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

**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
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 bacteri 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’- GGCCATTGCTCCTTCTGTTAT-3’ and reverse: 5’- CCGCTCTCAAGTGCTACAGTGAC-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 dNTPs 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
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 tested, 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
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

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

| Production type | Strains Code | Origin (province) | Time# | Clinical type | PRR size (aa) | Type | 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 | Respiratory signs | 19 | E | MZ558646 |
| Layer          | 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.
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.

| Production type | Strains | Origin (province) | Time# | Clinical typea | PRR size (aa) | Typeb | 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     |
| Layer 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 19HB01  | Anhui   | 2019.02           |       | Joint swollen   | 35  | L    | MZ558683     |
| Layer 19HB02    | Anhui   | 2019.02           |       | No clinical signs | 19  | E    | MZ558684     |
| Layer 19HB03    | Anhui   | 2019.02           |       | No clinical signs | 35  | L    | MZ558685     |
| Layer 19HB04    | Anhui   | 2019.02           |       | Respiratory signs | 19  | E    | MZ558686     |
| Layer 19HB05    | Anhui   | 2019.02           |       | No clinical signs | 35  | L    | MZ558687     |
| Layer 19HB06    | Anhui   | 2019.02           |       | EAA            | 35  | L    | MZ558688     |
| Layer 19HB07    | Anhui   | 2019.02           |       | Respiratory signs | 35  | L    | MZ558689     |
| Layer 19HB08    | Anhui   | 2019.03           |       | Joint swollen   | 38  | A    | MZ558690     |
| Layer 19HB09    | Anhui   | 2019.03           |       | No clinical signs | 35  | L    | MZ558691     |
| Layer 19HB10    | Anhui   | 2019.03           |       | Respiratory signs | 35  | L    | MZ558692     |
| Layer 19HB11    | Anhui   | 2019.03           |       | Joint swollen   | 35  | L    | MZ558693     |
| Broiler 19HB12  | Anhui   | 2019.03           |       | Joint swollen   | 35  | L    | MZ558694     |
| 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     |

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*aAbbreviation: EAA, eggshell apex abnormality. 
bGenotype based on the size of the PRR region in the *vlhA* gene.*
Figure 1. Genotype diversity of *Mycoplasma synoviae* strains from Central China between 2017 and 2019.

Figure 2. Phylogenetic tree of *vhlA* 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 genotyping is more by the size of PRR amino acid sequences is more precise and intuitive.

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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

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