Epidemiological investigations and locally determined genotype diversity of *Mycoplasma synoviae* in Central China from 2017 to 2019

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ABSTRACT *Mycoplasma synoviae* (***M. synoviae***) has been identified worldwide to cause respiratory diseases, infectious synovitis, airsacculitis, and eggshell apex abnormalities (**EAA**) in commercial chickens, which results in substantial economic losses to the poultry industry. Therefore, in this study, 258 flocks were investigated between 2017 and 2019 for *M. synoviae* by screening samples from Central China. Subsequently, 129 *M. synoviae* strains were isolated, with a positive rate of 50%. Moreover, a higher incidence of *M. Synoviae* infections was in layers (74.1%) than in broilers (20%) in this study. The 5′-end conserved segment of the variable lipoprotein hemagglutinin A (***vlhA***) gene of these isolates was then cloned and sequenced because it is a common genomic target identified so far for *M. synoviae* genotyping. Genotyping of all isolates was based on the phylogenetic analysis and length analysis of the proline-rich-repeat (**PRR**) regions, respectively. Phylogenetic analysis based on 5′-end conserved segment of the *vlhA* gene (76−421 nt) assigned the majority of the occurring strains as being from group 6, and others from groups 2 and 3. Results identified that these isolates were of 6 types: A (38aa), D (23aa), E (19aa), I (28aa), J (20aa), and L (35aa), based on the size of the PRR region analysis. Furthermore, most of the isolates (81.4% were identified as type L. Additionally, the epidemic types included only I and L in 2017; however, the types rose to 5 (A, D, E, I, L) in 2018 and rose to 6 (A, D, E, I, J, L) in 2019. These data showed the genotype diversity of *M. synoviae* in Central China. The high rate of positive flocks suggests the urgent need to take real-time supervisory controls of this Mycoplasma species in avian flocks.

Key words: epidemiological investigation, *Mycoplasma synoviae*, *vlhA*, genotyping, phylogenetic analysis

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INTRODUCTION

Mycoplasmas belong to a unique group of bacteria that lack a cell wall and possess the smallest genome among all independently replicating organisms (Razin et al., 1998). *M. synoviae* is one of the 4 major pathogenic avian mycoplasma species, which causes significant economic losses during intensive poultry production (Kleven, 1997). Infection of *M. synoviae* causes synovitis in breeders, thereby resulting in reluctance to move, which negatively affects their growth and breeding performances. This infection can therefore lead to body lesions with consequent higher condemnation rates of the birds. *M. synoviae* infections can also result in subclinical upper respiratory infection, infectious synovitis, airsacculitis in broiler, and EAA in breeder and layer (Kleven, 2008; Kursa et al., 2019). *M. synoviae* has been reported in Australia, South America, Asia, Europe, and Africa and with a high worldwide prevalence in the poultry industry (Catania et al., 2019). Meanwhile, the incidence of *M. synoviae* varied with age in white-feathered chickens, turkeys, and sparrows. (Dufour-Gesbert et al., 2006; Feberwee et al., 2008; Roussan et al., 2015). Some investigations have also found that *M. synoviae* has been widely circulating in Chinese native chickens (Sun et al., 2017a, 2017b; Xue et al., 2017).

The *M. synoviae vlhA* gene encodes an abundant immunodominant surface lipoprotein. This resulting *vlhA* protein cleaves the N-terminal part of the MSPB (major surface protein B) lipoprotein and the C-terminal part of the MSPA (major surface protein A), which mediates binding to erythrocytes (Bencina et al., 2001). Nowadays, several methods have been setup for *M. synoviae* genotyping. Reference to the length of the

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sequence encoding proline-rich-repeat (**PRR**) region has also shown *M. synoviae* to be divided into groups or types (A∼N) (Bencina et al., 2001; Hammond et al., 2009; Bayatzadeh et al., 2014; Limpavithayakul et al., 2016; Felice et al., 2020). Therefore, on the basis of these different isolation regions worldwide, *M. synoviae* isolates were typed to be A∼E in the United States and Canada, F∼K in Iran, L in Thailand, as well as M and N isolated from chicken and broiler breeders in Italy (Kreizinger et al., 2018). Furthermore, multilocus sequence analysis of *M. synoviae* loci was conducted to genotype isolates expressing the previously determined 5′-*vlhA* sequences (I. Cizelj et al., 2015). In addition to the above methods, amplified fragment length polymorphism and single-strand conformation polymorphism were used to type *M. synoviae* (Feberwee et al., 2005). Previous reports have genotyped Chinese isolates of *M. synoviae* from 2013 to 2014 based on size analysis of PRR at 5′-end of *vlhA* sequences by combining phylogenetic analysis for isolates. The most frequently identified group was proposed as group K (Sun et al., 2017a). Therefore, to help to trace back genotyping of *M. synoviae* isolates and to understand the variation and evolution of *M. synoviae* in China, we conducted an epidemiological investigation and genotyping, using the size of PRR and phylogenetic analysis of 5′-end-conserved segments of the *vlhA* of *M. synoviae*, using samples from the last 3 y in Central China.

