



## Comparison the pathogenicity of three *Mycoplasma synoviae* isolates from broiler flocks

GONG Xiao-Wei<sup>1</sup> CHEN Qi-Wei<sup>1</sup> Ferguson-Noel Naola<sup>2</sup> ZHENG Fu-Ying<sup>\*1</sup>  
Stipkovits Laszlo<sup>3</sup> Szathmary Susan<sup>3</sup> LIU Yong-Sheng<sup>\*1</sup>

<sup>1</sup> State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu 730046, China

<sup>2</sup> Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens 30602, USA

<sup>3</sup> RT-Europe Non-profit Research Center, Mosonmagyaróvár 9200, Hungary

**Abstract:** [Background] *Mycoplasma synoviae* (MS) is an important causative agent infecting chicken, which causes enormous losses to the poultry industry in China. [Objective] This study was designed to evaluate the pathogenicity of three isolates from MS outbreak farms, which could enrich our understanding about pathogenicity of MS isolates from different districts. [Methods] Systemic MS infection was induced experimentally in commercial broiler chickens with recent MS isolates (CHN-WF224-2016, CHN-BZJ2-2015 and CHN-JNB19-2016). The virulence of each strain was evaluated by detecting air sac and foot pad lesions, serologic response, tracheal mucosal thickness and MS isolation rates from the infected chicken at 10 and 21 days post-infection and comparing these results with those obtained from a live attenuated vaccine strain (MS-H) and uninfected controls. [Results] Isolates CHN-BZJ2-2015 and CHN-WF224-2016 induced foot pad lesions and typical infectious synovitis, especially CHN-BZJ2-2015 group. Serologic response and tracheal mucosal thickness measurements at 21 days post infection indicated that CHN-BZJ2-2015 isolate was significantly more virulent than the CHN-JNB19-2016 isolate and MS-H strain ( $P < 0.05$ ). [Conclusion] The variation in virulence of MS isolates emphasizes the importance of active, on-going control and prevention of MS in the chicken flocks of China and accumulates experimental data for vaccine development.

**Keywords:** Broiler, *Mycoplasma synoviae*, Pathogenicity

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\*Corresponding authors: Tel: 86-931-8342706

E-mail: ZHENG Fu-Ying: zhengfuying@caas.cn; LIU Yong-Sheng: liuyongsheng@caas.cn

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\*通信作者: Tel: 0931-8342706;

E-mail: 郑福英: zhengfuying@caas.cn; 刘永生: liuyongsheng@caas.cn

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### 3 株禽滑液囊支原体分离株致病性的比较和评价

官晓炜<sup>1</sup> 陈启伟<sup>1</sup> Ferguson-Noel Naola<sup>2</sup> 郑福英<sup>\*1</sup> Stipkovits Laszlo<sup>3</sup>  
Szathmary Susan<sup>3</sup> 刘永生<sup>\*1</sup>

1 中国农业科学院兰州兽医研究所 家畜疫病病原生物学国家重点实验室 甘肃 兰州 730046

2 美国佐治亚大学 人口健康部禽病诊断与研究中心 美国 雅典城 30602

3 生物防护和传染病防控欧洲三角中心 匈牙利 莫逊马雅罗瓦 9200

**摘要:**【背景】禽滑液囊支原体是感染鸡的一种重要病原体, 给我国的家禽养殖业带来了严重的经济损失。【目的】评价从病鸡中分离到的3株禽滑液囊支原体的致病性, 以期丰富不同区域来源的分离株对鸡致病性的认识。【方法】分别用分离株 CHN-WF224-2016、CHN-BZJ2-2015、CHN-JNB19-2016 感染商品肉鸡, 并在攻毒后第10天和21天进行实验室解剖, 通过气囊和足垫的临床病变、血清学检测结果及气管粘膜病理形态学改变来比较评价分离株的致病力。【结果】CHN-BZJ2-2015 和 CHN-WF224-2016 分离株能引起明显足垫肿胀和关节滑膜炎, 其中 CHN-BZJ2-2015 分离株致病力最强, 感染21 d后的血清学检测和气管粘膜厚度与 CHN-JNB19-2016 分离株和 MS-H 疫苗株比较差异显著( $P < 0.05$ )。【结论】本研究表明我国目前流行的禽滑液囊支原体菌株其致病力存在明显差异, 强调养殖场要积极控制和预防禽滑液囊支原体感染的重要性, 同时为今后该疫苗的研发积累了实验数据。

