Wang *et al. Animal Diseases* (2022) 2:28
https://doi.org/10.1186/s44149-022-00060-w

Animal Diseases

ORIGINAL ARTICLE

Open Access

Identification and antibiotic susceptibility evaluation of *Mycoplasma synoviae* isolated from chickens in central China

Chun Wang[1,2,3†], Naiji Zhou[4†], Haopeng Liu[1,3,4], Rongkun Yang[1,2,3], Weitao Cui[1,2,3], Qingrong Xu[1,2,3], Yuncai Xiao[1,2,3], Sishun Hu[1,2,3*], Rui Zhou[1,2,3], Zili Li[1,2,3] and Zutao Zhou[1,2,3*]

Abstract

Mycoplasma synoviae (*M. synoviae*) infections have become an increasingly serious concern in China because they cause huge economic losses to the poultry industry. Antibiotic treatment is one of control strategies that can be used to contain clinical outbreaks in *M. synoviae*-free flocks, especially because the bacteria can be transmitted through eggs. To understand *M. synoviae* infection status in farms of central China and the antibiotic susceptibility of the circulating strains *in vivo* and *in vitro*, 485 samples were collected from five provinces from 2019 to 2021. Fifty-two strains were isolated and identified. Determination of the minimum inhibitory concentration (MIC) of eight antibiotics (tylvalosin, tiamulin, tilmicosin, lincomycin, enrofloxacin, chlortetracycline, doxycycline and tylosin) for isolates showed that tylvalosin, doxycycline and tiamulin were effective against 52 clinical isolates (MIC values ≤ 0.0625–0.25 μg/mL, ≤0.0625–1 μg/mL, and 0.25–2 μg/mL, respectively). Tilmicosin, enrofloxacin and lincomycin had high MIC_{90} values (>32 μg/mL). An artificial *M. synoviae* infection model was established in chickens for evaluation of the short-term therapeutic effect of these antibiotics. After 5 days of medication, doxycycline (200 mg/L) showed a superior ability to inhibit *M. synoviae* compared with other groups, as did tylvalosin (200 mg/L). Furthermore, the therapeutic efficacy of tylvalosin (0.4 μg/mL) on intra-embryo-injected *M. synoviae* was higher than that of tiamulin at the same dose. A combination of MIC values determined *in vitro* and therapeutic effects observed *in vivo* revealed that tylvalosin and doxycycline had the best therapeutic effects. Tylvalosin also showed better inhibitory effects on the vertical transmission of *M. synoviae* than tiamulin.

Keywords: *Mycoplasma synoviae*, Antibiotics, Minimum inhibitory concentration, Therapeutic effect, Drug evaluation

Introduction

Mycoplasma synoviae (*M. synoviae*) is one of the main pathogens causing mycoplasma disease in poultry, which usually results in acute or chronic synovitis or air sacculitis (Lockaby et al. 1998). More importantly, *M. synoviae* infection can lead to reduced egg production, eggshell apex abnormality, or subclinical infections in commercial egg layers, which causes substantial economic losses (Feberwee et al. 2009; Catania et al. 2010). *M. synoviae* can be transmitted not only horizontally by contacting contaminated equipment and air but also vertically by breeding eggs (Landman and Feberwee 2012; Koutoulis et al. 2013). Compared with simple *M. synoviae* infections, *M. synoviae* mixed with other pathogen, such as avian influenza virus, infectious bronchitis virus, infectious bursal disease virus, and *Escherichia coli*, lead to more serious respiratory or whole-body symptoms, air

†Chun Wang and Naiji Zhou are co-first authors of the article.

*Correspondence: husishun74@mail.hzau.edu.cn; ztzhou@mail.hzau.edu.cn

[3] Key Lab of Preventive Veterinary Medicine of Hubei Province, Huazhong Agricultural University, Wuhan 430070, Hubei, China
Full list of author information is available at the end of the article