MATERIALS AND METHODS

Collection of Clinical Samples

Samples were acquired from 258 unvaccinated commercial chicken flocks in Henan (42 broiler and 59 layer flocks), Hubei (41 broiler and 35 layer flocks), and Anhui (32 broiler and 49 layer flocks) provinces of Central China from 2017 to 2019. Sampling source was chosen mainly based on clinical signs. Approximately 20 individual chickens were swabbed at the choanal cleft (for birds with EAA or without clinical signs), trachea (for birds with respiratory signs), or a joint (for birds with swollen joints) using a sterilized cotton swab. Then, *M. synoviae* strains were isolated and used to determine live-cell titers as described previously (Branton et al., 2008; Sun et al., 2017a). All *M. synoviae* cultures were stored at −80°C until further use.

DNA Extraction and Detection by PCR

The obtained *M. synoviae* culture (0.2 mL) of each clinical sample mixed with 25 μL Protease K were incubated in a shaking water bath at 55°C to complete bacteria lysis. DNA was extracted using the Easy Pure DNA Purification Kit (TransGene Biotech, Beijing, China) according to the manufacturer's instructions and subjected to partial *vlhA* gene amplification by PCR using the primer pair (forward: 5′- GGCCATTGCTCCTTC TGTTAT-3′ and reverse: 5′- CCCGTCTCAGTATA GTGTACG-3′) (Sun et al., 2017a). The PCR assay was performed in 20 μl reaction volume consisting of 1 μL DNA template; 0.4 μM primers; PCR buffer; 0.25 mM dNTIPs mixture and 1.5 U of PrimerSTAR HS DNA polymerase (TaKaRa Biotechnology, Dalian, China). The amplification protocol was performed as follows: Pre-denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52° C for 30 s, and extension at 72°C for 20 s, with a final extension at 72°C for 10 min. Additionally, partial sequences of *vlhA* from each positive sample were amplified and cloned into pMD18-T (TaKaRa Biotechnology Co., Ltd.) for Sanger sequencing in both directions (Syn-Biotechnology, Suzhou, China). PCR and sequencing were conducted at least 3 times.

Sequence Identity and Phylogenetic Analysis

Sequences of partial *vlhA* were assembled using the Seqman program of Lasergene 7.1 software package (DNA STAR Inc., Madison, WI). The *vlhA* gene of isolates and the reference sequence were multisequence aligned using the Clustal W model, and phylogenetic trees were subsequently generated via the neighbor-joining tree method with nucleotide sequence substitution of 5′-end *vlhA* with 1,000 bootstrap replicates using MEGA 7.0 software. The reference sequence was obtained from GenBank according to the published literature about genotyping of *M. synoviae* isolates.

Genotyping of M. synoviae Isolates

Besides phylogenetic analysis, typing of the *M. synoviae* strain was also conducted on the basis of PRR region's size as described previously. The size of PRR fragment of 38, 45, 32, 23, 19, 36, 51, 46, 28, 20, 12, 35, 30, and 41 amino acids were classified as type A, B, C, D, E, F, G, H, I, J, K, L, M, and N, respectively (Bencina et al., 2001).

RESULTS

Epidemic Information and Genotype of Clinical Samples

In this study, 258 flocks were investigated and 129 flocks were positive for *M. synoviae*; 129 *M. synoviae* strains were isolated in 2017 (n = 29), 2018 (n = 44), and 2019 (n = 56) from various broiler and layer farms in Anhui (65.3% for layers and 21.9% for broilers), Henan (74.6% for layers and 23.8% for broilers), and Hubei (85.7% for layers and 14.6% for broilers) provinces.

According to the samples used in this study, as shown in Tables 1−3, layers displayed a higher infection rate than broilers. Additionally, respiratory signs were more common in the clinic, and joint swellings were the main clinical signs. However, a few clinical cases manifested as EAA. Notably, chickens from 32.6% flocks that were

Table 1. Information on the isolates collected in Central China during 2017.

