Arch Virol (2015) 160:2629–2632
DOI 10.1007/s00705-015-2547-3

RAPID COMMUNICATION

Genomic characterization of a novel avian arthritis orthoreovirus variant by next-generation sequencing

Yi Tang[1] · **Huaguang Lu**[1]

Received: 22 April 2015 / Accepted: 20 July 2015 / Published online: 4 August 2015

Abstract By using next-generation sequencing (NGS) technology, we have identified a divergent avian orthoreovirus (ARV) field variant (Reo/PA/Broiler/15511/13, or PA15511), isolated from broiler chickens with viral arthritis in Pennsylvania in 2013. The complete genome of the PA15511 field strain was 23,495 bp in length with 10 dsRNA segments encoding 12 viral proteins. The lengths of the genomic segments ranged from 1192 bp (S4) to 3958 bp (L1). Genomic analysis has revealed that this virus is distinct from reference ARV strains and meets criteria for a new or novel strain.

Avian orthoreovirus (ARV), genus *Orthoreovirus*, family *Reoviridae* [1], is a non-enveloped double-stranded RNA (dsRNA) virus containing ten genome segments [9]. Frequent genetic reassortment among multiple genome segments could lead to the emergence of new serotypes, pathotypes or genotypes [6]. Most domestic and wild avian species are susceptible to ARV infections [3, 10], but ARV infection results in much severer disease symptoms in broiler chickens than in other avian species [2].

Newly emerging ARV variants have been causing major disease problems in broiler chickens in Pennsylvania (PA) since 2011. ARV-infected birds showed various disease conditions, as documented in the literature [7, 9]. Particularly, swollen legs, severe viral arthritis/tenosynovitis, runting-stunting syndrome, enteric disease, and malabsorption syndrome were commonly seen in ARV-affected broiler flocks. Between 2011 and 2014, more than 200 ARV field isolates were isolated, mostly from tendon and synovial tissues of broiler chickens showing symptoms of AIV infection, by virus isolation in LMH cell cultures (CRL-2113, ATCC, Manassas, VA) per routine cell culture procedures [4] in our laboratory. ARV-positive isolates were confirmed by ARV fluorescent antibody (FA) (Ref. No. 680, VDL 9501, NVSL, Ames, IA) staining of ARV-infected "bloom-like" giant cytopathic effect (CPE) cells from specimen-inoculated LMH cell cultures. By using next-generation sequencing (NGS) with the Illumina MiSeq, we sequenced the full genome of an ARV field strain (Reo/PA/Broiler/15511/13, or PA15511) isolated from an ARV-affected broiler flock in 2013. Full genomic sequence analysis indicated that the PA15511 field strain has unique genetic characteristics and thus should be considered a field variant or novel strain of ARV.

Sequencing was carried out on a Miseq platform (Illumina, San Diego, CA, USA) using total RNA samples extracted from supernatant of a cell culture infected with the PA15511 strain. As a result, a total of 825,865 of 35-151-mer sequencing reads were generated, yielding 271 Mb of fastq-format sequence data. After all reads had undergone quality trimming to remove non-target reads with similarities to chicken mRNA or rRNA sequences, a final yield of 78,540 (9.51 %) clean reads was obtained. All clean reads were assembled *de novo* using CLC Genomic Workbench V7.5 software (QIAGEN, Boston, MA, USA). After BLASTN searching, a total of ten contigs were generated by the software, corresponding to the ten genome segments. For each of the ten contigs, the entire each

Electronic supplementary material The online version of this article (doi:10.1007/s00705-015-2547-3) contains supplementary material, which is available to authorized users.

✉ Huaguang Lu
hxl15@psu.edu

[1] Wiley Lab/Avian Virology, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802, USA

segment was correctly assembled as a single contig, and the coverage of reads was calculated at 131× on average.

The full genome of the PA15511 strain was 23,495 bp in length, with ten dsRNA genome segments encoding 12 viral proteins. The lengths of the PA11551 strain genome segments, ranging from 1192 bp (S4 segment) to 3958 bp (L1 segment), were similar to those of ARV reference strains. ORF analysis of nucleotide (nt) sequences indicated that nine of the ten genome segments, with the exception of S1, were monocistronic and encoded one single ORF (Table 1). The untranslated regions (UTR) were located at the 5' end (12 to 30 nt in length) and 3' end (33 to 98 nt in length) of each segment. All of the ten fragments shared a GCUUUU motif in their 5' UTR and a UCAUC motif in their 3' UTR. The full-length genome sequences of all ten segments of the PA15511 strain were deposited in the GenBank database in February 2015 (GenBank accession numbers: KP731611-KP731620) (Table 1).