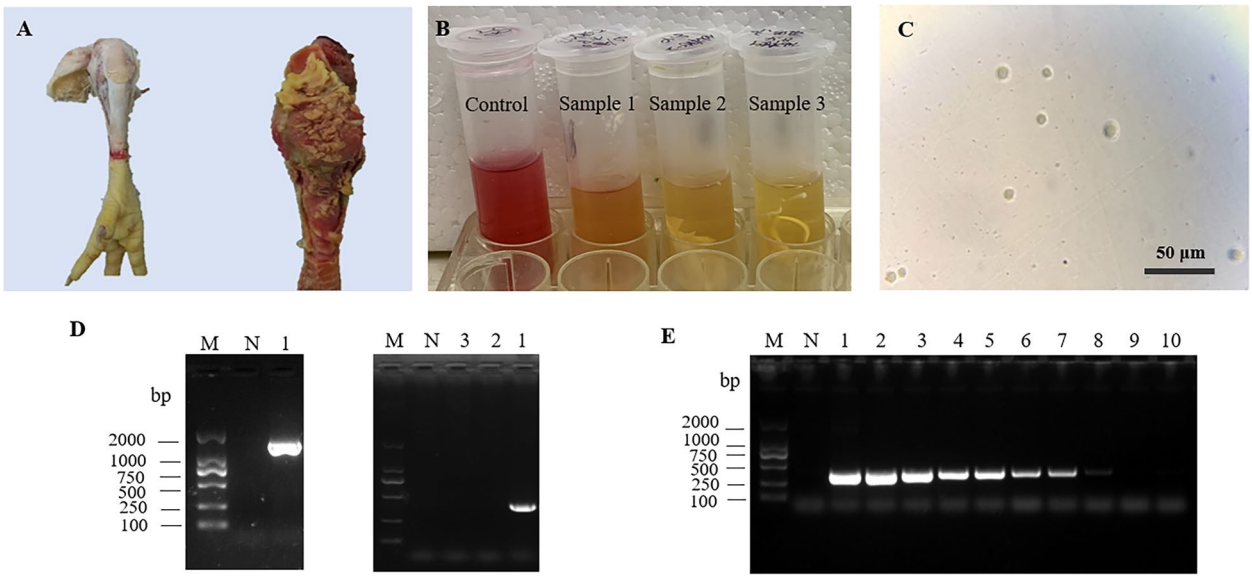

Fig. 1 Isolation and identification of *M. synoviae*. **A** Treatment of suspected diseased chicken tarsal joint; **B** Culture of isolates; **C** Colony morphology of isolated strains (40× magnification); **D** Specificity test; 1: MS-HB1 strain; 2: *M. gallisepticum* strain; 3: *E. coli* strain; **E** Susceptibility test; 1-10: dilutions from 10^{-1} to 10^{-10}. M: DL2000 DNA Marker (Tsingke Biotechnology Co., Ltd., Beijing, China). *vlh*A gene product fragment size: 392 bp; 16S rDNA product fragment size: 1500 bp

sac lesions, and eggshell apex abnormalities (Raviv et al. 2007; Fiorentin et al. 2013).

The main methods currently used to the long term control of the disease strict biosecurity measures, eradication programs involving the depopulation of infected animals, and vaccination; while short-term control methods usually applies antibiotic treatment (Dijkman et al. 2017; Feberwee et al. 2017; Kreizinger et al. 2017; Shahid et al. 2018), such as tilmicosin, enrofloxacin and oxytetracycline. However, antibiotic treatment can only mitigate the clinical symptoms and is not sufficient for the eradication of infection in affected flocks (Kleven 2008). This may be due to the chronic infection of *M. synoviae* and the high levels of microorganisms in the environment (Marois et al. 2000). Molecular mechanism underlying the acquired antibiotic resistance of *M. synoviae* has been reported (Le Carrou et al. 2006; Lysnyansky et al. 2013); therefore, understanding antibiotic susceptibility of prevailing strains and therapeutic effects of antibiotics are critical to improve management by drug therapy.

In this study, we conducted antibiotic susceptibility tests on 52 *M. synoviae* strains isolated and identified in central China from 2019 to 2021. We also evaluated the efficacy of tylvalosin and tiamulin in blocking *M. synoviae* infection in chicken embryos to provide a basis for clinical drug selection for the short-term control of poultry infections in central China.

Results

Isolation and identification of clinical samples

In total, 52 *M. synoviae* isolates (52/485, 10.72%) were purified from 485 tarsal tissue and secretion samples collected from (Fig. 1A-C) five provinces [Henan, Hubei, Anhui, Hunan, and Jiangxi (16.67%, 11.71%, 9.09%, 10%, and 6.25%, respectively)] in China (Fig. 2). The specificity (Fig. 1D) and susceptibility (Fig. 1E) were verified by PCR. Homology was 93–99% between these 52 samples and MS-H strain registered in GenBank (Registration No. CP021129.1). Titers of the 52 strains ranged from 10^5 to 10^7 color change units (CCU)/mL.

Determination of the minimum inhibitory concentration

The MIC results sorted by drug are shown in Table 1. All isolates showed a concordance of MIC results among replicates.

Most of the 36 *M. synoviae* isolates (69.2%) had tylosin MIC values of <1 μg/mL, eight isolates (15.4%) had MIC values of 1 μg/mL, and eight isolates (15.4%) were between 1-4 μg/mL. MIC values of tylosin for the strains isolated in Anhui Province (2-4 μg/mL) were higher than those isolated from the other provinces.