Production type	Strains	Origin (province)	Time#	Clinical type[a]	PRR size (aa)	Type[b]	Accession No.
Layer	17HB01	Hubei	2017.01	Joint swollen	35	L	MZ558595
Layer	17HB02	Hubei	2017.01	EAA	35	L	MZ558596
Broiler	17HB03	Hubei	2017.01	Joint swollen	35	L	MZ558597
Layer	17HB04	Hubei	2017.01	Respiratory signs	35	L	MZ558598
Broiler	17HB05	Hubei	2017.01	No clinical signs	35	L	MZ558599
Layer	17HB06	Hubei	2017.01	Respiratory signs	35	L	MZ558600
Layer	17HB07	Hubei	2017.02	No clinical signs	35	L	MZ558601
Layer	17HB08	Hubei	2017.03	Respiratory signs	35	L	MZ558602
Layer	17HB09	Hubei	2017.03	EAA	35	L	MZ558603
Broiler	17AH01	Anhui	2017.03	Joint swollen	35	L	MZ558604
Layer	17AH02	Anhui	2017.03	No clinical signs	35	L	MZ558605
Layer	17AH03	Anhui	2017.04	Respiratory signs	35	L	MZ558606
Broiler	17AH04	Anhui	2017.04	No clinical signs	35	L	MZ558607
Layer	17AH05	Anhui	2017.04	Joint swollen	35	L	MZ558608
Layer	17AH06	Anhui	2017.04	Respiratory signs	35	L	MZ558609
Broiler	17AH07	Anhui	2017.04	Respiratory signs	35	L	MZ558610
Layer	17AH08	Anhui	2017.04	EAA	35	L	MZ558611
Layer	17AH09	Anhui	2017.04	Joint swollen	28	I	MZ558612
Layer	17HN01	Henan	2017.05	No clinical signs	35	L	MZ558613
Layer	17HN02	Henan	2017.05	Joint swollen	35	L	MZ558614
Layer	17HN03	Henan	2017.05	Respiratory signs	35	L	MZ558615
Layer	17HN04	Henan	2017.05	Joint swollen	28	I	MZ558616
Layer	17HN05	Henan	2017.05	EAA	35	L	MZ558617
Broiler	17HN06	Henan	2017.05	No clinical signs	35	L	MZ558618
Layer	17HN07	Henan	2017.05	Joint swollen	35	L	MZ558619
Layer	17HN08	Henan	2017.05	Respiratory signs	35	L	MZ558620
Layer	17HN09	Henan	2017.05	Respiratory signs	35	L	MZ558621
Broiler	17HN10	Henan	2017.05	Joint swollen	35	L	MZ558622
Layer	17HN11	Henan	2017.05	EAA	35	L	MZ558623

[a]Abbreviation: EAA, eggshell apex abnormality.
[b]Genotype based on the size of the PRR region in the *vlhA* gene.

infected with *M. synoviae* did not display clinical signs, similar to all the other 129 flocks negative for *M. synoviae*.

Partial *vlhA* genes of the 129 isolated *M. synoviae* strains were sequenced and analyzed. These strains were identified as A, D, E, I, J, and L types by size analysis of the PRR regions expressing *vlhA* sequences from samples obtained in Central China between 2017 and 2019 (detailed clinical information and GenBank accession numbers of each strain are displayed in Tables 1–3); 81.4% strains were classified as type L. In 2017, only 2 genotypes of *M. synoviae* isolates were tesed, after which the genotype numbers increased to 5 in 2018 and 6 in 2019 (Figure 1).

Analysis of the Size of PRR

According to PRR length analysis results, most of the strains in the most extensive group (group 6) belonged to type L. The detailed genotype of isolates from different areas is shown in Figure 1. This study also indicated that the Anhui, Henan, and Hubei provinces had the same diversity of genotypes. Furthermore, type J was identified only in Henan Province (1 isolate), whereas, type D, the second-largest genotype was identified only in Hubei Province (4 isolates). Contrarily, type E occupied the second-largest genotype in Anhui and Henan provinces (4 and 7 isolates, respectively).

Phylogenetic Analysis

As shown in the phylogenetic tree analysis (Figure 2), all *M. synoviae* strains in this study were divided into 6 groups, including the reference strains. Most isolated strains of this study belonged to group 6, whereas other strains belonged to groups 2 and 3 (Figure 2). Furthermore, while isolates 18HB04, 19HB07, and 19HB08 (shown in Figure 2 with the red label in group 2) belonged to type D, the isolates 17HN04, 17AH09, 18AH11, 19HB06, and 19HN06 had a distant relationship with the current reference strains (detailed information of each reference strain is presented in Supplement 1).

DISCUSSION

This research was conducted to determine the genotype of *M. synoviae* isolates in Central China based on the phylogenetic tree and analysis of the size of PRR. Analyzing the size of the PRR region showed that the *M. synoviae* isolates identified in Central China were genotypes A (38aa), D (23aa), E (19aa), I (28aa), J (20aa), and L (35aa). This finding indicated that at least 6 genotypes of *M. synoviae* were circulating in Central China, with a genotype diversity increase observed over time. We propose that this increase was caused by antibody pressure from persistent infection and exchanges with other provinces or countries. Furthermore, in the 3 provinces of Anhui, Henan, and Hubei, the proportion of

Table 2. Information on the isolates collected in Central China during 2018.

