1). This group of strains, originally isolated in feral, domestic, and wild pigeons as well as in doves, all from the family Columbidae (Ujvári et al. 2003), is currently a global threat because of its expanded geographic occurrence coupled with a host range that is increasingly diverse and includes non-Columbidae such as waterfowl (Anseriformes), robins (Turdidae), partridges (Phasianidae), and domestic fowl (Phasianidae, *Gallus* spp.) (Jindal et al. 2009; Vidanovic et al. 2011). The strain is responsible for the growing number of outbreaks in chickens in a variety of locations (Alexander 2011). In Kenya, PPMV-1 has not been associated with epidemics in poultry.

Mass deaths of wild birds in the order Columbiformes have occurred annually in several parts of Kenya, with NDV associated with the deaths (Kenya Wildlife Service unpubl.). However, little information is known on the genotype, pathotype, or genomic information of the causal strains in Kenya.

MATERIALS AND METHODS

Sampling area and sample collection

Die-offs of Laughing Doves (*Streptopelia senegalensis*) have occurred since 2006 in several locations, mainly in the semiarid and arid regions of Kenya (Fig. 1). The locations are inhabited by both agro-pastoralists and nomadic-pastoralist communities who mainly keep cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra aegagrus hircus*). The regions are also home to a rich diversity of wildlife, particularly hosting the largest populations of Kenya's African elephants (*Loxodonta africana*). Although these sites are in the dry regions of Kenya (Fig. 1), permanent bodies of water are common. For example, the Tsavo River traverses the Tsavo National Park while natural springs provide permanent water for animals in the Amboseli ecosystem. Kinna, a community area adjacent to the Bisanadi National Reserve, has a rich network of permanent rivers and streams. No other wildlife species were found moribund or dead in the die-off sites. All these die-off sites had a common characteristic in that they were around stagnant pools or slow-flowing water that appeared greenish due to algal growth. Moribund Laughing Doves and their carcasses were scattered in water perimeters at lengths ranging from 1–40 m from the river/stream banks. The moribund Laughing Doves were on the ground or perched on low canopy vegetation. We observed Laughing Doves flying in and drinking water, and thereafter we saw them lose flight capacity and progress to being listless and drowsy. By the time we responded to these outbreaks in Milgis, Melako, Kina, and Biliqo Bulesa (Fig. 1), 3–5 wk after the initial outbreak reports, we found the sites littered with carcasses and some Laughing Doves that were displaying neurologic disorders. Die-offs occurred in March, May, and August–November of 2012. Because the die-off occurred in remote regions, viable samples were collected from only one site at Biliqo Bulesa, Merti Division, Isiolo County (0°32′41″N; 38°24′51″E). At this site a troop of olive baboons (*Papio anubis*) was found feeding, capturing the moribund birds and feeding on them. In an estimated area of 200 × 35 m, we collected and counted 977 carcasses, which we burned on site to sanitize the area. We captured 12 moribund Laughing Doves by hand and weighed and examined them for overall body condition and presence of ectoparasites. An inadequate quantity of dry ice at the time prevented us from transporting all 12 Laughing Doves after their death. Nevertheless, we collected two of the dead doves, preserved their whole carcass in dry ice, and airlifted them to the Veterinary Laboratory at the Kenya Wildlife Service in Nairobi. The two birds were necropsied and portions of the intestines, liver, spleen, lung, heart, brain, and proventriculus were excised, placed in separate labeled cryovials, and immediately frozen in liquid nitrogen. The organ samples were then transported in liquid nitrogen to the Animal Bioscience