MIC values of tylvalosin and tiamulin were ≤0.25 μg/mL and 2 μg/mL for all *M. synoviae* isolates, respectively, of which 28 isolates (53.8%) had tylvalosin MIC values lower than or equal to the lowest concentration of the antibiotic present in the test (0.0625 μg/mL). Doxycycline MIC values were ≤1 μg/mL for all *M. synoviae* isolates,

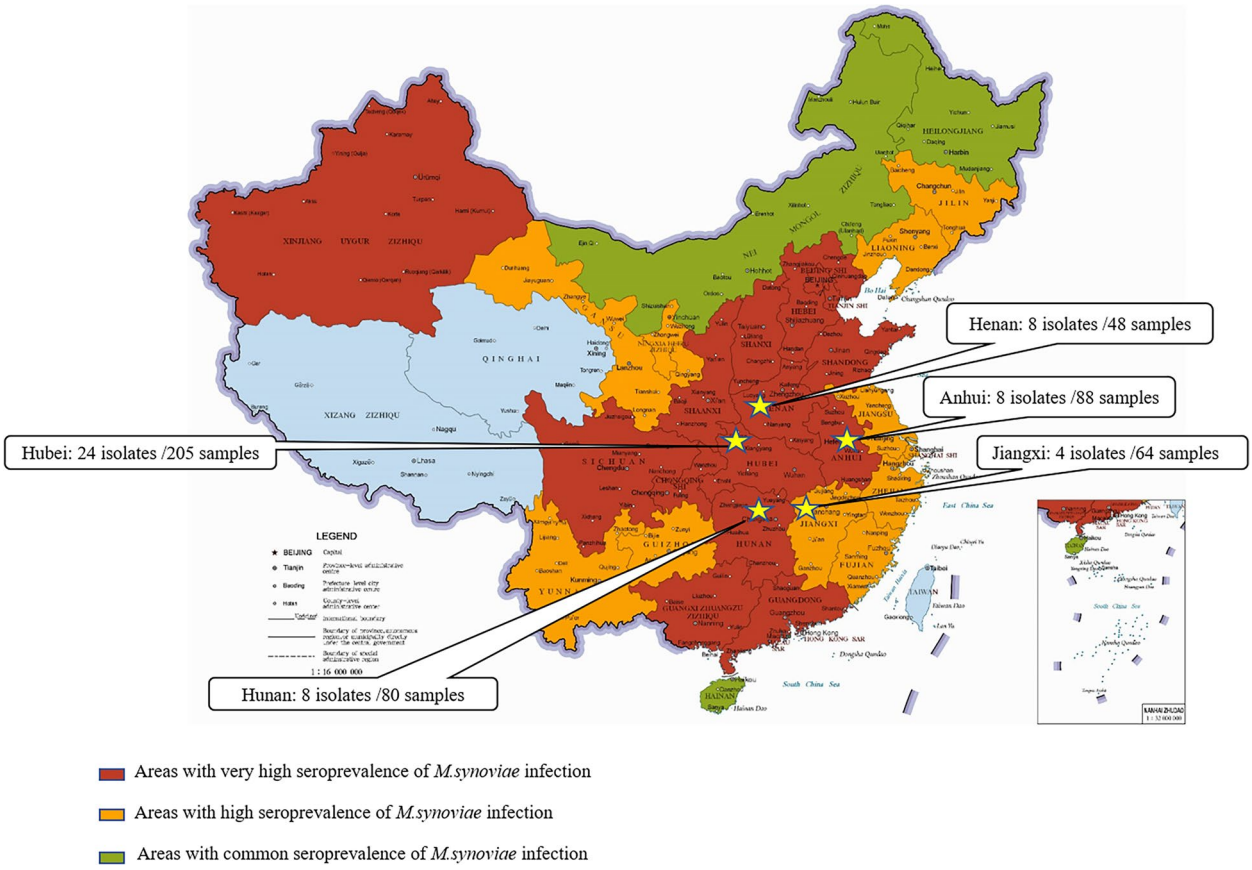

Fig. 2 Regional distribution of *M. synoviae* isolates and seroprevalence of *M. synoviae* infection in different provinces of China. The number of samples collected in each province and the isolates are shown in boxes. Censor code of this map: GS (2019) 1695

Table 1 Distribution of minimum inhibitory concentration (MIC, μg/mL) of antibiotics for strains isolated from different provinces of China

Antibiotics	Provinces					MIC_{50}	MIC_{90}
	Hubei	Henan	Hunan	Anhui	Jiangxi		
Tylosin	0.125–1	1	0.25	2–4	0.25–0.5	0.25	4
Tylvalosin	≤0.0625–0.25	0.125	≤0.0625	0.25	≤0.0625	≤0.0625	0.25
Tiamulin	0.25–1	0.5	0.5–1	0.25–2	0.25	0.5	1
Tilmicosin	1–8	>32	8	>32	2–4	2	>32
Enrofloxacin	16–>32	8	16	16->32	16	16	>32
Lincomycin	0.5–2	>32	0.5–1	>32	0.5–1	1	>32
Doxycycline	≤0.0625–0.25	≤0.0625	0.5–1	≤0.0625–0.25	0.5–1	0.125	0.5
Chlortetracycline	0.25–4	0.25	8–16	0.5–1	4–8	1	8

Antibiotics were tested in the concentration range 0.0625–32 μg/mL. All MIC values are expressed in μg/mL and values that were below or above the dilution range are marked as ≤ or >, respectively. MIC_{50} values indicate the concentration that inhibited 50% isolates. MIC_{90} values indicate the concentration that inhibited 90% isolates

of which 30 (57.7%) had MIC values of 0.0625 μg/mL. The MIC_{50} and MIC_{90} values of tylvalosin were the lowest registered, together with those of doxycycline and tiamulin.