Production type	Strains	Origin (province)	Time#	Clinical type[a]	PRR size (aa)	Type[b]	Accession No.
Broiler	18HB01	Hubei	2018.06	Respiratory signs	35	L	MZ558624
Layer	18HB02	Hubei	2018.06	Respiratory signs	35	L	MZ558625
Layer	18HB03	Hubei	2018.06	EAA	38	A	MZ558626
Layer	18HB04	Hubei	2018.06	No clinical signs	23	D	MZ558627
Layer	18HB05	Hubei	2018.07	Respiratory signs	35	L	MZ558628
Layer	18HB06	Hubei	2018.07	No clinical signs	35	L	MZ558629
Broiler	18HB07	Hubei	2018.07	Joint swollen	35	L	MZ558630
Layer	18HB08	Hubei	2018.07	Respiratory signs	35	L	MZ558631
Layer	18HB09	Hubei	2018.07	No clinical signs	23	D	MZ558632
Layer	18HB10	Hubei	2018.07	Respiratory signs	35	L	MZ558633
Layer	18HB11	Hubei	2018.08	EAA	35	L	MZ558634
Layer	18HB12	Hubei	2018.08	Joint swollen	35	L	MZ558635
Layer	18AH01	Anhui	2018.08	No clinical signs	35	L	MZ558636
Layer	18AH02	Anhui	2018.09	Respiratory signs	35	L	MZ558637
Layer	18AH03	Anhui	2018.09	EAA	35	L	MZ558638
Layer	18AH04	Anhui	2018.09	Respiratory signs	35	L	MZ558639
Broiler	18AH05	Anhui	2018.09	No clinical signs	35	L	MZ558640
Layer	18AH06	Anhui	2018.09	Joint swollen	35	L	MZ558641
Layer	18AH07	Anhui	2018.09	No clinical signs	35	L	MZ558642
Layer	18AH08	Anhui	2018.10	EAA	19	E	MZ558643
Layer	18AH09	Anhui	2018.10	No clinical signs	35	L	MZ558644
Layer	18AH10	Anhui	2018.10	Respiratory signs	19	E	MZ558645
Layer	18AH11	Anhui	2018.10	Joint swollen	28	I	MZ558646
Broiler	18HN01	Henan	2018.10	No clinical signs	19	E	MZ558647
Layer	18HN02	Henan	2018.10	No clinical signs	35	L	MZ558648
Layer	18HN03	Henan	2018.10	Respiratory signs	19	E	MZ558649
Layer	18HN04	Henan	2018.10	EAA	19	E	MZ558650
Layer	18HN05	Henan	2018.10	No clinical signs	35	L	MZ558651
Layer	18HN06	Henan	2018.10	Respiratory signs	35	L	MZ558652
Layer	18HN07	Henan	2018.10	EAA	35	L	MZ558653
Layer	18HN08	Henan	2018.10	Joint swollen	35	L	MZ558654
Broiler	18HN09	Henan	2018.11	No clinical signs	35	L	MZ558655
Layer	18HN10	Henan	2018.11	Respiratory signs	35	L	MZ558656
Layer	18HN11	Henan	2018.11	No clinical signs	35	L	MZ558657
Layer	18HN12	Henan	2018.11	No clinical signs	35	L	MZ558658
Broiler	18HN13	Henan	2018.11	Respiratory signs	35	L	MZ558659
Layer	18HN14	Henan	2018.11	No clinical signs	35	L	MZ558660
Layer	18HN15	Henan	2018.11	Respiratory signs	35	L	MZ558661
Layer	18HN16	Henan	2018.11	No clinical signs	35	L	MZ558662
Layer	18HN17	Henan	2018.11	No clinical signs	35	L	MZ558663
Layer	18HN18	Henan	2018.11	No clinical signs	19	E	MZ558664
Broiler	18HN19	Henan	2018.11	EAA	35	L	MZ558665
Layer	18HN20	Henan	2018.11	Joint swollen	35	L	MZ558666
Layer	18HN21	Henan	2018.12	Respiratory signs	35	L	MZ558667

[a]Abbreviation: EAA, eggshell apex abnormality.
[b]Genotype based on the size of the PRR region in the *vlhA* gene.

the L-type strain was as high as 81.4%. Therefore, genotype results showed that the L type was the most prevalent in Central China.

Different chicken products displayed different incidences postinfection by *M. synoviae*. These findings further indicated that *M. synoviae* had a lower incidence in broilers (20%, 23/115) than in layers (74.1%, 106/143) (Chaidez-Ibarra et al., 2021), thereby leading to the speculation that the broiler's lower incidence was due to the short feeding cycle. Additionally, based on the fact that *vlhA* gene encodes 2 main membrane antigens of *M. synoviae* that is, MSPA and MSPB, we propose that the complex genotypes and clinical signs in the egg layers were related to gene mutations in *M. synoviae* in flocks with common infections, and the frequent use of antibiotics during prolonged feeding (Bencina et al., 2001). Meanwhile, some *M. synoviae*-infected chicken displayed no clinical signs, which led to the spread of *M. synoviae*, thereby increasing the probability of infection and coinfection with other pathogens.

According to the phylogenetic tree analysis, the results showed that majority of these isolates belonged to group 6 according to group K in Sun's report; meanwhile, some isolates were typed as groups 2 and 3 (Bencina et al., 2001; Sun et al., 2017a). Isolates of group 2 caused no clinical signs, and all isolates of group 3 showed clinical signs in swollen joints; meanwhile, respiratory signs were the dominant symptom caused by isolates in group 6. Interestingly, although most of the strains had close genetic distances, they were isolated from different provinces. Some strains of *M. synoviae*, also harbored genetic distances, although they were isolated from the same region. These findings indicated that geography was not significantly related to genotype.