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

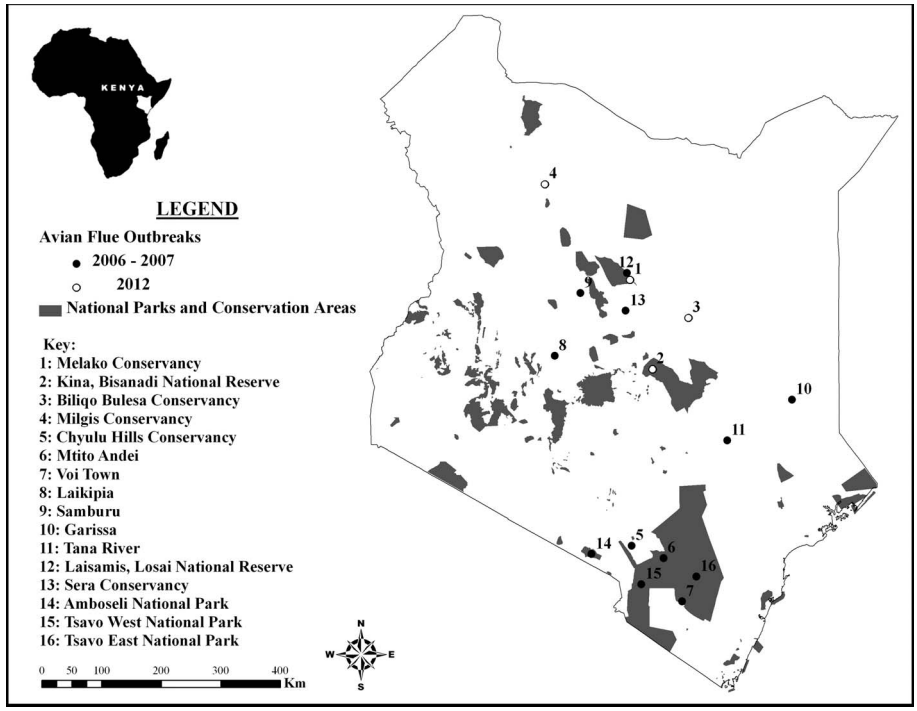

FIGURE 1. Sites of mass deaths of wild doves in Kenya (2006, 2007, and 2012).

Laboratory at the International Livestock Research Institute in Nairobi for analysis.

RNA isolation

We isolated RNA using the Roche MagNA Pure LC instrument (Roche, Mannheim, Germany) with the MagNA Pure LC RNA isolation tissue kit III (Roche). The analyzed tissue samples from the two Laughing Doves were from the intestines, liver, spleen, lung, heart, brain, and proventriculus. A pea-size tissue sample was placed in a 2-mL screw-cap tube and 400 μL of lysis buffer from the MagNA Pure LC RNA isolation kit III was added to the tube. Silicon beads (1-mm) were added to the tube covering the tissue and were crushed for 1 min using a bead beater. The tube was centrifuged for 1 min at 10,621 × G using a refrigerated bench-top centrifuge (Eppendorf 5417R, New York, New York, USA). We carefully withdrew 200 μL of supernatant without unsettling the beads. The supernatant was loaded onto a Roche MagNA Pure LC sample cartridge and processed following the manufacturer's instructions.

cDNA synthesis, amplification, and sequencing

We used the amplification protocol of Chen et al. (2011) for cDNA synthesis with the following modifications of the primers. The first and second strands of cDNA synthesis were primed using modified primer A (MID1-AGTCACGATCNNNNNNNNN) where the Multiplex IDentifier (MID) was a unique 10-base pair (bp) sequence used as a bar code for the different tissue. Different MID sets were used for each tissue. The second strand cDNA was further amplified using modified primer B (MID1-AGTCACGATC) where the MID was the same unique 10-bp sequence as used for the first and second strand cDNA synthesis. The reaction mixtures and PCR conditions are provided in the upcoming text.

First strands cDNA synthesis

In synthesizing the first strand cDNA, 4 μL of RNA and 1 μL of 40 pmol/μL modified Primer A (MID tagged) were mixed in a 200-μL PCR tube A and heat denatured for 5 min at 65 C and placed on ice for 1 min. To the PCR tube, 7.2 μL master mix was added, comprising 5X RT 1st strand buffer (4 μL), 0.1M DTT (2 μL), 10 mM dNTPs (1 μL), and 400U/μL RNase OUT (0.2 μL) and then incubated at 25 C for 5 min. Subsequently, 0.5 μL of superscript III enzyme was added and the reaction incubated at 25 C for 10 min, 50 C for 50 min, and 70 C for 15 min.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

Second strand cDNA synthesis

The contents of tube A (first strand cDNA synthesis) were denatured at 94 C for 2 min and cooled to 10 C followed by addition of 5 μL Sequenase™ mix (USB, Cleveland, Ohio, USA; 5X Sequenase buffer [1 μL], Sequenase enzyme [0.2 μL], and sterile water [3.8 μL]). The reaction mixture was taken through one cycle, which involved ramping from 10 C to 37 C in 8 min; a hold at 37 C for 8 min (a 2% increase in ramp speed); 94 C for 2 min (2% increase in ramp speed); and a hold at 10 C for 5 min. During the 10 C hold, 0.6 μL of the Sequenase mixture (Sequenase dilution buffer and Sequenase enzyme mixed in the ratio of 4:1) was added and the reaction mixture taken through the single cycle described earlier.

Amplification of cDNA

AccuPrime™ Taq polymerase (Invitrogen, Carlsbad, California, USA) was used for amplification of 5 μL of double stranded cDNA in a reaction mix containing 10X PCR buffer (5 μL), 12.5 mM dNTP (1.2 μL), 100 pmol/μL Primer B (1μL), AccuPrime Taq polymerase (0.5 μL), and sterile water (37.3 μL). Initial activation was done at 94 C for 1 min followed by 30 cycles of 94 C for 30 s, 50 C for 30 s, 72 C for 1 min, and a final hold at 72 C for 7 min. The products were cleaned, and libraries were prepared and sequenced on a Roche GS FLX 454 sequencer according to Roche manuals. The sequences obtained were demultiplexed into individual tissue samples and the adaptors, MIDs, and primer sequences trimmed off using Roche sff file tools. Trimmed read quality was checked using NGS Toolkit v2.3.2 (Patel and Jain 2012), where reads <100 bases and those with quality scores <20 were discarded. High-quality reads were checked for the closest match with sequences in GenBank.