**关键词:** 肉鸡, 禽滑液囊支原体, 致病力

#### 1 Introduction

*Mycoplasma synoviae* (MS) is an important pathogen that causes economic losses to poultry industry<sup>[1]</sup>. Infection can also be associated with upper respiratory disease, airsacculitis, synovitis, tenosynovitis and bursitis, which lead to lameness, pale comb and retarded growth of chickens and turkeys. Severe swelling of joints and feet may be observed in infected poultry and usually noticed as disease progresses<sup>[2]</sup>. Besides, eggshell apex abnormality described as a novel presentation of MS infection. Disease severity has been influenced by other respiratory pathogens, such as Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and avian influenza virus (AIV). A number of studies have demonstrated a synergistic role of viruses and *E. coli* in avian mycoplasma disease in increasing the pathogenicity of mycoplasma and causing serious clinical manifestations and mortality rates in poultry worldwide<sup>[3-5]</sup>.

Infectious synovitis was first described and associated with mycoplasma infection in the early 1950's<sup>[6]</sup>, before the organism, MS, was identified<sup>[7]</sup>. Subsequently, since the expansion of the poultry

industry, MS appeared to have worldwide distribution and its importance has been highlighted in several studies and there is an increased consciousness to generate MS-free poultry<sup>[8-9]</sup>. In China, the prevalence of MS infection was investigated from 2013 to 2014, and 110 MS strains which belong to K group through *vlhA* gene sequencing were isolated from 16 Chinese provinces. While MS constitutes a single serotype, there are significant differences in the virulence and pathogenicity of strains. Some MS strains appeared subclinical or inapparent infection of the upper respiratory tract and exhibited lower morbidity rate, and some MS strains can cause synovitis and result in a decrease of egg production rate, hatchability rates and high morbidity rates<sup>[10-11]</sup>.

The evaluation of pathogenicity of MS strains is based on clinical signs, the pathological changes of tracheal mucosal thickness, analysis of foot-pad lesion, specific serology, isolation and molecular diagnosis<sup>[12]</sup>. As data are lacking in China, the aim of this study was to compare and characterize the pathogenicity of three MS isolates (CHN-WF224-2016, CHN-BZJ2-2015, CHN-JNB19-2016) from Shandong province and the attenuated live vaccine strain (MS-H).

## 2 Materials and Methods

### 2.1 MS isolates

Three field strains (CHN-WF224-2016, CHN-BZJ2-2015, CHN-JNB19-2016) were isolated from MS-infected Chinese native broiler chickens from different farms located in 3 cities (one isolate per city) of Shandong province between 2015 to 2016 according to the procedures as described previously<sup>[13-14]</sup>. Three isolates were subject to 16S rRNA gene (from PDRC, UGA, unpublished) and portion of *vlhA* gene PCR<sup>[1]</sup>. The nucleotide sequences of 16S rRNA and *vlhA* gene were determined and have been deposited in GenBank under following accession numbers: KY750311–KY750313 and MF073022–MF073024, and BLAST analysis of these sequences showed that three field isolates belong to MS. MS-H live vaccine strain was deposited in our laboratory at Lanzhou veterinary research institute. Approximately 1 mL of growing culture was sprayed per chicken, 100  $\mu$ L of broth culture was inoculated into the left foot pad, and NDV/IBV live nasal vaccine which was purchased from QiLu DOBIO Co., Ltd. was inoculated 30  $\mu$ L. The MS titer of each inoculum (color changing units (CCU)/mL) was determined as previously described<sup>[15]</sup>.

### 2.2 Experimental birds and designs

All animal procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Lanzhou veterinary research institute, CAAS. Ninety-six 1-day-old broiler chicks were purchased from Lanzhou Zhengda Chicken Co., Ltd. and randomly divided into six groups with each group of 16 at 11 days age. All chickens in this study were provide with feed and water ad libitum and were euthanized by carbon dioxide before necropsy. At 21 days of age, 15 chickens were randomly selected and tested by ELISA and culture of the trachea swabs to confirm that chicks were *Mycoplasma* negative, and same time, four groups of 16 chicks each were inoculated (aerosol and foot pad) with broth culture of CHN-WF224-2016 ( $2.2 \times 10^8$  CCU/mL), CHN-BZJ2-2015 ( $2.65 \times 10^8$  CCU/mL), CHN-JNB19-2016 ( $3.36 \times 10^8$  CCU/mL) and MS-H ( $7.2 \times 10^8$  CCU/mL). The live attenuated temperature-sensitive MS-H vaccine strain used in this experiment was cultured at 33 °C, other isolates at 37 °C. These MS-inoculated groups and a control group of 16 chickens were also infected with