Ten isolates (19.2%) had tilmicosin MIC values of ≤1 μg/mL and 36 isolates (69.2%) were ≤2 μg/mL. MIC value of 22 isolates (42.3%) were 2 μg/mL, and 20 isolates (38.5%) were over 4 μg/mL, of which 16 (30.8%) were greater than the highest concentration of antibiotic used in this test (32 μg/mL). There were marked differences in the lincomycin MIC values for all 52 strains, with a difference of at least seven serial dilutions between the maximum MIC value (>32 μg/mL) and the minimum MIC value (0.5 μg/mL). MIC values of enrofloxacin were ≥8 μg/mL for all isolates. MIC_{50} and MIC_{90} values of enrofloxacin were the highest registered, together with those of tilmicosin and lincomycin. MIC values of tilmicosin, enrofloxacin and lincomycin for the strains isolated from Anhui and Henan were higher than those isolated from the other provinces.

A marked variation was observed in MIC values of chlortetracycline for these strains, ranging from 0.25 μg/mL to 16 μg/mL. MIC values of chlortetracycline for isolates from Hunan and Jiangxi provinces were higher than those isolated from the other provinces.

Evaluation of antibiotic treatment effect

The treatment effects of antibiotics were evaluated by measuring changes in number and percentage of *M. synoviae*-positive and *M. synoviae*-negative chickens before and after treatment. One day before treatment, 9/20 to 12/20 of the chickens in each group were infected with *M. synoviae*.

On the first day after drug withdrawal and in all treatment groups except the tylvalosin low-dose group, number and percentage of *M. synoviae*-positive chickens decreased noticeably compared with that of the day before treatment ($P < 0.05$). In the tylvalosin medium-dose, tylvalosin high-dose, tylosin, doxycycline, tiamulin and enrofloxacin groups, the number of *M. synoviae*-positive chickens decreased significantly ($P < 0.01$) (Fig. 3A).

Seven days after drug withdrawal, number and percentage of *M. synoviae*-positive chickens increased in the tylvalosin medium-dose, tylosin and tiamulin groups, but these decreased compared with that of the day before treatment ($P < 0.05$) (Fig. 3B).

Fourteen days after drug withdrawal, *M. synoviae*-positive chickens increased in doxycycline group but decreased significantly compared with the day before treatment ($P < 0.01$) (Fig. 3C).

Evaluation of the ability of antibiotics to block vertical transmission of *M. synoviae*

On the third day of treatment, the number and percentage of *M. synoviae*-positive individuals noticeably decreased in the 0.4 μg/mL tylvalosin group compared with the infection group ($P < 0.05$) (Fig. 4A).

On the fifth day of treatment, the number and percentage of *M. synoviae*-positive animals noticeably decreased in each treatment group ($P < 0.05$) but decreased significantly in the 0.4 μg/mL tylvalosin group ($P < 0.01$) (Fig. 4B).

Discussion

In a serological survey of *M. synoviae* infections among chickens in different regions of China from 2010 to 2015, the overall seropositive rate of *M. synoviae* infections in 21 provinces was 41.19% (Xue et al. 2017), which confirmed that *M. synoviae* infections are very common among poultry in China. Antibiotic treatment represents an effective strategy for the control of *M. synoviae* infections in chickens (Sun et al. 2017; Sui et al. 2022). In the context of the global concerns surrounding antibiotics, it is essential to use antibiotics correctly and effectively.

In this study, we investigated the incidence and regional distribution of *M. synoviae* infections in 485 samples collected from poultry-breeding farms in central China. By the end of the sampling period, 52 strains of *M. synoviae* were isolated. The relatively low rate of isolations may indicate that ongoing antibiotic treatments are effective or the infections were in a chronic state. The fact that *Mycoplasma* cultures are easily contaminated is also a potential reason.

In recent years, drug resistance and the rational use of antibiotics have attracted an increasing amount of attention all over the world. In this study, the antibiotic susceptibility of 52 *M. synoviae* strains isolated from central China were analyzed and found that tiamulin, tylvalosin and doxycycline had low MIC_{90} for isolates from all regions, while MIC values were higher for the macrolide tilmicosin against *M. synoviae*. The macrolides show good activity against *M. synoviae* strains worldwide, with higher MIC values (> 2 μg/mL) identified in Europe (Hannan 2000; Kreizinger et al. 2017; Abd El-Hamid et al. 2019). MIC values of enrofloxacin, tilmicosin and lincomycin in this study were similar to those reported by Catania in a study of the effects of 10 antibiotics on 154 *M. synoviae* isolates collected from Italy during 2012 to 2017. In this report, seven isolates showed MIC values of >32 μg/mL for lincomycin, and they also showed high MIC values for other tested drugs. The highest MIC values (>32 μg/mL) for lincomycin always coincided with high MIC values for tilmicosin. Strains isolated from Anhui and Henan had similar profiles to these seven strains. Enrofloxacin had high MIC values for all *M. synoviae* isolates (Catania et al. 2019). Zhang found that enrofloxacin generally had high MIC values for some of the *M. synoviae* isolates (Zhang et al. 2022). A comparison of the *par*C quinolone