Phylogenetic analysis

Two alignments were prepared; the Fusion gene (n=78, 1,681 bp) and the complete virus genome (n=61, 15,344 bp). Sequences were chosen for the alignments through a GenBank BLAST search and by including other, more-diverged African and non-African type VI strains that would help compare phylogenetic relationships with our target sequence.

Sequences were aligned in Seaview v.4.2.11 (Gouy et al. 2010) under ClustalW2 (Larkin et al. 2007) default settings. The most appropriate substitution model for the Bayesian inference analysis was determined by the Bayesian Information Criterion (BIC) in jModeltest v.0.1.1 (Posada 2008). The tree was constructed using the Bayesian inference optimality criteria under the best fitting model (GTR+I+G, fusion gene tree: -lnL 1907.50481, BIC 40625.64114; and complete virus genome tree: -lnL 104579.82889, BIC 210403.021674). All computations were run through the CIPRES gateway (Miller et al. 2010). MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001) was used with default priors, Markov chain settings, and with random starting trees. Each run consisted of four chains of 40,000,000 generations, sampled each 1,000 generations. A plateau was reached after a few generations, and 25% of the trees resulting from the analyses were discarded as "burn in."

RESULTS

The 12 moribund Laughing Doves had sharp keels indicating emaciation and their weights averaged 65.2 g. The birds were infested with hippoboscid flies (Diptera: Hippoboscidae); death occurred shortly after capture. Field necropsy of the 12 Laughing Doves revealed variable conditions in different carcasses which included dark spots on the lungs, empty crop, cloudy air sacs, apparently enlarged heart and liver, and congested brain. The two Laughing Doves that were necropsied in the laboratory and had their organ samples analyzed were overtly emaciated (sharp keels). The lungs were congested with dark spots and air sacs appeared cloudy. We also noted congestion of the brain and liver and the heart appeared enlarged. The intestines had a greenish content. There was cloacal matting due to greenish fecal material and the birds were infested with hippoboscid flies. We obtained positive virus identification aligning to PPMV-1 from one of the Laughing Doves (bird 2) whose intestinal samples had high-quality reads of 15.4% (5, 007/32, 427). From the intestine of bird 2, 39,386 reads were obtained with quality distributed as follows; read length <100 bp 2,154 (5.47%), low-quality score 4,805 (12.20%), high-quality score 32,427 (82.33%), and average read length 261 bp. The five 007 reads were assembled to five contigs using Newbler assembler version 2.5.3 (Roche Applied Science, Mannheim, Germany). The contigs arrangements were ordered by mapping to PPMV-1 strain AV324/96, complete genome

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

(GenBank: GQ429292.1), using CLCBio Workbench version 5.1 (CLC bio A/S, Aarhus, Denmark). No sequences from the other tissues aligned to PPMV-1. Other sequences had hits to host and other microbial organisms, mainly bacteria and Archaea (data not shown).

Cleavage site and phylogeny

The fusion protein from the isolated virus had the sequence motif of ^{110}GGRRQKRF^{117}, which is similar to isolates from other countries such as the US (accessions; gi/217323225 and gi/45511229), Italy (gi/21780303), France (gi/81991862), and Tanzania (gi/58201052) (Table 1). Phylogenetic comparison of our isolate with GenBank F gene sequences of classes I and II NDVs indicated that the Kenyan Laughing Dove isolate belongs to the class II genotype VI (Fig. 2).

The phylogenetic relationships of the fusion gene revealed a terminal taxa positioning of the Kenyan sequence. The highest affinity was to Nigerian pigeon fusion type VI strain, but it showed unique lineage differentiation from the rest of the Nigerian fusion gene sequences (Fig. 2). Within the monophyletic clade (supported by Bayesian posterior probability: 1.00), the Kenyan sequences were sister to the Nigerian samples. All strain VI were terminal to the tree, mostly from pigeon and dove hosts. Strains VII and VIII were basal to strain VI and were mostly associated to a chicken host.

Genomic data and phylogeny

The genomic sequence length of our isolate was partially complete with 15,114 bp (GenBank JX518532). Phylogenetic relationships of the complete genomic sequences revealed a basal positioning of the Kenyan strain (Fig. 3). The Kenyan Laughing Dove strain did not group with any other strain VI virus and showed high genetic divergence to all others (Fig. 3). Unlike the Kenyan strain, most other strains showed high monophyly to strains from the same country and host (e.g., US, Belgium, China), as supported by the robust Bayesian posterior probabilities.