As reported previously, types C, E, F, and L isolates of *M. synoviae* induced EAA syndrome-like Dutch *M. synoviae* type C and E isolates, however, their forms

Table 3. Information on the isolates collected in Central China during 2019.

Production type	Strains	Origin (province)	Time#	Clinical type[a]	PRR size (aa)	Type[b]	Accession No.
Layer	19HB01	Hubei	2019.01	Respiratory signs	35	L	MZ558668
Layer	19HB02	Hubei	2019.01	Respiratory signs	35	L	MZ558669
Layer	19HB03	Hubei	2019.01	No clinical signs	35	L	MZ558670
Layer	19HB04	Hubei	2019.01	EAA	35	L	MZ558671
Broiler	19HB05	Hubei	2019.01	No clinical signs	35	L	MZ558672
Layer	19HB06	Hubei	2019.01	Joint swollen	28	I	MZ558673
Layer	19HB07	Hubei	2019.01	No clinical signs	23	D	MZ558674
Layer	19HB08	Hubei	2019.01	No clinical signs	23	D	MZ558675
Layer	19HB09	Hubei	2019.01	Joint swollen	35	L	MZ558676
Layer	19HB10	Hubei	2019.01	EAA	35	L	MZ558677
Layer	19HB11	Hubei	2019.01	Respiratory signs	35	L	MZ558678
Broiler	19HB12	Hubei	2019.01	Respiratory signs	35	L	MZ558679
Layer	19HB13	Hubei	2019.02	Joint swollen	35	L	MZ558680
Layer	19HB14	Hubei	2019.02	No clinical signs	35	L	MZ558681
Layer	19HB15	Hubei	2019.02	Respiratory signs	35	L	MZ558682
Broiler	19AH01	Anhui	2019.02	Joint swollen	35	L	MZ558683
Layer	19AH02	Anhui	2019.02	No clinical signs	19	E	MZ558684
Layer	19AH03	Anhui	2019.02	No clinical signs	35	L	MZ558685
Layer	19AH04	Anhui	2019.02	Respiratory signs	19	E	MZ558686
Layer	19AH05	Anhui	2019.02	No clinical signs	35	L	MZ558687
Layer	19AH06	Anhui	2019.02	EAA	35	L	MZ558688
Layer	19AH07	Anhui	2019.02	Respiratory signs	35	L	MZ558689
Layer	19AH08	Anhui	2019.03	Joint swollen	38	A	MZ558690
Layer	19AH09	Anhui	2019.03	No clinical signs	35	L	MZ558691
Layer	19AH10	Anhui	2019.03	Respiratory signs	35	L	MZ558692
Layer	19AH11	Anhui	2019.03	Joint swollen	35	L	MZ558693
Broiler	19AH12	Anhui	2019.03	Joint swollen	35	L	MZ558694
Layer	19AH13	Anhui	2019.03	No clinical signs	38	A	MZ558695
Layer	19AH14	Anhui	2019.03	No clinical signs	35	L	MZ558696
Layer	19AH15	Anhui	2019.03	Respiratory signs	35	L	MZ558697
Layer	19AH16	Anhui	2019.04	No clinical signs	35	L	MZ558698
Broiler	19AH17	Anhui	2019.04	Joint swollen	35	L	MZ558699
Layer	19AH18	Anhui	2019.04	Joint swollen	35	L	MZ558700
Layer	19AH19	Anhui	2019.04	Respiratory signs	35	L	MZ558701
Layer	19HN01	Henan	2019.04	No clinical signs	35	L	MZ558702
Layer	19HN02	Henan	2019.04	No clinical signs	35	L	MZ558703
Layer	19HN03	Henan	2019.04	Respiratory signs	35	L	MZ558704
Layer	19HN04	Henan	2019.04	EAA	35	L	MZ558705
Layer	19HN05	Henan	2019.04	Respiratory signs	35	L	MZ558706
Broiler	19HN06	Henan	2019.04	Joint swollen	28	I	MZ558707
Layer	19HN07	Henan	2019.04	No clinical signs	19	E	MZ558708
Layer	19HN08	Henan	2019.04	Joint swollen	35	L	MZ558709
Layer	19HN09	Henan	2019.04	No clinical signs	20	J	MZ558710
Layer	19HN10	Henan	2019.04	Respiratory signs	19	E	MZ558711
Layer	19HN11	Henan	2019.04	No clinical signs	35	L	MZ558712
Layer	19HN12	Henan	2019.04	Respiratory signs	19	E	MZ558713
Layer	19HN13	Henan	2019.05	EAA	35	L	MZ558714
Broiler	19HN14	Henan	2019.05	Joint swollen	35	L	MZ558715
Layer	19HN15	Henan	2019.05	No clinical signs	35	L	MZ558716
Layer	19HN16	Henan	2019.05	Respiratory signs	35	L	MZ558717
Layer	19HN17	Henan	2019.05	Joint swollen	35	L	MZ558718
Layer	19HN18	Henan	2019.05	No clinical signs	35	L	MZ558719
Broiler	19HN19	Henan	2019.06	Joint swollen	35	L	MZ558720
Broiler	19HN20	Henan	2019.07	Joint swollen	35	L	MZ558721
Layer	19HN21	Henan	2019.07	EAA	35	L	MZ558722
Layer	19HN22	Henan	2019.07	Respiratory signs	35	L	MZ558723