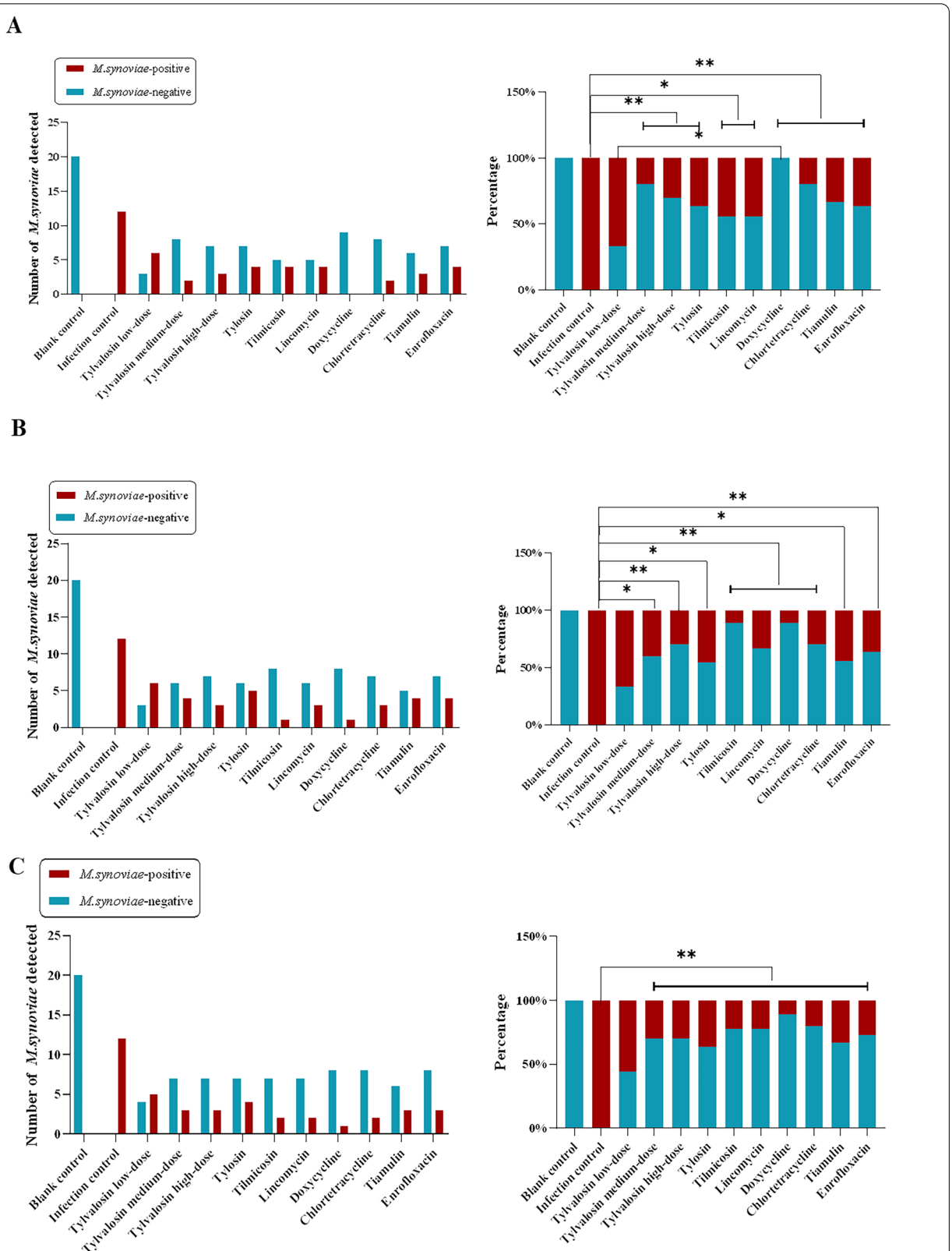

Fig. 3 Number and percentage of *M. synoviae* detected after drug withdrawal. **A** First day after drug withdrawal; **B** Seventh day after drug withdrawal; **C** Fourteenth day after drug withdrawal. Asterisks in the figures indicate significant differences (*$P < 0.05$; ** $P < 0.01$)