DISCUSSION

Although NDV-associated die-offs in wild birds have previously occurred in Kenya, herein we report the genotype and genomic information of a PPMV-1 strain that is circulating in Laughing Doves in Kenya. The die-offs occurred mainly in xeric regions of Kenya during the dry months, and mainly affected the Laughing Doves. We could not ascertain whether the die-offs were caused by a single strain of PPMV-1. Elsewhere, in West and South Africa, multiple strains of PPMV-1 tend to cocirculate within a region or country (Abolnik 2007; Snoeck et al. 2013). In South Africa, it was reported that most of the PPMV-1 isolates were mainly from several species of doves in multiple regions (Abolnik et al. 2008), which tends to agree with our observation and suggests host-specific susceptibility. This is in spite of the presence of other sympatric dove species in Kenya such as African Mourning Dove (*Streptopelia decipiens*), Ring-necked Dove (*Streptopelia capicola*), Emerald-spotted Wood Dove (*Turtur chalcospilos*), and Namaqua Dove (*Oeana capensis*). It would be of interest to investigate whether these other sympatric Columbiformes asymptomatically carry the isolated dove strain or perhaps different strains. Nevertheless, our study suggests that Laughing Doves are either highly susceptible or they are reservoirs and that other factors trigger the outbreak. Winter and drought are some of the stressful environmental conditions associated with NDV outbreaks in wild birds (Goekjian et al. 2011).

Hippoboscid flies comprise numerous species, are considered normal ectoparasites of diverse avian taxa, and are associated with negative health consequences (Lloyd 2002), but their role in NDV transmission is not clear and requires further investigation.

Pathogenicity of Newcastle disease is based on an intracerebral pathogenicity index of at least 0.7 in 1-day-old chicks or by multiple basic amino acids at the F protein cleavage site (World Organisation for Animal Health 2012). The sequence of the cleavage site of our isolate had a characteristic motif

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

TABLE 1. List of amino acid sequences of the fusion gene cleavage site of the Kenyan Laughing Dove (*Streptopelia senegalensis*) pigeon paramyxovirus type-1 (PPMV-I) isolate (in bold) and those from other regions sourced from GenBank.

Accession number	Isolate	Host species	Country	Amino acid sequence at Fusion gene cleavage site											
				110				115						120	
gi|18700395	APMV1/Pigeon/Tochigi/1995	Domestic pigeon (*Columba livia domestica*)	Japan	G	V	R	R	K	K	R	F	I	G	A	I
gi|18700403	APMV1/Pigeon/Fukushima/1996	Domestic pigeon (*C. l. domestica*)	Japan	G	V	R	R	K	K	R	F	I	G	A	I
gi|18700409	APMV1/Pigeon/Saitama/1997	Domestic pigeon (*C. l. domestica*)	Japan	G	V	R	R	K	K	R	F	I	G	A	I
gi|40556831	PPMV1/Chicken/ZA469/2002	Chicken (*Gallus gallus*)	S. Africa	G	V	R	R	K	K	R	F	I	G	A	I
gi|21780291	PPMV1/Eurasian dove/2736/2003	Eurasian collared dove (*Streptopelia decaocto*)	Italy	G	V	R	R	K	K	R	F	I	G	A	I
gi|21780308	PPMV1/Eurasian dove/177/01/2003	Eurasian collared dove (*S. decaocto*)	Italy	G	V	R	R	K	K	R	F	I	G	A	I
gi|18700425	APMV1/Pigeon/Gunma/2000	Domestic pigeon (*C. l. domestica*)	Japan	G	A	R	R	K	K	R	F	I	G	A	I
gi|15011277	PPMV1/Pigeon/Ch/98-1/2001	Domestic pigeon (*C. l. domestica*)	China	G	E	K	R	Q	K	R	F	I	G	A	I
gi|18042229	APMV1-Goose/JS/2/98/Go/2003	Domestic goose (*Anser* spp.)	China	G	E	K	R	Q	K	R	F	I	G	A	I
gi|4585251	APMV1/Fowl/GB/1168/84/2000	Fowl (*G. gallus* and *Meleagris gallopavo*)	UK	G	G	G	R	Q	K	R	F	I	G	A	I
gi|21780303	PPMV1/Eurasian dove/1811/00/2003	Eurasian collared dove (*S. decaocto*)	Italy	G	G	G	R	Q	K	R	F	I	G	A	I
gi|81991862	APMV1/Racing pigeon/99106/2003	Domestic pigeon (*C. l. domestica*)	France	G	G	R	R	Q	K	R	F	I	G	A	I
gi|21780311	PPMV1/Eurasian dove/7007/01/2003	Eurasian collared dove (*S. decaocto*)	Italy	G	G	R	R	Q	K	R	F	I	G	A	I
gi|21780305	PPMV1/Pigeon/4400/00/2003	Domestic pigeon (*C. l. domestica*)	Italy	G	R	G	R	Q	K	R	F	I	G	A	I
gi|409677451	**Laughing Dove/Kenya/Isiolo/B2/2012**	**Laughing Dove (*Streptopelia senegalensis*)**	**Kenya**	**G**	**G**	**R**	**R**	**Q**	**K**	**R**	**F**	I	G	A	I
gi|45511229	APMV1/Chicken/California/1083/72/2004	Chicken (*G. gallus*)	USA	G	G	R	R	Q	K	R	F	I	G	A	I
gi|217323225	PPMV-1/Pigeon/New York/1984/2009	Domestic pigeon (*C. l. domestica*)	USA	G	G	R	R	Q	K	R	F	I	G	A	I
gi|32395025	APMV1/AV 1300/95 TB0224D/2003	Domestic duck (*Anas platyrhynchos domesticus*)	Tanzania	G	G	R	R	Q	K	R	F	V	G	A	V
gi|58201052	APMV1/Chicken/Morogoro/MG10.5c/1996	Chicken (*G. gallus*)	Tanzania	G	G	R	R	Q	K	R	F	V	G	A	V