Newcastle disease (La Sota strain) and infectious bronchitis virus (H120 strain) by nasal drop. A sixth group of 16 chickens served as negative control. The chickens were observed by air sac and foot pad lesion scoring, bled for serology, tracheal swabs and laryngeal wash were obtained for culture and real-time quantitative PCR (qPCR), and tracheal sections for histopathology at 10 days and 21 days post infection (DPI).

### 2.3 Isolation and identification of mycoplasma

Cotton swabs from tracheal and air sacs of chickens were inoculated onto modified Frey's agar and broth at 37 °C. Colonies were confirmed as MS using portion of *vlhA* gene PCR and sequencing<sup>[1]</sup>.

### 2.4 Serology

The commercial mycoplasma synoviae or gallisepticum antibody test kit (IDEXX, Westbrook, Maine) was used to detect antibody titers following the manufacture's instruction.

### 2.5 qPCR

At 10 days and 21 days post challenge, the larynxes of individual birds were collected in 5 mL sterile PBS. qPCR was carried out using primers and a procedure described by Raviv<sup>[16]</sup>. Genomic DNA was extracted from 200  $\mu$ L of the laryngeal wash using the QIAamp cador Pathogen Mini Kit. Real-time PCR was performed using a Agilent Technologies Stratagene Mx3000P and a cycle threshold (Ct) value  $\leq 36$  was considered positive, and the standard plasmid was constructed containing MS genome target (16S-23S rDNA ISR).

### 2.6 Evaluation of lesions

The lesions in chickens necropsied during the study were evaluated grossly by foot-pad lesions scoring on a scale from 0 to 4<sup>[17]</sup>. The tracheal lesions were evaluated microscopically by measuring the width of the tracheal mucosa. A section was collected from the upper third of trachea and fixed in 4% paraformaldehyde. The tracheal mucosa thickness was measured at four equidistant points on histological slides of cross sections of tracheas<sup>[18]</sup>.

### 2.7 Statistical analysis

The air sac lesion scores, foot-pad lesion scores and MS isolation were analyzed using the Kruskal-Wallis Rank Sums test. The mean tracheal thickness and mean copy numbers (MCNs) Log<sub>10</sub> were analyzed using the Tukey-kramer honest significant difference test. These analyses were performed using SPSS software (version

19.0) and R software, respectively.

### 3 Results and Analysis

#### 3.1 Serology

The serological results for this trial are presented in Table 1. All chickens tested pre-inoculation and the groups that were uninfected with MS strain were negative for mycoplasma gallisepticum (MG) and MS antibodies. The group inoculated with MS-H and CHN-JNB19-2016 did not seroconvert strongly, with the exception of the chickens that were infected with CHN-WF224-2016 and CHN-BZJ2-2015 at 10 or 21DPI.

#### 3.2 Footpad lesions

As presented in Table 2, foot pad lesions were observed in all of the infected groups at 10 or 21 DPI. The most severe lesions were observed in the group inoculated with CHN-BZJ2-2015. Mean footpad lesion scores were significantly greater compare with CHN-JNB19-2016 and MS-H group ( $P<0.05$ ). There were no footpad lesions in the control groups (Figure 1).

#### 3.3 Air sac lesions

The mean air sac lesion scores are summarized in Table 2. Although the air sac lesion were more severe in the CHN-WF224-2016 and CHN-BZJ2-2015 group, the mean lesion scores of infected groups were not significant difference after pairwise comparison ( $P>0.05$ ), but were significantly higher than control groups ( $P<0.05$ ).