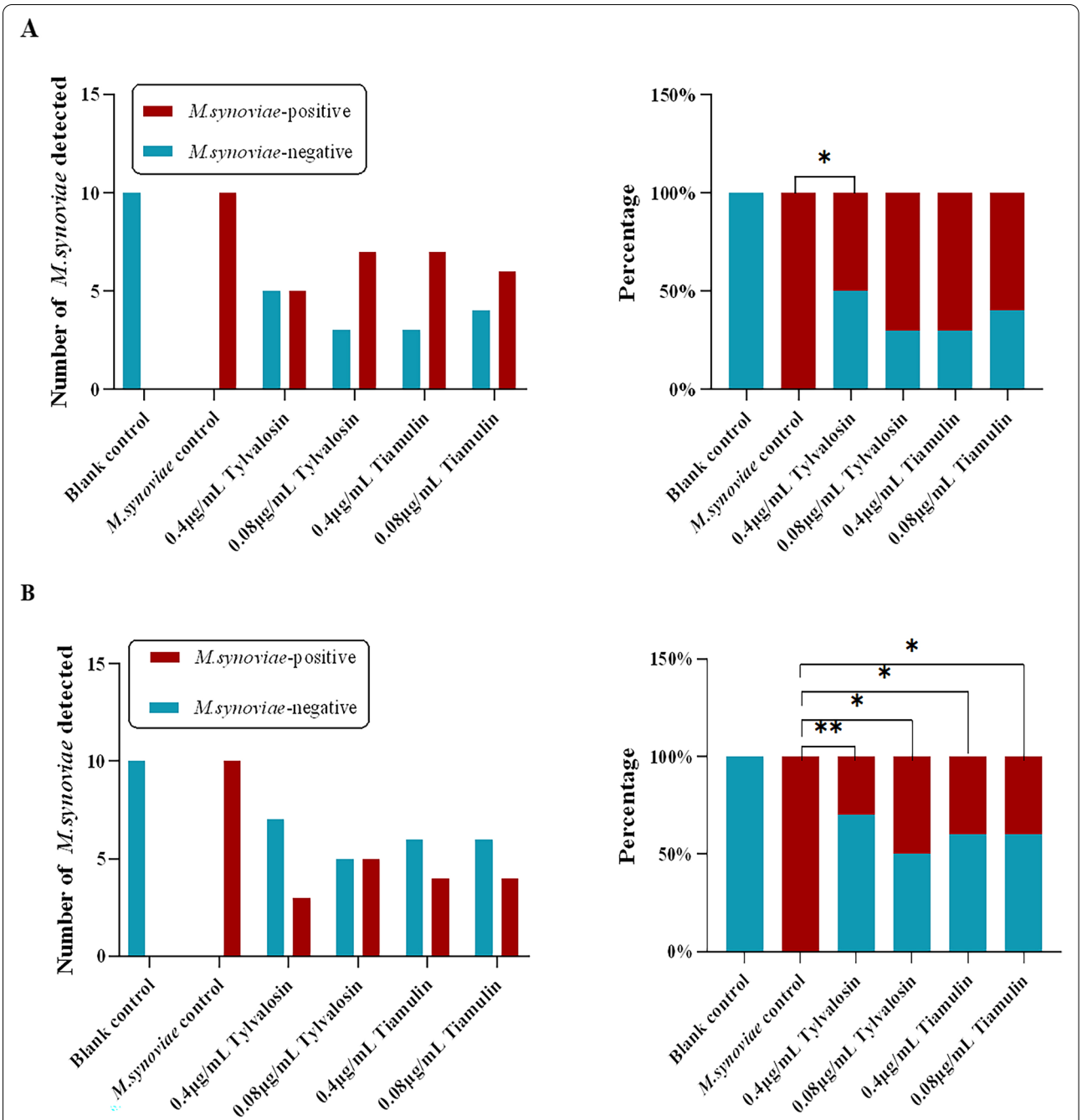

Fig. 4 Number and percentage of *M. synoviae* detected during treatment. **A** Third day of treatment; **B** Fifth day of treatment. Asterisks in the figures indicate significant differences (*$P < 0.05$; ** $P < 0.01$)

resistance determining region (QRDR) identified a mutation at nucleotide position 254 (C254T), resulting in a Thr 85 to Ile amino acid change in all *M. synoviae* isolates and the reference strain ATCC 25204, which is resistant to enrofloxacin. The mutations in the QRDR of *gyr*A and *par*E genes are also related to enrofloxacin resistance.

Using an *in vivo* model of *M. synoviae* infection in chickens, we found that all treatment groups achieved good therapeutic effects 14 days after drug withdrawal, and doxycycline had one of the best treatment effects, along with tylvalosin. However, the detection of *M. synoviae* infection in some of chickens after drug withdrawal suggested that antibiotic treatment did not achieve

complete elimination of the pathogen. Therefore, antibiotics can only control *Mycoplasma* over a short time, and vaccines are needed for long-term control.

M. synoviae can be transmitted through eggs, thus inhibiting transmission *via* eggs is an effective control method. Drug treatment can reduce the level of egg-borne infection. Studies have shown that treatment (dipping or injection) of eggs with tylosin and gentamicin or heat prevents the transmission of *M. synoviae* through eggs in breeding flocks (Yoder Jr 1970). In this study, we showed that tylvalosin inhibited *M. synoviae* growth in chicken embryos, and the inhibitory effect was superior to that of tiamulin at the same dose. Experiments involving in the blocking of vertical transmission need to be carried out in breeding chickens at a later stage.

When we examined the results of MIC *in vitro* experiments and *in vivo* therapeutic effects, *in vitro* susceptibility of some drugs were inconsistent with the clinical efficacy, mainly due to the different pharmacokinetic and pharmacodynamic characteristics of the antibiotics, as well as internal and external differences *in vivo* (Gill et al. 2021). Furthermore, the components of the *in vitro* culture medium have an impact on MIC, growth rates, and kill rates (Mouton 2018). Therefore, the results of *in vitro* antibiotic susceptibility tests should be combined with the observations on clinical treatment effects to select the best antibacterial drugs and improve the effects of clinical medication.

