Infection, Genetics and Evolution 17 (2013) 260–268

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Successful establishment and global dispersal of genotype VI avian paramyxovirus serotype 1 after cross species transmission

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ARTICLE INFO

Article history:
Received 23 February 2013
Received in revised form 8 April 2013
Accepted 22 April 2013
Available online 27 April 2013

Keywords:
Evolution
Pigeon
Newcastle disease
Phylogeography
Cross-species transmission
Avian paramyxovirus

ABSTRACT

The evolutionary history of avian paramyxovirus serotype 1 (PMV1), which includes the agents of Newcastle disease (ND), is characterized by a series of strain emergence events since viruses in this family were first recognized in the 1920s. Despite the importance of ND to the poultry industry, little is known about PMV1 strain emergence events and the subsequent dispersal and evolution of new strains. The genotype VI-PMV1 was first identified in the 1980s and has been named pigeon paramyxovirus-1 (PPMV1) because of unusual host specificity with Columbiformes (Collins et al., 1996); it has been responsible for panzootics in both chickens and pigeons during that time. Here, we used evolutionary analyses to characterize the emergence of this contemporary PMV1 lineage. We demonstrate that GVI-PMV1 arose through cross-species transmission events from Galliformes (i.e. chicken) to Columbiformes, and quickly established in pigeon populations. Our studies revealed a close association between the time of viral emergence and panzootic events of this virus. The virus appeared first in Southeastern Europe and quickly spread across the European continent, which became the epicenter for global virus dissemination. With new viral gene sequences, we show that GVI-PMV1 viruses currently circulating in North America resulted from multiple invasion events from Europe, one associated with an exotic European Columbiformes species, and that extant lineages have diversified locally. This study extends our understanding of successful viral emergence subsequent to cross-species transmission and dispersal patterns of newly emerged avian viruses, which may improve surveillance awareness and disease control of this and other important avian pathogens.

1. Introduction

The number of emergent or re-emergent infectious viral diseases that are linked to cross-species transmission from reservoirs to naive hosts is increasing in both animals and humans (Woolhouse, 2008). Notable examples include emergence of avian and swine influenza viruses, henipaviruses, and the severe acute respiratory syndrome coronavirus. The mechanisms in viral emergence are complex and include ecological, immunological, and genetic features of both virus and host (Lloyd-Smith et al., 2009; Parrish et al., 2008). Although the dispersal of viruses in humans following zoonotic transmission can be documented by integrating epidemiological data with virus sequence data, the events that precede productive viral infection in a new host are more difficult to untangle (Lloyd-Smith et al., 2009). Similarly, cross-species transmissions among animal species leading to successful strain emergence are poorly understood. With the increased availability of genetic data and associated temporal and geographic information from improved surveillance effort, it is now possible to obtain insights of inter-species transmission dynamics that give rise to new viral strains, which pose health risks in animals.

Avian paramyxovirus serotype 1 (PMV1) is a negative-sense single stranded RNA virus in the family *Paramyxoviridae*. It is best known for causing outbreaks of Newcastle disease (ND), a source of significant economic loss to the poultry industry worldwide. In the 1950s, 30 years after the first reported PMV1 outbreak, modified live vaccines derived from the dominant circulating strain were deployed to prevent and control the disease (Alexander, 1988a). Subsequently, a series of new strains have emerged from unknown sources that contribute to the ND global burden. There are now at least ten reported genotypes of PMV1 contributing to the extensive genetic diversity in avian populations worldwide (Aldous et al., 2004; Barbezange and Jestin, 2003, 2005; Diel et al., 2012; Kim

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http://dx.doi.org/10.1016/j.meegid.2013.04.025

et al., 2008; Kommers et al., 2001; Liu et al., 2006; Mase et al., 2009; Meulemans et al., 2002; Panshin et al., 1999; Servan de Almeida et al., 2009; Sleeman, 2010; Tsai et al., 2004; Ujvari et al., 2003; Zhu et al., 2010, http://www.worldpoulty.net/). Understanding the mechanism of successful strain emergence in PMV1 can inform efforts to control ND and other viral diseases with similar evolutionary dynamics that threaten domestic and wild birds.

In the late 1970s, a distinct strain of PMV1 was sporadically isolated from pigeons in proximity to ND outbreaks in chickens (Alexander et al., 1984; Alexander et al., 1985a; Kaleta et al., 1985; Kaleta and Baldauf, 1988; Stewart, 1971). The new virus, called pigeon-associated PMV1 (PPMV1), was initially presumed to be of chicken origin and to infect pigeons only as a result of sporadic spill over events. However, in 1984, multiple ND outbreaks in Great Britain were initiated by PPMV1 and spread from pigeons to chickens (Alexander et al., 1985b). Subsequently, PPMV1 has been responsible for other chicken ND outbreaks worldwide (Aldous et al., 2004; Capua et al., 2002; Kommers et al., 2001; Liu et al., 2006; OIE, 2011a,b; Toro et al., 2005; Werner et al., 1999) and has caused extensive panzootic infections in racing pigeons in European countries despite vaccination efforts (Alexander et al., 1997; Kommers et al., 2001; Meulemans et al., 2002; Werner et al., 1999; Zanetti et al., 2001). Genetic analysis of PPMV1 demonstrated that this strain, named genotype VI-PMV1 (GVI-PMV1) (Aldous et al., 2004), constituted a distinct monophyletic lineage with considerable genetic heterogeneity.