A comparison of the nt sequences of all ten genome segments (Table 1) of the PA15511 strain with those of six ARV reference strains (Table S1, GenBank accession numbers) showed that the PA15511 strain shared highest sequence similarity (65.5 %-94.1 % identity in the L1, M1 and S1 segments) with strain AVS-B; 89.9 % in the L2 segment with ARV strain 176; 91.9 % in segment L3 and 90.6 % in segment S3 with strain ARV138; 91.9 % in S2 with ARV strain 1733; and 81.4 %-99.4 % in segments M2, M3 and S4 with the Reo/PA/broiler/05682/12 strain (PA05682) (Table 2). PA05682 is another PA broiler ARV field strain, which was isolated in 2012, and its full genomic characterization results were published recently [8].

Phylogenetic analysis was carried out on the genes encoding the outer capsid proteins (μB, σB, σC) using

Table 1 General genome features of the novel avian arthritis orthoreovirus (ARV) strain (Reo/PA/Broiler/15511/13) isolated in Pennsylvania poultry in the USA

Genome segment	Protein name	Size	Length (bp) of the			Strain in GenBank with the highest similarity	Identity (%)	GenBank accession number
			5' end	ORF	3' end			
L1	λA	3958	20	3882	56	AVS-B (FR694191)	92	KP731611
L2	λB	3829	14	3780	36	176 (EU707936)	90	KP731612
L3	λC	3907	12	3870	37	138 (EU707937)	92	KP731613
M1	μA	2283	12	2199	72	AVS-B (FR694194)	94	KP731614
M2	μB	2158	29	2031	98	PA05682 (KM877329)	99	KP731615
M3	μNS	1996	24	1908	64	1017-1 (AY573905)	90	KP731616
S1	p10	1645	22	300	33	RAM-1 (OTOSIGMAC)	82	KP731617
	p17			441				
	σC			981				
S2	σA	1324	15	1251	58	GX110058 (KF741743)	92	KP731618
S3	σB	1203	30	1104	68	138 (AF059721)	91	KP731619
S4	σNS	1192	23	1104	65	PA05682 (KM877334)	99	KP731620

Table 2 Sequence identities (%) of all 10 genome segments of the PA11551 strain (Reo/PA/Broiler/15511/13) to those of avian arthritis orthoreovirus (ARV) reference stains

Genome segment	ARV reference strain							
	PA05682	S1133	1733	138	176	AVS-B	MN9	J18
L1	90.9	88.5	88.7	91.2	88.7	92.1	83.5	77.4
L2	83.3	89.5	89.8	83.7	89.9	83.0	82.3	76.7
L3	88.3	72.8	72.9	91.9	72.9	88.8	83.5	70.3
M1	87.3	87.4	87.4	88.9	87.6	94.1	85.2	74.2
M2	99.4	84.9	84.9	89.7	85.8	76.0	74.9	75.9
M3	81.4	80.7	81.0	80.6	80.6	80.2	80.2	71.0
S1	62.4	54.0	54.1	55.1	54.2	65.5	53.3	43.2
S2	89.4	91.7	91.9	90.8	91.8	89.8	87.1	77.1
S3	89.4	85.5	85.5	90.6	85.6	90.2	70.5	65.6
S4	99.3	80.8	81.0	89.9	80.7	92.6	78.5	78.2