Conclusion

In this study, tylvalosin, doxycycline, and tiamulin were effective against *M. synoviae in vivo* and *in vitro*. Tylvalosin also showed good inhibitory effects on the vertical transmission of *M. synoviae*. These results provide valuable information for the short-term control of *M. synoviae* mycoplasma disease in central China. Subsequent studies are necessary to determine what methods can be used to effectively block the vertical transmission of *M. synoviae*.

Materials and methods

Isolation, purification, and identification of *M. synoviae strains*

The study was carried out from 2019 to 2021, during which 485 samples were taken from 5 to 25 week old chickens suspected of having *M. synoviae* infection in the farms based on clinical symptoms of air sacculitis, chest cyst, footpads and joint swelling. We collected samples from the footpads and joints in this study.

For isolation of *M. synoviae*, chicken feet were wiped repeatedly with alcohol-soaked cotton swabs and dried before aseptic collection of joint fluid and the contents of the swollen tarsal joint cavity in a biologically clean safety cabinet. The samples were then placed into 3 mL *Mycoplasma* medium (pH 7.8) [Modified Frey Medium Base (Chinese Veterinary Pharmacopoeia) with 15% porcine serum, 1% glucose, 1% 100 mg/L L-cysteine, 3% 400 mg/L L-arginine, 1% 100 mg/L β-nicotinamide adenine dinucleotide trihydrate (β-NAD), 0.1% 100 mg/mL ampicillin (Amp), and 0.02% phenol red] and cultured at 37°C in a 5% CO_2 incubator (Thermo Fisher Scientific, USA). When the medium became yellow, the isolates were transferred to fresh medium. After three passages, 100 µL liquid culture was plated onto *Mycoplasma* solid medium (*Mycoplasma* medium + 1.5% Agar) and cultured at 37°C in a 5% CO_2 incubator. Colony growths with a characteristic "fried egg-like" bulge in the center were observed under a low-power microscope after about 7 days. Bacteria from single colonies were selected and cultured in liquid medium.

At the third passage in liquid medium, the strains were identified by PCR and sequenced using 16S rDNA primers. To amplify the conservative region of *M. synoviae vlh*A gene (accession no. AF035624.1), a pair of specific primers (F: 5′–TTAGCAGCTAGTGCAGTGGCC–3′; R: 5′–GTAACCGATCCGCTTAATGC–3′) were designed using Primer Premier 5.0 software. The 16S rDNA primers (Karita et al. 2003) (27F: 5′–AGAGTTTGATCCTGG CTCAG–3′; 1492R: 5′–GGTTACCTTGTTACGACTT–3′) were synthesized by Shenggong Biotechnology Co., Ltd. (Shanghai, China).

The 50 µL reaction mixture consisted of 25 µL 2× Taq Master Mix (Vazyme Biotechnology Co., Ltd. Nanjing, China), 2 µL forward primer (10 µM), 2 µL reverse primer (10 µM), 2 µL sample culture, and 19 µL sterile ddH_2O. Amplification was performed on a PCR instrument (Bio-Rad, Hercules, CA, USA) with the following thermal cycling conditions: 94°C for 5 min, then 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C (depending on primers) for 2 min (16S rDNA) or 30 s (*vlh*A gene).

M. gallisepticum and *E. coli* strains stored in the laboratory and sterile ddH2O were used as negative controls for the specificity test. DNA was extracted from isolates using the TIANamp Bacteria DNA Kit DP302 (Tiangen Biotech Co., Ltd., Beijing, China), 10-fold serial diluted to 10^{-10}, then used to perform PCR amplification according to the above conditions and evaluate the susceptibility.

Amplified DNA products were separated on a 1.5% agarose gel in tris-acetic acid-ethylene diamine tetraacetic acid (EDTA-TAE) buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA, pH 8.3) for 30 min at a constant voltage of 110 V. Amplified products were detected with a gel imaging system (Bio-Rad). The amplified 16S rDNA products were sequenced by Tsingke Biotechnology Co., Ltd. (Beijing, China). Sequencing results were compared with BLAST software on the NCBI website.

Growth titration of isolates – color change units

The growth titers (in CCUs) of isolated strains were determined using the microdilution technique. Briefly, 200 µL liquid medium was added to the 12th well of each row in a 96-well plate, and 180 µL was added to wells 1–11 of each row. Subsequently, 20 µL bacterial solution was added into the 1st well and used to prepare 10-fold serial dilutions to the 11th well. The 1st well was set up as the positive control (180 µL medium + 20 µL bacterial solution), and the 12th well as the negative control (200 µL medium). The plate was sealed with parafilm (PM-996 Parafilm, USA) and incubated at 37°C in a 5% CO_2 incubator for approximately 2 weeks. Three repetitions were prepared for each experiment. The concentration of the last well of test wells 1–11 showing yellow color was identified as the CCU of the strain and deemed to be the titer of the strain (CCU/mL).