**Table 1 Serological response from broiler chickens at 10 and 21 DPI infection with MS isolates<sup>1</sup>**

表 1 感染 MS 第 10 天和第 21 天的血清学反应<sup>1</sup>

MS strain	NDV/IBV	DPI	ELISA <sup>2&amp;3</sup>
None	No	10	0/8 (0.28) <sup>a</sup>
None	Yes	10	0/8 (0.29) <sup>a</sup>
MS-H	Yes	10	1/8 (0.39) <sup>ab</sup>
CHN-WF224-2016	Yes	10	8/8 (0.92) <sup>bc</sup>
CHN-BZJ2-2015	Yes	10	8/8 (1.05) <sup>c</sup>
CHN-JNB19-2016	Yes	10	3/8 (0.43) <sup>ab</sup>
None	No	21	0/8 (0.26) <sup>a</sup>
None	Yes	21	0/8 (0.31) <sup>a</sup>
MS-H	Yes	21	5/8(0.55) <sup>ab</sup>
CHN-WF224-2016	Yes	21	8/8 (1.75) <sup>bc</sup>
CHN-BZJ2-2015	Yes	21	8/8 (2.1) <sup>c</sup>
CHN-JNB19-2016	Yes	21	7/8(0.94) <sup>ab</sup>

Note: <sup>1</sup>: Values within a column with a different lower case superscript are significantly different ( $P<0.05$ ); <sup>2</sup>: No. of positive samples/No. of tested samples (ELISA: S/P>0.5); <sup>3</sup>: S/P ratio.

注: <sup>1</sup>: 最后一列数字的上标, 小写字母不同代表有显著性差异 ( $P<0.05$ ); <sup>2</sup>: 阳性样本数量/检测样本总数(ELISA 检测结果, S/P>0.5 为阳性); <sup>3</sup>: S/P 的平均值。

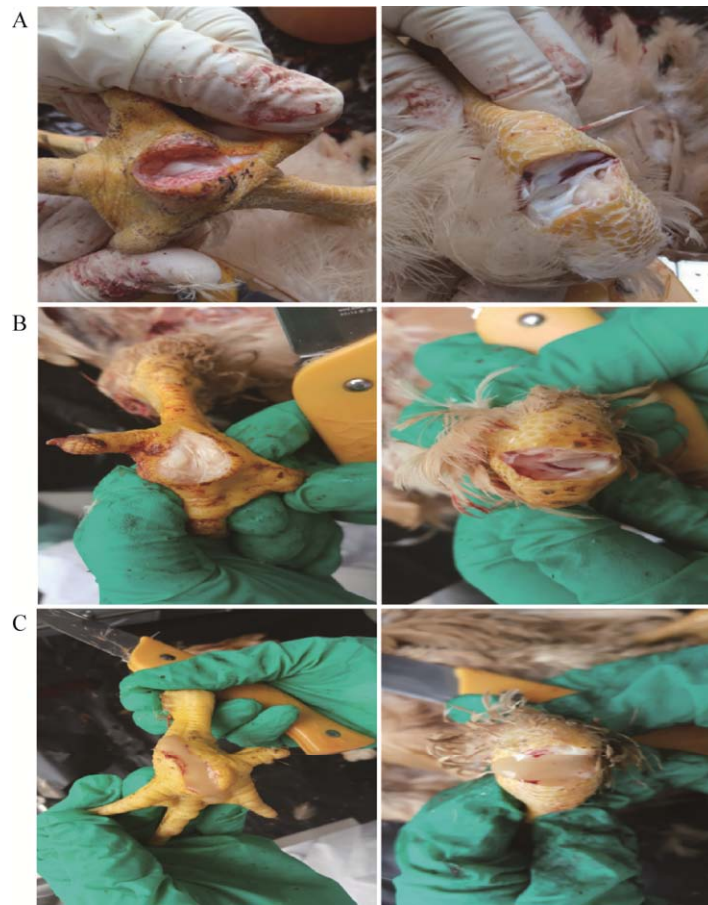
**Table 2 Air sac and footpad lesion scores, tracheal mucosa thickness, MS isolation and qPCR results from broiler chickens at 10 and 21 DPI infection with MS isolates<sup>1</sup>**