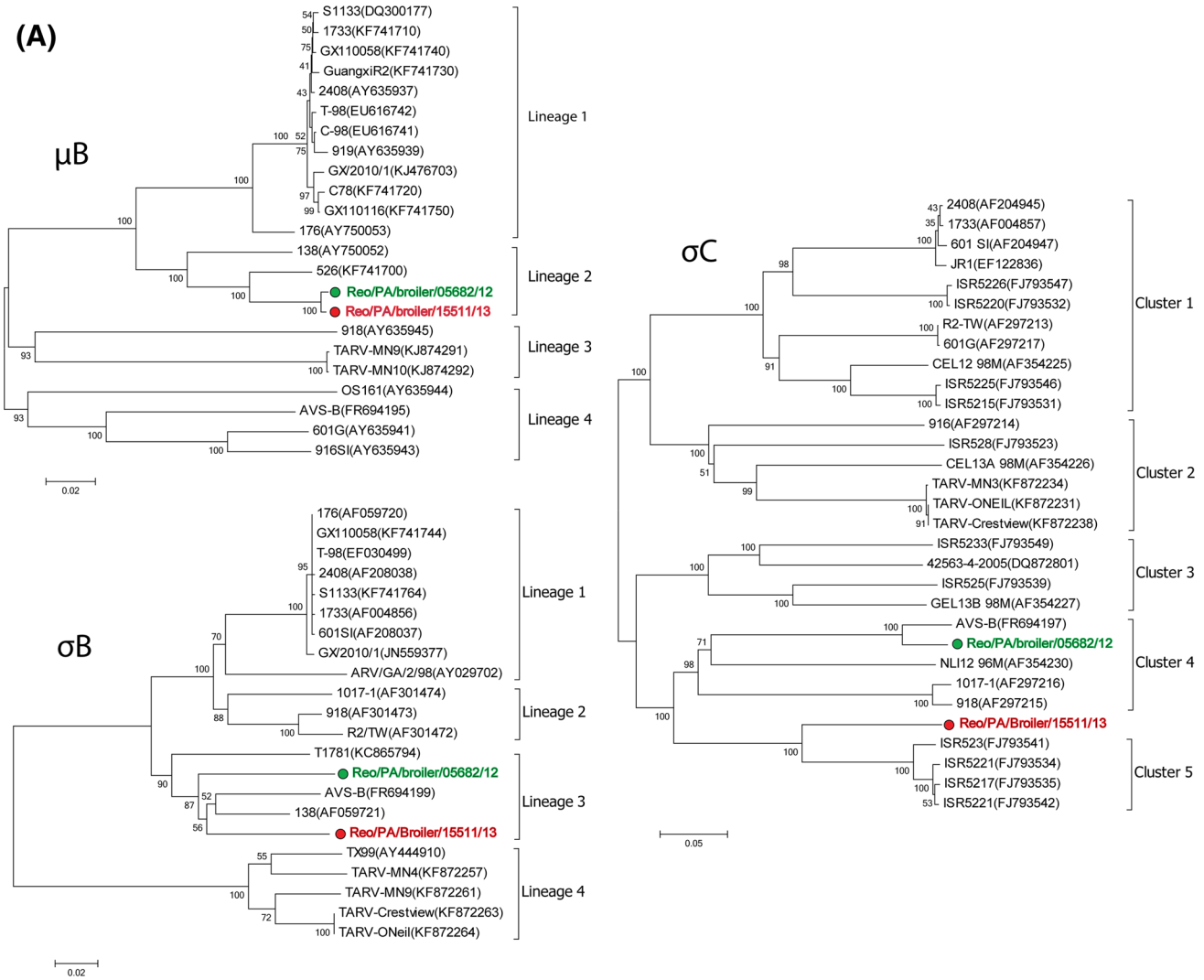

Fig. 1 A. Phylogenetic trees constructed based on amino acid sequences of μB, σB and σC of avian orthoreoviruses (ARV) using the neighbor-joining method implemented in MEGA5.0. Bootstrap values for 1000 replicates are shown at the nodes. The bar indicates genetic distance. The PA11551 broiler ARV strain (Reo/PA/Broiler/15511/13) is highlighted in red; the PA05682 (Reo/PA/broiler/05682/12) strain is highlighted in green. **B.** Nucleotide sequence alignment of genome segments by mVISTA software, comparing the PA11551 broiler ARV strain (Reo/PA/Broiler/15511/13) with PA05682, S1133, 1733, 138, AVS-B, and 176. The pink-colored areas indicate ≥90 % conservation; blank areas indicate <90 % conservation. The scale bar at the bottom shows the length of the genome (color figure online)

amino acid (aa) sequences. In the μB gene, all ARV strains could be divided into four lineage groups (Fig. 1A, μB). Clearly, the two PA broiler ARV field strains, PA15511 and PA05682, were almost identical and both were closely related to ARV 526 and 138 in lineage 2. Additionally, both PA broiler ARV field strains were genetically different from all other reference strains in lineages 1, 3, and 4. However, the σB phylogenetic tree showed that the PA15511 strain was more closely related to ARV 138 and AVS-B than it was to PA05682 in lineage 3 (Fig. 1A, σB). Phylogenetic analysis of the σC-encoding genes resulted in five clusters as described by Kent et al. [5]. The PA15511 strain was clearly separated from the four reference strains (aa identity $\leq$ 84.8 %), although they were in the same genotype cluster (cluster 5; Fig. 1A, σC). Obviously, PA11551 was evolutionarily divergent from the reference strains in clusters 1 through 4, including the PA05682 field strain (in cluster 4) isolated in PA poultry in 2012 [8]. In addition, when σC aa sequences of 55 other ARV strains retrieved from GenBank were analyzed, none of them were closely related to the PA15511 strain. The aa identities between the PA15511 strain and the other 55 strains ranged from 49.3 % to 85.1 %.