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

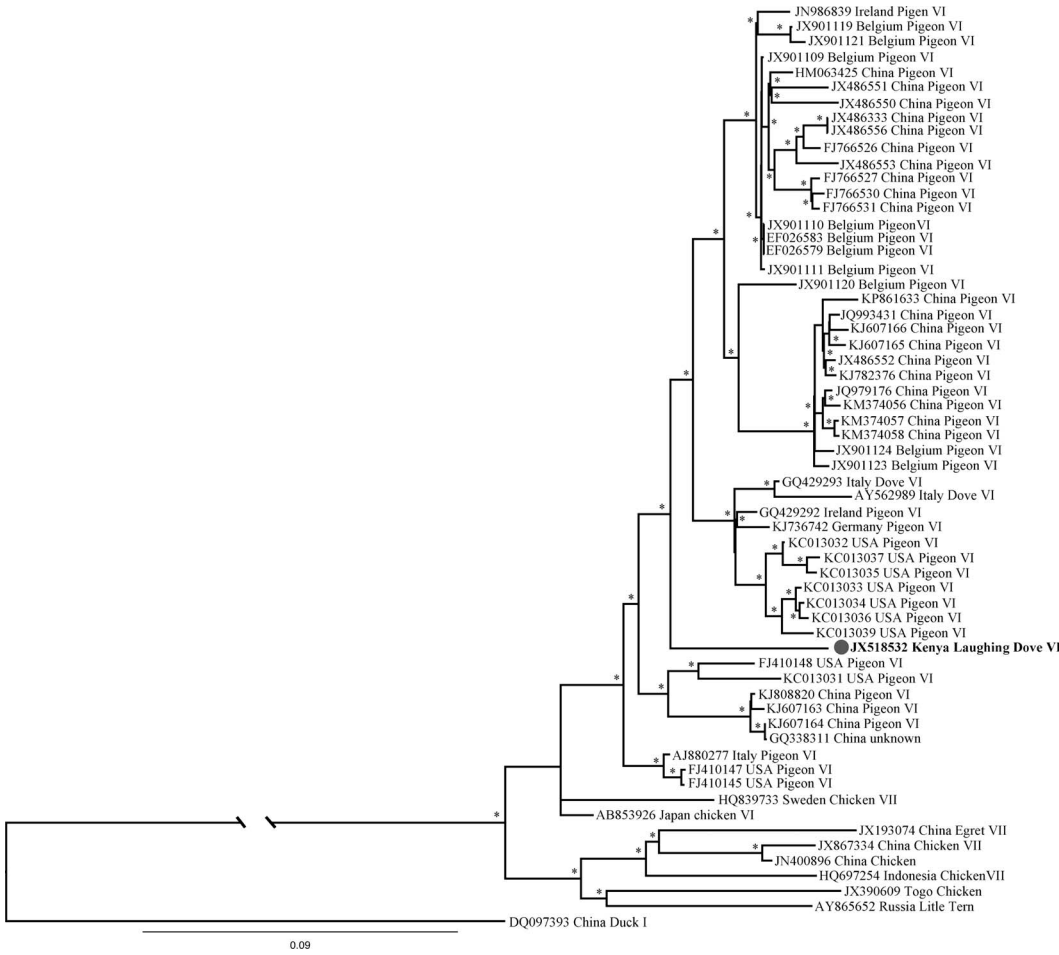

FIGURE 2. Bayesian inference tree of the fusion gene of pigeon paramyxovirus type-1virus isolate from Laughing Dove (*Streptopelia senegalensis*) in Kenya (bold dot) and those from GenBank. All terminal taxa have the GenBank accession number, locality, host, and strain type. Asterisks by nodes indicate Bayesian posterior probabilities higher than 95%.