表 2 感染 MS 第 10 天和第 21 天的气囊及足垫临床打分、粘膜厚度、MS 分离率和 qPCR 结果<sup>1</sup>

MS strain	NDV/IBV	DPI	Air sac lesion score <sup>2&amp;3</sup>	Foot pad lesion score <sup>2&amp;3</sup>	Tracheal mucosal thickness <sup>4</sup>	MS isolation <sup>2</sup>	qPCR <sup>2&amp;5</sup>
None	No	10	0/8 (0±0) <sup>a</sup>	0/8 (0±0) <sup>a</sup>	116±38 <sup>a</sup>	0/8 <sup>a</sup>	0/8 (0.0) <sup>a</sup>
None	Yes	10	0/8(0±0) <sup>a</sup>	0/8 (0±0) <sup>a</sup>	128±52 <sup>a</sup>	0/8 <sup>a</sup>	0/8 (0.0) <sup>a</sup>
MS-H	Yes	10	2/8 (0.3±0.5) <sup>ab</sup>	2/8 (0.2±0.4) <sup>a</sup>	159±35 <sup>ab</sup>	0/8 <sup>a</sup>	6/8 (4.7±1.9) <sup>b</sup>
CHN-WF224-2016	Yes	10	6/8 (1.1±0.8) <sup>b</sup>	7/8 (1.1±0.6) <sup>bc</sup>	183±71 <sup>ab</sup>	6/8 <sup>b</sup>	8/8 (6.7±0.5) <sup>c</sup>
CHN-BZJ2-2015	Yes	10	7/8 (1.4±0.9) <sup>b</sup>	8/8 (1.6±0.7) <sup>c</sup>	242±58 <sup>b</sup>	8/8 <sup>b</sup>	8/8 (6.9±0.4) <sup>c</sup>
CHN-JNB19-2016	Yes	10	4/8 (0.6±0.7) <sup>ab</sup>	3/8 (0.4±0.5) <sup>ab</sup>	164±92 <sup>ab</sup>	3/8 <sup>ab</sup>	8/8 (7.1±0.6) <sup>c</sup>
None	No	21	0/8 (0±0) <sup>a</sup>	0/8(0±0) <sup>a</sup>	111±43 <sup>a</sup>	0/8 <sup>a</sup>	0/8 (0.0) <sup>a</sup>
None	Yes	21	0/8(0±0) <sup>a</sup>	0/8 (0±0) <sup>a</sup>	146±60 <sup>a</sup>	0/8 <sup>a</sup>	0/8 (0.0) <sup>a</sup>
MS-H	Yes	21	0/8 (0±0) <sup>a</sup>	4/8 (0.5±0.5) <sup>ab</sup>	163±53 <sup>ab</sup>	2/8 <sup>a</sup>	6/8 (4.6±1.9) <sup>b</sup>
CHN-WF224-2016	Yes	21	5/8 (1.0±0.9) <sup>ab</sup>	8/8 (1.8±0.7) <sup>bc</sup>	200±61 <sup>ab</sup>	8/8 <sup>b</sup>	8/8 (6.0±0.7) <sup>b</sup>
CHN-BZJ2-2015	Yes	21	7/8 (1.5±0.7) <sup>b</sup>	8/8 (2.6±0.7) <sup>c</sup>	304±76 <sup>c</sup>	8/8 <sup>b</sup>	8/8 (6.1±0.6) <sup>b</sup>
CHN-JNB19-2016	Yes	21	4/8 (0.6±0.7) <sup>ab</sup>	5/8 (0.6±0.5) <sup>ab</sup>	148±62 <sup>a</sup>	5/8 <sup>ab</sup>	6/8 (4.7±2.9) <sup>b</sup>

Note: <sup>1</sup>: Values within a column with a different lower case superscript are significantly different ( $P<0.05$ ); <sup>2</sup>: No. of positive samples/No. of tested samples; <sup>3</sup>: Mean score±SD; <sup>4</sup>: Mean thickness for the group in micrometers±SD; <sup>5</sup>: Mean DNA copy number (MCN) Log<sub>10</sub>±SD.

注: <sup>1</sup>: 每列数字的上标, 小写字母不同代表有显著性差异 ( $P<0.05$ ); <sup>2</sup>: 阳性样本数量/检测样本总数; <sup>3</sup>: 均数±标准差; <sup>4</sup>: 每组的平均气管粘膜厚度±标准差; <sup>5</sup>: DNA 平均拷贝数(log<sub>10</sub>)±标准差。



**Figure 1** Foot pad lesions in MS strains challenged and non-challenged chicks

**图 1** MS 攻毒组和未攻毒组的足垫损伤

Note: A: Lesion score 0, No foot-pad lesion was observed; B: Lesion score 1, Foot-pad slightly inflamed, with a slightly increased amount of cloudy joint fluid; C: Lesion score 3, Foot-pad markedly inflamed, with thickened and hyperemic footpad tissue.