To identify reassortment events between different ARV strains, the whole genome of the PA15511 strain was aligned with five ARV reference strains using the mVISTA online platform (http://genome.lbl.gov/vista/mvista/submit.shtml). Considerable genetic relatedness of the PA15511 strain and reference strains was found in nine of the ten genome segments, the exception being S1 (Fig. 1B). Although most of the genome segments were conserved between PA15511 and ARV 138, their M3 and S1 segments were very distantly related to each other, especially the σC-encoding nt genes in the S1 segment (from nt 631 to 1611). The greatest sequence similarity values were seen in the M2 and S4 genome segments between the two PA broiler ARV field strains PA11551 and PA05682. These findings suggested that reassortment among different ARV strains, including vaccine strains, could play a critical role in the emergence of this novel ARV strain.

In conclusion, we have determined the sequences of all ten genome segments and have completed the full genomic sequence characterization of the novel PA11551 strain. Phylogenetic analysis and nucleotide sequence comparisons have demonstrated that there are close genetic relationships in several genome segments but considerable differences in other segments (e.g., the S1 segment σC coding gene) between this novel strain and reference ARV strains, indicating a high probability that the PA15511 strain resulted from reassortment of other ARV field strains or vaccine strains circulating in PA poultry. The great genetic divergence of the σC genes between the PA11551 and conventional vaccine strains (S1133, 1733 and 2408) reveals why vaccination failed to protect against ARV infections. Vaccination against ARV field strains with conventional vaccines prior to the observed outbreaks had been practiced in broiler breeders, turkey breeders and layer chickens in PA. However, these conventional vaccines did not appear to confer any protection against ARV infections. Since the detection of ARV field variants in commercial broiler and turkey flocks in PA, killed autogenous vaccines of ARV field variants have been used for vaccination of breeder flocks, significantly improving protection of progeny against ARV infections.

Acknowledgments This research project was funded by the Pennsylvania Department of Agriculture, Animal Health and Diagnostic Commission's research program in 2015, and the Pennsylvania Soybean Board's research program in 2015.

References

1. Bagust TJ, Westbury HA (1975) Letter: Isolation of reoviruses associated with diseases of chickens in Victoria. Aust Vet J 51:406–407
2. Benavente J, Martinez-Costas J (2007) Avian reovirus: structure and biology. Virus Res 123:105–119
3. Dandar E, Balint A, Kecskemeti S, Szentpali-Gavaller K, Kisfali P, Melegh B, Farkas SL, Banyai K (2013) Detection and characterization of a divergent avian reovirus strain from a broiler chicken with central nervous system disease. Arch Virol 158:2583–2588
4. Heggen-Peay CL, Cheema MA, Ali RA, Schat KA, Qureshi MA (2002) Interactions of poult enteritis and mortality syndrome-associated reovirus with various cell types in vitro. Poultry Sci 81:1661–1667
5. Kant A, Balk F, Born L, van Roozelaar D, Heijmans J, Gielkens A, ter Huurne A (2003) Classification of Dutch and German avian reoviruses by sequencing the sigma C protein. Vet Res 34:203–212
6. Liu HJ, Lee LH, Hsu HW, Kuo LC, Liao MH (2003) Molecular evolution of avian reovirus: evidence for genetic diversity and reassortment of the S-class genome segments and multiple cocirculating lineages. Virology 314:336–349
7. Sterner FJ, Rosenberger JK, Margolin A, Ruff MD (1989) In vitro and in vivo characterization of avian reoviruses. II. Clinical evaluation of chickens infected with two avian reovirus pathotypes. Avian Dis 33:545–554
8. Tang Y, Lu H (2015) Genomic characterization of a broiler reovirus field strain detected in Pennsylvania. Infect Genet Evol 31C:177–182
9. Van Der Heide L (2000) The history of avian reovirus. Avian Dis 44:638–641
10. Zhang Y, Liu M, Shuidong O, Hu QL, Guo DC, Chen HY, Han Z (2006) Detection and identification of avian, duck, and goose reoviruses by RT-PCR: goose and duck reoviruses are part of the same genogroup in the genus Orthoreovirus. Arch Virol 151:1525–1538