(^{110}GGRRQKRF^{117}) that is indicative of virulence. The F protein cleavage position for virulent or mesogenic strains entail a furin recognition site that is made up of various basic amino acids (arginine [R] or lysine [K]) surrounding the glutamine (Q) at position 114 and phenylalanine (F) at position 117 (Collins et al. 1993; Hines and Miller 2012). Cleavage of the F protein and virulence of the NDV strain depends on one or both 'R' at positions 112 and 115 or 'F' at position 117 (Hines and Miller 2012). The sequence motif of the F gene cleavage site is not sufficient to distinguish between velogenic and mesogenic strains (Hines and Miller 2012). However, we consider our isolate as virulent because of its fusion gene sequence, which conforms to the characteristic motif of virulent strains (Table 1). Phylogenetic comparison of our isolate indicates that it belongs to class II genotype VI (Fig. 2). Genotype VI was originally from the Middle East and Asia during the 1980s and 1990s (Kwon et al. 2003) but has since spread to other continents (Kaleta et al. 1985). Genotype VI is highly diverse and includes several subgenotypes, but many PPMV-1 strains from Columbidae cluster as a monophyletic group of genotype VIb (Ujvári et al. 2003). This genotype is both antigenetically

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

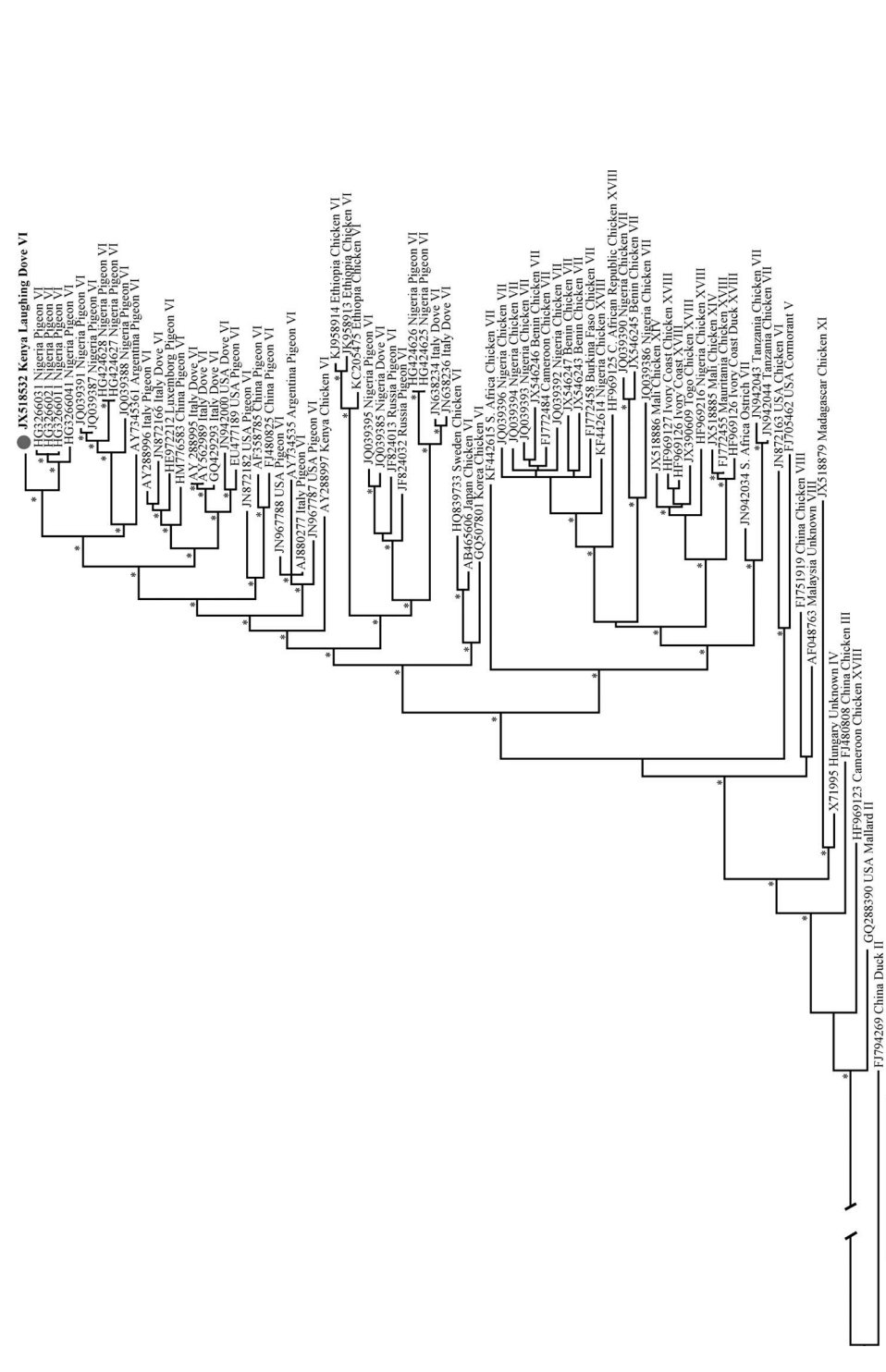

FIGURE 3. Bayesian inference tree of complete genomes of pigeon paramyxovirus type-1 virus isolate from Laughing Dove (*Streptopelia senegalensis*) in Kenya (bold dot) and those retrieved from GenBank. All terminal taxa have the GenBank accession number, locality, host, and strain type. Asterisks by nodes indicate Bayesian posterior probabilities higher than 95%.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

and genetically dissimilar from NDV isolates common in poultry (Collins et al. 1993); however, with exposure to domestic poultry and subsequent adaptation (Dortmans et al. 2011), the genotype has been reported to cause severe disease in domestic poultry (Abolnik 2007).

The interhost species transmission of virulent strains between wild and domestic fowl (Vidanovic et al. 2011) is viewed as an epidemiologic risk predicted to influence the rate of evolution and emergence of novel genotypes (de Almeida et al. 2013), diagnostic failures, and weak efficacy of first-generation vaccines (Samuel et al. 2013). Phylogenetic relationships showed a close association of the Kenyan strain to pigeon (Columbidae) strains, with the fusion gene matching closely to African strains from Nigeria (Fig. 2). The strain is basal to strains from pigeons in Europe and the US (Fig. 2). The genomic information of our isolate (PPMV1/Laughing Dove/Kenya/Isiolo/B2/2012) suggests that it is ancestral to most known New and Old World strains but with high genetic divergence to such strains (Fig. 3). The nongrouping of the Laughing Dove genome with other available strains shows a unique evolutionary lineage. Further studies would help assess the reasons for such divergence and the implications from an epidemiologic perspective.

