Development of Multi-locus Sequence Typing (MLST) Scheme for *Avibacterium paragallinarum*

Mostafa Ghanem [1, *], Alyssa Harris[1], Dhiraj Chundru [1],Madhusudan Timilsina[1], Michele Williams [1], Amro Hashish[2], Shankar Mondal[3], Daniel Bautista[4], Mohamed El-Gazzar[2]and Yan Zhang[5]

[1] Department of Veterinary Medicine, Virginia-Maryland College of Veterinary Medicine, University of Maryland, College Park, Maryland, USA
[2]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA
[3] Salisbury Animal Health Laboratory, Maryland Department of Agriculture, Annapolis, Maryland, USA
[4] Zoetis, Inc., Durham, NC 27703
[5]Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, Ohio, USA

Abstract

Avibacterium paragallinarum (*AP*) is the causative agent of Infectious Coryza (IC), a respiratory disease affecting chickens. The prevalence of IC has recently increased in both commercial and non-commercial poultry. Existing strain differentiation methods, such as classical and molecular serotyping, have limited discriminatory power for epidemiological investigations and outbreak tracking. Therefore, a more precise and practical strain-typing method is needed to enhance epidemiological studies and inform control strategies.

This study aimed to develop a Multilocus Sequence Typing (MLST) scheme for *AP* to improve strain differentiation, elucidate transmission pathways, and analyze population structure dynamics. We analyzed all available *AP* whole genome sequences (WGS) in GenBank and identified 18 candidate housekeeping genes for the MLST scheme. After screening these candidates, we selected six loci that provided the highest discriminatory power. Primers were designed and successfully amplified across a subset of isolates and PCR-positive clinical samples. Phylogenetic relationships among 75 *AP* samples, including WGS and clinical specimens, were assessed using MLST, ad hoc core genome MLST (cgMLST), and HPG2-based methods. Our MLST scheme, based on six loci, demonstrated superior discriminatory power compared to HPG2-based typing and aligned closely with ad hoc cgMLST.

In conclusion, the newly developed MLST scheme offers a robust and reliable tool for *AP* strain differentiation. It enhances epidemiological investigations, provides a standardized and portable typing system, and supports global efforts in IC prevention and control.

Key words: **MLST; *Avibacterium paragallinarum*; Coryza; Sequence typing.**

Table 1 The final six loci selected for the *A. paragallinarum* MLST scheme, their forward and reverse primers, amplicons size, final segment size, start and end point from start codon.

Gene	Locus Tag	Primer Sequence (5' to 3')	Amplicon Size (bp)	Final Segment		
				Size (bp)	Start	End
metF	EIA51_RS06635	F: GATTGATGCCACGCCTGAAG	568	495	315	810
		R: GCTGCGGTTTAGGGTGTAGA				
prfC	EIA51_RS08435	F: AGGGGCATCGAACGAATTTG	635	549	759	1308
		R: CAAATTGCAGCACACCAACC				
rpsA	EIA51_RS08385	F: AGATCCTTGGGTTGCGATTG	638	534	852	1386
		R: AACCTTCAACTCCGCCATCT				
rseA	EIA51_RS00965	F: TGATGGCGAACAAATCAGYGA	455	408	51	459
		R: GCTCAACTTGCTCTGGTGTAA				
ufp1	EIA51_RS10630	F: AGGGCTTGCTCAATTTGGTG	742	669	441	1110
		R: TAGGCGATTTCCACTGGTGT				
xseA	EIA51_RS08215	F: CGTATCGGGGCATTGGTATC	574	513	162	675
		R: AATAACGGGAATGGCGGAGT				