注: A: 0分, 正常足垫; B: 1分, 足垫有轻微的肿胀, 伴有少量的炎性渗出; C: 3分, 足垫明显肿胀, 有明显的炎性渗出和充血.

### 3.4 Tracheal lesions

As shown in Table 2, the Mean tracheal mucosal thickness measurements of the infected groups were not significantly different from each other at 10 DPI ( $P>0.05$ ). The CHN-BZJ2-2015 group had a significantly higher measurement than other infected groups and control groups ( $P<0.05$ ) at 21 DPI (Figure 2).

### 3.5 MS isolation

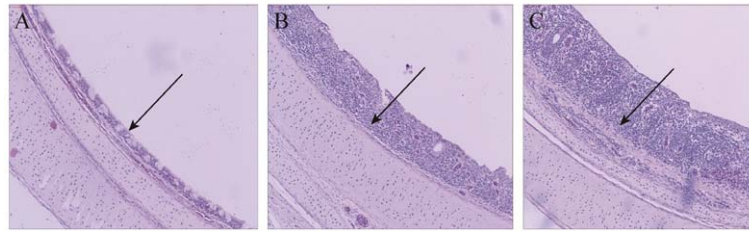
MS was isolated from the joint fluid of 75% (6/8), 100% (8/8) and 37.5% (3/8) of the chickens in the CHN-WF224-2016, CHN-BZJ2-2015 and CHN-JNB19-2016 groups at 10 DPI, respectively. MS isolated from MS-H group were significantly fewer compared to CHN-WF224-2016 or CHN-BZJ2-2015 groups ( $P<0.05$ ) at 21 DPI.

### 3.6 qPCR

MS was detected in the all chicken laryngeal wash of CHN-WF224-2016 and CHN-BZJ2-2015 groups by Real-time PCR at 10 and 21 DPI, while the 87.5% (7/8) of MS-H group was positive at 10 and 21 DPI. CHN-JNB19-2015 group was tested 100% (8/8) and 75% (6/8) positive at 10 and 21 DPI, respectively. Mean DNA copy numbers in the CHN-WF224-2016, CHN-BZJ2-2015 and CHN-JNB19-2015 were significantly higher than the MS-H group at 10 DPI ( $P<0.05$ ).

## 4 Discussion and Conclusion

Over the last decade, China has become the second largest poultry production country in the world. Almost 22% of meat in China is from chickens, behind pork. Based on literature data<sup>[11,19]</sup>, the number



**Figure 2 The tracheal mucosa thickness observation in MS strains challenged and non-challenged chicks**

**图 2 MS 攻毒组和未攻毒组的气管粘膜厚度观察**

Note: A: Normal tracheal mucosa; B: Tracheal mucosa of low pathogenicity MS strains challenged chicks, showing normal ciliated columnar epithelial lining and goblet cell with a single focus of leucocyte infiltration; C: Tracheal mucosa of high pathogenicity MS strain challenged chicks, showing squamous to cuboidal epithelium and marked thickening of mucosa associated with severe diffuse leucocyte infiltration.

注: A: 正常粘膜; B: 低致病力 MS 攻毒组, 支气管粘膜可见较正常的假复层纤毛柱状上皮细胞和杯状细胞, 有少量白细胞浸入; C: 高致病力 MS 攻毒组, 可见气管黏膜表面假复层纤毛柱状上皮结构丧失, 出现鳞状上皮化生, 有大量炎性细胞浸入, 气管粘膜明显增厚。

of MS infection in veterinary clinic has risen since 2008 in China, with an increasing trend on the year by year. MS can result in a variety of different signs such as depression, decreased feed intake, breathing difficulty, nasal discharge, diarrhea, weight loss and lameness. Between 2013 and 2014, MS infection was circulating in Chinese native-type chickens, affecting their productivity and causing the loss of millions of chickens in Chinese poultry farms. Unlike USA, UK and Netherlands<sup>[20]</sup>, China Lacks eradication programmes to establish MS-free chicken flock, so antimicrobial drugs, such as tiamulin, tylosin, enrofloxacin and florfenicol were used for treatment of MS infection in chickens. The live, attenuated, temperature-sensitive MS vaccine strain MS-H is used to control virulent MS infection in commercial chicken flocks, but rarely used in China. Research shows that although MS-H vaccination may help to reduce or prevent clinical signs, it does not prevent infection with field MS strain<sup>[21-22]</sup>.