Although virulent PMV1 is considered to be exotic to US poultry, serological data evidenced GVI-PMV1 in ND outbreaks in pigeons during the 1980–1990s (Barton et al., 1992; Pearson et al., 1987; Tangredi, 1988). A recent molecular study (part of the West Nile Virus Surveillance program) (Kim et al., 2008) also confirmed that GVI-PMV1 is circulating in North American pigeon populations. The evolutionary dynamics of this pathogen in North America remains unclear because of limited sequence data and absence of coordinated national surveillance.

In this study, we aim to understand the events leading to the emergence and the molecular evolution and phylogeography of the new strain. We inferred the evolutionary timescale for the entire GVI-PMV1 lineage with statistical confidence. The chicken-to-pigeon transmission events leading to the establishment of the major GVI-PMV1 sub-lineage in Columbiformes populations was studied in details. Using advanced phylogenetic methods, we estimated the time and geographic origin of this important cross-species event. To better understand the viral spread in North America, we sequenced the F genes of 74 PMV1 isolates collected in northeastern United States (US) from 2001 to 2009. We demonstrate that there were at least three introductions of GVI-PMV1 into North America, including one associated with Eurasian collared doves. We also provide evidence for viral population expansion in one of the two circulating lineages.

2. Materials and methods

2.1. PMV1 field isolates

The Animal Diagnostic Laboratory at The Pennsylvania State University (PSU-ADL) conducted virus isolation tests on avian cases submitted from Pennsylvania and other states in the US. Bird tissue specimens and swab samples were processed for virus isolation in specific pathogen free (SPF) embryonated chicken eggs (ECE) and/or chicken embryo fibroblast (CEF) cell cultures following described procedures [(Alexander, 1988b) and OIE Terrestrial Manual, 2009 (Manual, 2009)]. Hemagglutination (HA) and hemagglutination-inhibition (HI) tests were conducted for PMV1 identification. PMV1 field strains isolated from various avian species at PSU-ADL between 1996 and 2009 were retrieved for this study.

2.2. Viral RNA extraction and sequencing

Viral RNA was extracted using the QIAamp Viral RNA extraction kit (Qiagen, Valencia, CA-USA) and was reverse transcribed using a specific PMV1 oligonucleotide targeting the 3′ untranslated region of the genome (5′-ACGGGTAGAAGGTGTGAATC-3′) with the AffinityScript™ Multiple Temperature Reverse Transcriptase Kit (Stratagene, Santa Clara, CA-USA). PCR amplification was performed targeting the entire coding region of the F gene using two pairs of specific primers (GVI-forward primer: 5′-TGCTCGGACCTTCTGTG CTTGTGA-3′; GVI-reverse primer: 5′-TGCGGACCTTGTTCTTGCTGC-TAC-3′; GII-forward primer: 5′-TGACCGCCGACCACGAG-3′; GII-reverse primer: 5′-TCAGGAGAGGCCGATTCAAGTATT-3′) with the TaKaRa Ex Taq Hot Start PCR kit (Clontech, Mountain View, CA-USA). These primer sets also amplify the entire coding region of the HN gene. A total of 74 PMV1 isolates were amplified. PCR products were gel purified using the QIAquick Gel Extraction kit (Qiagen, Valencia, CA-USA) and sequenced. Sequences were assembled and edited using the Lasergene DNASTAR software package (DNASTAR Inc. WI, USA). Sequences have been deposited in GenBank (Accession numbers JX901304–JX901377; Table S1).

2.3. Sequence collection and alignment

A total of 3631 F gene sequences of PMV1 were downloaded from GenBank in April, 2011 and aligned using Muscle program (Edgar, 2004). Sequences with length shorter than 240 bp were removed. The 74 PMV1 sequences generated in this study were combined with GenBank sequences, and tested for recombination by RDP2 (Martin et al., 2005). Putative recombinants, which were confirmed by GARD method (Kosakovsky Pond et al., 2006), were removed. This resulted in a final data set of 3469 sequences, which included sequences ranging from 240 to 1662 bp. Of these 710 were full-length.

2.4. Phylogenetic analyses

A maximum likelihood (ML) panoramic phylogeny was built from the F gene sequence data set using FastTree v2.1 program (Price et al., 2010). The GVI-PMV1 lineage ($n = 534$, with known isolation dates; 512 were longer than 350nt and 108 were full-length) was identified and extracted from the panoramic phylogeny for further analyses (detailed taxon names are shown in Table S2). A refined ML phylogenetic tree of GVI-PMV1 was reconstructed by PhyML v3 program (Guindon et al., 2010), where the best tree was selected from subtree pruning and regrafting (SPR) and nearest-neighbor interchange (NNI) topological optimizations. The general time reversible with gamma distribution ($GTR + \Gamma$) nucleotide substitution model was used. Local topological supports were assessed by Shimodaira–Hasegawa (SH) tests (Guindon et al., 2010). Among the new PMV1 sequences generated in this study, 61 isolates were GVI and the remaining 13 isolates were grouped in genotype II (GII; the genotype to derive vaccine strain). These strains are indicated in Fig. 1A.

2.5. Evolutionary timescale and population dynamics of GVI-PMV1

The time-scaled phylogeny of GVI-PMV1, along with various evolutionary parameters including substitution rate and population size, were estimated simultaneously by the Bayesian Markov Chain Monte Carlo (BMCMC) method implemented in BEAST v1.6.1 program (Drummond and Rambaut, 2007). Relaxed clock model with uncorrelated lognormal distributed rates was used.