[a]Abbreviation: EAA, eggshell apex abnormality.
[b]Genotype based on the size of the PRR region in the *vlhA* gene.

were different from those of Thai *M. synoviae* isolates (Limpavithayakul et al., 2016). In this study, 106 *M. synoviae* strains included 6 genotypes, including genotypes A, D, E, I, J, and L (35aa). Some reports have found that a more extended PRR region was associated with higher invasiveness (Bencina et al., 2001; Sun et al., 2017a). Furthermore, pathogenic analysis of animal experiments in Sun's paper found that highly pathogenic strains, CHN-HN03 (KU572344) and CHN-QZ-ZZX (KU572380) with a size of 35aa, were larger than those of the mildly pathogenic CHN-GX-NN01 (KU572288) with a size of 19aa. However, the pathogenicity of CHN-FJ-ZZ01 (KU572307) was opposite to that of CHN-HN03 (KU572344) and CHN-QZ-ZZX (KU572380) (Sun et al., 2017a). A previous study reported that *M. synoviae* for type L causes infectious synovitis in chickens besides type B, which has a long PRR region (Limpavithayakul et al., 2016). The clinical signs induced by isolates in this study indicated that *M. synoviae* for all Type D with a PRR size of 23 aa caused no clinical signs; Some isolates of type E (19 aa) caused no clinical signs and others induced respiratory signs

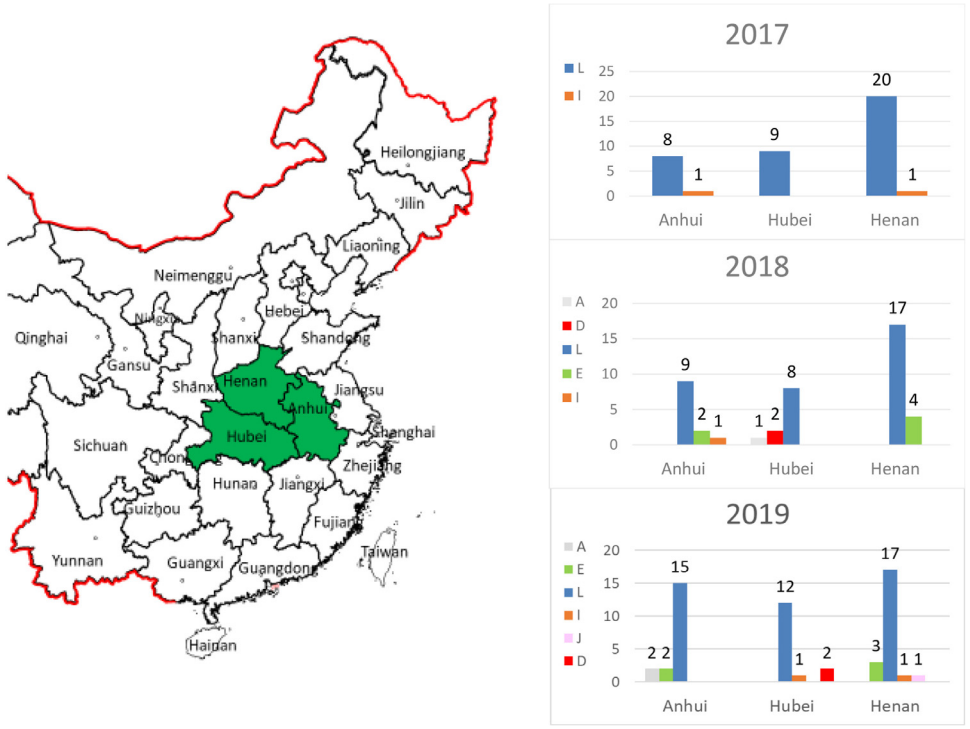

Figure 1. Genotype diversity of *Mycoplasma synoviae* strains from Central China between 2017 and 2019.