Presently, MS outbreaks majorly in poultry industry and causes enormous economic loss in China. However, no efficient vaccine is successfully developed and put into use in China. In order to screen candidate for further studying the live attenuated or inactivated vaccine, the virulence of the epidemic MS strains were evaluated in this study. The systematic analysis of pathogenicity of Chinese MS isolates can provide experimental data and materials to develop more effective MS vaccine.

Obtained results showed that three isolates (CHN-WF224-2016, CHN-BZJ2-2015, CHN-JNB19-

2016) originally obtained from Chinese native chickens are significant differences in virulence. Although these isolates belonged to same genotype, K group, there was no correlation between pathogenicity and genotype for MS, which is consistent with the findings of previous study<sup>[11]</sup>.

In this study, chickens were infected systemically by a combination of the left footpad and aerosol routes to evaluate respiratory and synovitis virulence. Besides, MS strains challenge with live NDV/IBV vaccination was employed in this trial, and a NDV/IBV control group was incorporated. This challenge method has been demonstrated to increase the incidence and severity of mycoplasma lesions<sup>[23]</sup>. All groups challenged with MS in this study showed mild respiratory signs, but joint and footpad swelling were more severe in CHN-WF224-2016 and CHN-BZJ2-2015 group. The chickens from CHN-BZJ2-2015 group showed lameness and had significantly lower weight gains than other group at 21DPI, suggesting relatively high virulence.

Serological titers (ELISA), footpad lesion scores, tracheal mucosal thickness measurements, MS isolation and qPCR copy numbers was highest in the CHN-BZJ2-2015 group, compare with MS-H and control groups. While air sac lesion scores were numerically greatest in the CHN-BZJ2-2015 group, this difference lacked statistical significance, which may be related with the number of birds and wide variation in scores in each of the respiratory challenge groups.

Footpad inoculation is an accepted method to

determining the ability of MS to cause synovitis. Lesions of synovitis were reproduced in groups challenged with the MS isolates, but footpad lesion scores were greatest in the CHN-BZJ2-2015 group, which was consistent with the clinical symptom when we sampled in the field.

MS was readily recovered from the tracheas from MS-infected groups. In this study, MS isolated earlier from the trachea of chickens in CHN-BZJ2-2015 and CHN-WF224-2016 groups at 10 or 21 DPI, compared with the MS-H or CHN-JNB19-2015 group, which was the same as the results of serological test, chickens were serologically positive earlier and s/p ratio were higher in the CHN-BZJ2-2015 and CHN-WF224-2016 group. The “Hot” field strains of MS elicited stronger serological reactions than less virulent MS strains<sup>[24]</sup>, so these trends likely reflect that CHN-BZJ2-2015 and CHN-WF224-2016 had more invasiveness and fecundity, compared with CHN-JNB19-2015 or MS-H.

The qPCR conducted on laryngeal wash from each group was positive in CHN-BZJ2-2015 and CHN-WF224-2016 groups at 10 and 21 DPI except for MS-H or CHN-JNB19-2015 group at 10 or 21 DPI, even though MS isolation, which may suggest that CHN-JNB19-2015 strain similar to MS-H strain had low virulence and did not colonize the upper respiratory system completely. The previous study has shown that the difference in ability of a temperature-sensitive mycoplasma vaccine to colonize the respiratory system was related to the different administered routes which may result from variation in the number of organisms deposited in the respiratory system<sup>[25]</sup>. MS-H vaccine can colonize the respiratory system of chickens after eyedrop inoculation<sup>[26]</sup>.

In conclusion, the recent MS isolates had strong virulence, and especially CHN-BZJ2-2015 isolate was clearly capable of inducing synovitis and weight loss. Although these isolates had a limited ability to induce lesion and changes in air sac, this result may not be representative of field condition. Further work will be required to fully elucidate the transmission of these isolate which may spread into susceptible chickens through horizontal transmission and influence egg production through vertical

transmission. In the meantime, it is an important that MS control and eradication programme should be enforced in China based on early detection of infected flocks by regular monitoring and a series of control measures.

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