A PPMV-1 strain circulates in wild birds within Kenya, which is likely different from strains currently circulating in domestic poultry. Because Laughing Doves are found in farms and backyards where they interact closely with free-ranging domestic poultry, specifically sharing feed lot and watering points, there is a probability of exposure and cross-transmission of this virulent strain to domestic poultry.

ACKNOWLEDGMENTS

We are grateful to the Director, Kenya Wildlife Service, for supporting the investigation of wild bird mortality events, field sample collection, and laboratory analysis. We appreciate all the individuals who were involved in making the activities of this work successful.

LITERATURE CITED

Abolnik C. 2007. *Molecular epidemiology of Newcastle disease and avian influenza in South Africa*. University of Pretoria, Pretoria, South Africa, 286 pp.

Abolnik C, Gerdes G, Kitching J, Swanepoel S, Romito M, Bisschop S. 2008. Characterization of pigeon paramyxoviruses (Newcastle disease virus) isolated in South Africa from 2001 to 2006. *Onderstepoort J Vet Res* 75:147–152.

Alexander DJ. 2011. Newcastle disease in the European Union 2000 to 2009. *Avian Pathol* 40:547–558.

Byarugaba DK, Mugimba KK, Omony JB, Okitwi M, Wanyana A, Otim MO, Kirunda H, Nakavuma JL, Teillaud A, Paul MC. 2014. High pathogenicity and low genetic evolution of avian paramyxovirus type I (Newcastle disease virus) isolated from live bird markets in Uganda. *Virol J* 11:1–13.

Chen EC, Miller SA, Derisi JL, Chiu CY. 2011. Using a pan-viral microarray assay (Virochip) to screen clinical samples for viral pathogens. *J Vis Exp* (50). pii: 2536.

Collins M, Bashiruddin J, Alexander D. 1993. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Arch Virol* 128:363–370.

Courtney SC, Susta L, Gomez D, Hines NL, Pedersen JC, Brown CC, Miller PJ, Afonso CL. 2013. Highly divergent virulent isolates of Newcastle disease virus from the Dominican Republic are members of a new genotype that may have evolved unnoticed for over 2 decades. *J Clin Microbiol* 51:508–517.

de Almeida RS, Hammoumi S, Gil P, Briand F-X, Molia S, Gaidet N, Cappelle J, Chevalier V, Balança G, Traoré A, et al. 2013. New avian paramyxoviruses type I strains identified in Africa provide new outcomes for phylogeny reconstruction and genotype classification. *PLoS One* 8:e76413.