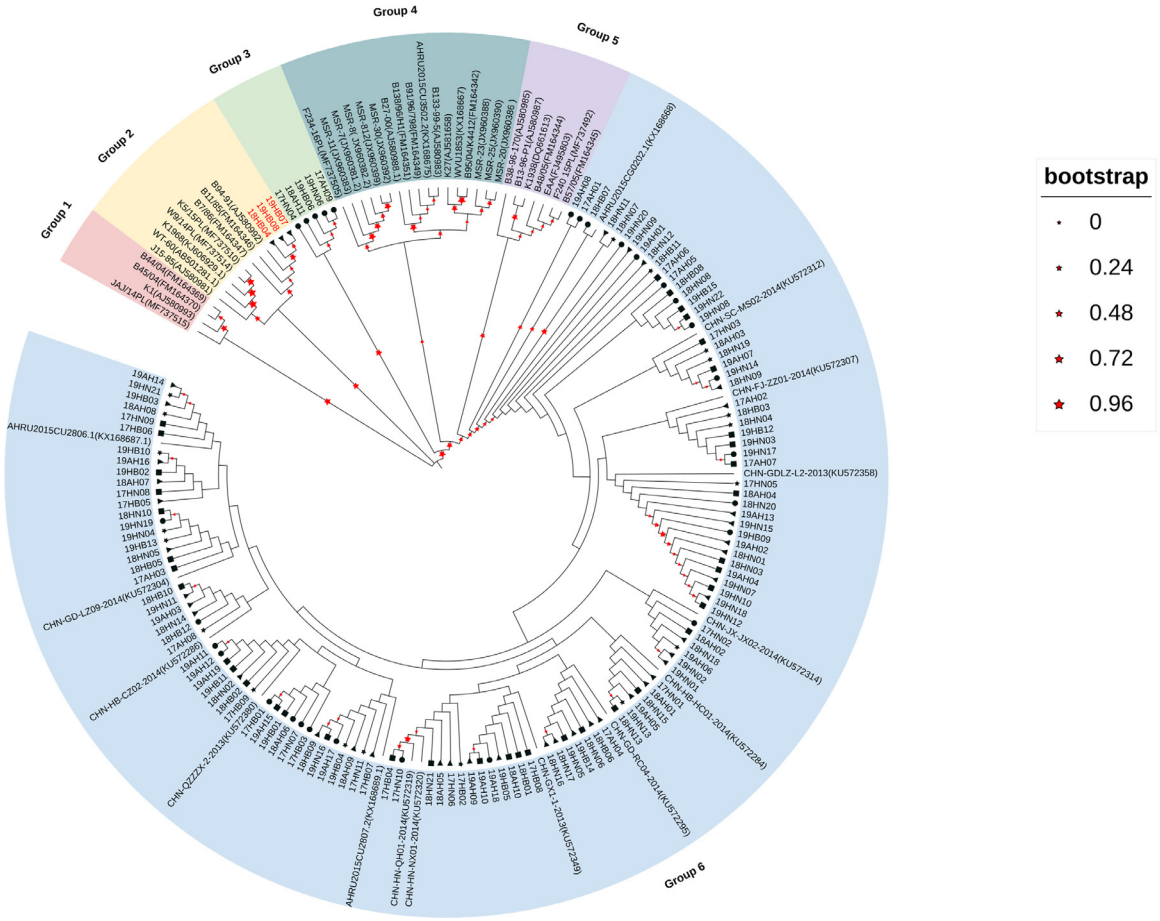

Figure 2. Phylogenetic tree of *vlhA* partial sequences of *Mycoplasma synoviae* isolated in Central China from 2017 to 2019. The filled squares for respiratory disease, filled circles for synovitis, filled stars for EAA and right pointing triangle for no clinical signs.

and EAA. Type I (28 aa) showed only swollen joints, and respiratory signs are the main clinical signs of the L type along with swollen joints, EAA *M. synoviae* for type A has a long PRR region (38 aa) that caused swollen joints and EAA. Meanwhile, this investigation proposes that the Central China *M. synoviae* types E and L isolates in layers caused EAA and respiratory tract diseases, which is consistent with other research (Limpavithayakul et al., 2016). These results suggested the size of the PRR region was related to pathogenicity. By contrast, layers infected by *M. synoviae* isolate (19AH13) for type A (38 aa) displayed no clinical signs. It was also speculated that other genes are also involved in the pathogenicity of *M. synoviae*.

In conclusion, this study provides a theoretical basis and technical guidance for genotyping *M. synoviae* strains from Central China. Nevertheless, the diversity of genotypes suggests that adequate control methods are required in this country. We also established that genotype analysis by the size of PRR amino acid sequences is more precise and intuitional.

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Ethical Statement: The autopsy and sampling protocols for dead birds were approved by the South China Agricultural University Committee for Animal Experiments (approval ID: SYXK-2014-0136).

Data Availability Statement: All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

DISCLOSURES

The authors have no competing interests to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101522.

REFERENCES

Bayatzadeh, M. A., S. A. Pourbakhsh, A. Ashtari, A. R. Abtinand, and M. Abdoshah. 2014. Molecular typing of Iranian field isolates Mycoplasma synoviae and their differentiation from the live commercial vaccine strain MS-H using vlhA gene. Br. Poult. Sci. 55:148–156.

Bencina, D., M. Drobnic-Valic, S. Horvat, M. Narat, S. H. Klevenand, and P. Dovc. 2001. Molecular basis of the length variation in the N-terminal part of Mycoplasma synoviae hemagglutinin. FEMS Microbiol. Lett. 203:115–123.

Branton, S. L., S. A. Leigh, W. B. Roush, J. L. Purswell, H. A. Olanrewaju, and S. D. Collier. 2008. Effect of selected water temperatures used in Mycoplasma gallisepticum vaccine reconstitution on titer at selected time intervals. Avian Dis 52:291–296.

Catania, S., M. Bottinelli, M. Gastaldelli, A. Fincato, and G. F. Barberio A. 2019. Evaluation of Minimum Inhibitory Concentrations for 154 Mycoplasma synoviae isolates from Italy collected during 2012-2017. PLoS One 14:e0224903–2017.

Chaidez-Ibarra, M. A., D. Z. Velazquez, D. C. N. C. Enriquez-Verdugo Idalia, M. A. Rodriguez-Gaxiola Miguel Angel, and G. S. M. Diaz Daniel. 2021. Pooled molecular occurrence of Mycoplasma gallisepticum and Mycoplasma synoviae in poultry: a systematic review and meta-analysis [e-pub ahead of print]. Transboundary Emerg. Dis., doi:10.1111/tbed.14302, accessed November 15, 2021.

Cizelj, I., R. L. Berčič, M. Narat, B. Slavecand, P. Dovč, and D. Benčina. 2015. Multilocus sequence analysis for Mycoplasma synoviae molecular genotyping. Br. Poult. Sci. 56:658–665.

Dufour-Gesbert, F., A. Dheilly, C. Maroisand, and I. Kempf. 2006. Epidemiological study on Mycoplasma synoviae infection in layers. Vet. Microbiol. 114:148–154.

Feberwee, A., T. S. de Vriesand, and W. J. Landman. 2008. Seroprevalence of Mycoplasma synoviae in Dutch commercial poultry farms. Avian Pathol 37:629–633.

Feberwee, A., J. R. Dijkstra, T. E. von Banniseht-Wysmuller, A. L. Gielkensand, and J. A. Wagenaar. 2005. Genotyping of Mycoplasma gallisepticum and M. synoviae by Amplified Fragment Length Polymorphism (AFLP) analysis and digitalized Random Amplified Polymorphic DNA (RAPD) analysis. Vet. Microbiol. 111:125–131.

Felice, V., C. Lupini, G. Mescolini, F. Silveira, A. Guerrini, E. Catelliand, and A. Di Francesco. 2020. Molecular detection and characterization of Mycoplasma gallisepticum and Mycoplasma synoviae strains in backyard poultry in Italy. Poult. Sci. 99:719–724.

Hammond, P. P., A. S. Ramírez, C. J. Morrowand, and J. M. Bradbury. 2009. Development and evaluation of an improved diagnostic PCR for Mycoplasma synoviae using primers located in the haemagglutinin encoding gene vlhA and its value for strain typing. Vet. Microbiol. 136:61–68.

Kleven, S. H. 2008. Control of avian mycoplasma infections in commercial poultry. Avian Dis 52:367–374.

Kleven, S. H 1997. Mycoplasma synoviae infection. Pages 220−228 in Diseases of Poultry. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald and Y. M. Saif, eds. Iowa State University Press, Ames, IA.

Kreizinger, Z., K. M. Sulyok, K. Bekő, Á. B. Kovács, D. Grózner, O. Felde, S. Marton, K. Bányai, S. Catania, D. Benčinaand, and M. Gyuranecz. 2018. Genotyping Mycoplasma synoviae: development of a multi-locus variable number of tandem-repeats analysis and comparison with current molecular typing methods. Vet. Microbiol. 226:41–49.

Kursa, O., G. Tomczykand, and A. Sawicka. 2019. Prevalence and phylogenetic analysis of Mycoplasma synoviae strains isolated from Polish chicken layer flocks. J. Vet. Res. 63:41–49.

Limpavithayakul, K., J. Sasipreeyajanand, and S. Pakpinyo. 2016. Characterization of Thai Mycoplasma synoviae isolates by sequence analysis of partial vlhA gene. Avian Dis 60:810–816.

Razin, S., D. Yogevand, and Y. Naot. 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62:1094–1156.

Roussan, D. A., G. Khawaldehand, and I. A. Shaheen. 2015. A survey of Mycoplasma gallisepticum and Mycoplasma synovaie with

avian influenza H9 subtype in meat-type chicken in Jordan between 2011−2015. Poult. Sci. 94:1499–1503.

Sun, S., X. Lin, J. Liu, Z. Tian, F. Chen, Y. Cao, J. Qin, and T. Luo. 2017a. Phylogenetic and pathogenic analysis of Mycoplasma synoviae isolated from native chicken breeds in China. Poult. Sci 96:2057–2063.

Sun, S. K., X. Lin, F. Chen, D. A. Wang, J. P. Lu, J. P. Qinand, and T. R. Luo. 2017b. Epidemiological investigation of Mycoplasma Synoviae in native chicken breeds in China. BMC Vet. Res. 13:115.

Xue, J., M. Y. Xu, Z. J. Ma, J. Zhao, N. Jinand, and G. Z. Zhang. 2017. Serological investigation of Mycoplasma synoviae infection in China from 2010 to 2015. Poult. Sci. 96:3109–3112.