Diel DG, Susta L, Garcia SC, Killian ML, Brown CC, Miller PJ, Afonso CL. 2012. Complete genome and clinicopathological characterization of a virulent Newcastle disease virus isolate from South America. *J Clin Microbiol* 50:378–387.

Dortmans JC, Rottier P, Koch G, Peeters BP. 2011. Passaging of a Newcastle disease virus pigeon variant in chickens results in selection of viruses with mutations in the polymerase complex enhancing virus replication and virulence. *J Gen Virol* 92:336–345.

Goekjian VH, Smith JT, Howell DL, Senne DA, Swayne DE, Stallknecht DE. 2011. Avian influenza viruses and avian paramyxoviruses in wintering and breeding waterfowl populations in North Carolina, USA. *J Wildl Dis* 47:240–245.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024

Hines NL, Miller CL. 2012. Avian paramyxovirus serotype-1: A review of disease distribution, clinical symptoms, and laboratory diagnostics. *Vet Med Int* 2012:708216.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Jindal N, Chander Y, Chockalingam AK, De Abin M, Redig PT, Goyal SM. 2009. Phylogenetic analysis of Newcastle disease viruses isolated from waterfowl in the upper midwest region of the United States. *Virol J* 6:191.

Kaleta EF, Alexander DJ, Russell PH. 1985. The first isolation of the avian PMV-1 virus responsible for the current panzootic in pigeons? *Avian Pathol* 14:553–557.

Kwon H-J, Cho S-H, Ahn Y-J, Seo S-H, Choi K-S, Kim S-J. 2003. Molecular epidemiology of Newcastle disease in Republic of Korea. *Vet Microbiol* 95:39–48.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.

Lloyd J. 2002. Louse flies, keds, and related flies (Hippoboscoidea). *Med Vet Entomol* 2:331–344.

Lomniczi B, Wehmann E, Herczeg J, Ballagi-Pordany A, Kaleta E, Werner O, Meulemans G, Jorgensen P, Mante A, Gielkens A. 1998. Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). *Arch Virol* 143:49–64.

Mayo M. 2002. A summary of taxonomic changes recently approved by ICTV. *Arch Virol* 147:1655–1656.

Miller M, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, Institute for Electrical and Electronics Engineers, New Orleans, Louisiana, 14 November 2010, pp. 1–8.

Miller PJ, Haddas R, Simanov L, Lublin A, Rehmani SF, Wajid A, Bibi T, Khan TA, Yaqub T, Setiyaningsih S. 2015. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infect Genet Evol* 29:216–229.

Patel RK, Jain M. 2012. NGS QC Toolkit: A toolkit for quality control of next generation sequencing data. *PLoS One* 7:e30619.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256.

Saif Y, Fadly A, Glisson J, McDougald L. 2008. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: *Diseases of poultry*, 12th Ed. Iowa State University Press, Ames, Iowa, pp. 75–93.

Samuel A, Nayak B, Paldurai A, Xiao S, Aplogan GL, Awoume KA, Webby RJ, Ducatez MF, Collins PL, Samal SK. 2013. Phylogenetic and pathotypic characterization of Newcastle disease viruses circulating in West Africa and efficacy of a current vaccine. *J Clin Microbiol* 51:771–781.

Snoeck CJ, Owoade AA, Couacy-Hymann E, Alkali BR, Okwen MP, Adeyanju AT, Komoyo GF, Nakouné E, Le Faou A, Muller CP. 2013. High genetic diversity of Newcastle disease virus in poultry in West and Central Africa: Cocirculation of genotype XIV and newly defined genotypes XVII and XVIII. *J Clin Microbiol* 51:2250–2260.

Ujvári D, Kaleta EF, Werner O, Savić V, Nagy É, Czifra G, Lomniczi B. 2003. Phylogenetic analysis reveals extensive evolution of avian paramyxovirus type 1 strains of pigeons (*Columba livia*) and suggests multiple species transmission. *Virus Res* 96:63–73.

Vidanovic D, Šekler M, Ašanin R, Milic N, Nišavic J, Petrovic T, Savic V. 2011. Characterization of velogenic Newcastle disease viruses isolated from dead wild birds in Serbia during 2007. *J Wildl Dis* 47: 433–441.

World Organisation for Animal Health. 2012. *Manual of diagnostic tests and vaccines for terrestrial animals: Mammals, birds and bees*. World Organisation for Animal Health, Paris, France, pp. 1–19.

Xiao S, Paldurai A, Nayak B, Samuel A, Bharoto EE, Prajitno TY, Collins PL, Samal SK. 2012. Complete genome sequences of Newcastle disease virus strains circulating in chicken populations of Indonesia. *J Virol* 86:5969–5970.

Submitted for publication 28 July 2015.
Accepted 6 December 2015.

Downloaded from http://meridian.allenpress.com/jwd/article-pdf/52/3/599/2248270/2015-07-199.pdf by guest on 15 September 2024