Antibiotics

The following eight antibiotics from five different groups were purchased from Hvsen Biotechnology Co., Ltd. (Wuhan, China). The macrolides tylvalosin (lot number 201903024), tylosin (lot number AEC80138KM), and tilmicosin (lot number TMC1908048); the pleuromutilin tiamulin (lot number 01971807063); the fluoroquinolone enrofloxacin (lot number 190107-4); the lincosamide lincomycin (lot number 190322097); and the tetracyclines doxycycline (lot number YD190401097) and chlortetracycline (lot number S1906003). For each antibiotic, a stock solution was prepared using the appropriate solvents and diluents following the manufacturer's instructions. The antibiotic solutions were diluted to 64 µg/mL and passed through a 0.22 µm membrane filter to sterilize the diluted solution. Antibiotics were tested in the concentration range of 0.0625–32 µg/mL.

Minimum inhibitory concentration for *M. synoviae*

The MIC for the isolated strains was determined using the microdilution technique based on the guidelines of Hannan (2000). Briefly, 200 µL liquid medium was added to the 12th well of each row in a 96-well plate, and 100 µL was added to wells 1–11 of each row. After dilution to 64 µg/mL with liquid medium, 100 µL diluted antibiotic was added in the 1st well and used to prepare 2-fold serial dilutions to the 10th well. Subsequently, 100 µL bacterial solution diluted to 10^5 CCU/mL was added to wells 1–11. The 11th well was set up as the positive control (100 µL medium + 100 µL bacterial solution), and the 12th well as the negative control (200 µL medium). The plate was sealed with parafilm (PM-996 Parafilm, USA) and incubated at 37°C in a 5% CO_2 incubator for about 10 days. Three repetitions were set up for each experiment. After the yellow color was observed in the positive-control well, the concentration of the first well with no color change in test wells 1–10 was defined as MIC of the antibiotic for each strain.

The lowest concentration of antibiotic that completely inhibited the growth or metabolism of the isolate *in vitro* was considered to be the MIC value of the tested antibiotic. MIC_{50} and MIC_{90} were taken as the MIC of the antibiotic on 50% and 90% bacterial isolation.

When the highest antibiotic concentration did not inhibit growth, MIC value indicated that it was greater than (>) the highest antibiotic concentration in the plate. Conversely, if the minimum antibiotic concentration present in the plate inhibited growth, the MIC value was expressed as being lower than or equal to (≤) that concentration.

Evaluation of antibiotic treatment effects

Specific pathogen-free chickens ($n = 240$ aged 1 day) were randomly divided into the following groups ($n = 20$ per group): blank control (uninfected), infection control, tylvalosin low-dose (50 mg/L), tylvalosin medium-dose (100 mg/L), tylvalosin high-dose (200 mg/L), tylosin (200 mg/L), tilmicosin (200 mg/L), lincomycin (200 mg/L), doxycycline (200 mg/L), tiamulin (200 mg/L), and enrofloxacin (200 mg/L) groups. On day 14, chickens in the 10 treatment groups and infection control group were inoculated with 0.1 mL 10^6 CCU/mL of the MS-HB1 strain via the intranasal route. The blank control group was inoculated with sterile PBS.

Tracheal samples were collected 14 days after infection (1 day before medication). The 10 experimental groups of chickens were then treated with the corresponding doses of drugs, which were provided in their drinking water continuously for 5 days. Tracheal samples were collected 1, 7 and 14 days after drug withdrawal. DNA was extracted from samples using the TIANamp Bacteria DNA Kit DP302 (Tiangen Biotech Co.). The numbers of *M. synoviae*-positive chickens based on PCR amplification in the different antibiotic groups were compared according to the protocol described for identification of *M. synoviae* strains. The therapeutic effects were expressed as the number and percentage of *M. synoviae*-positive and *M. synoviae*-negative chickens.

Evaluation of the ability of tylvalosin and tiamulin to block vertical transmission of *M. synoviae*

Chicken embryos ($n = 120$ at the 5-day stage) were randomly divided into six groups ($n = 20$ per group): 0.4 µg/mL (5 MIC) tylvalosin group, 0.08 µg/mL (1 MIC) tylvalosin group, 0.4 µg/mL tiamulin group, 0.08 µg/mL tiamulin group, *M. synoviae* control group, and blank

control group. With the exception of the blank control group, all groups were inoculated with 10^4 CCU/0.1 mL of the MS-HB1 strain through the yolk sac. Three days after infection, the four experimental groups were inoculated with 100 μL drugs of corresponding doses. Each group was placed in a 37°C incubator. On the third and fifth days of treatment, 10 chicken embryos were selected from each of the experimental and control groups before the extraction of the whole vitelline membrane. These samples were inoculated into 5 mL liquid medium and incubated at 37°C under 5% CO_2. After 3 days, the vitelline membrane culture was centrifuged at 8000 rpm for 5 min, then 100 μL supernatant was added to 900 μL fresh liquid medium, and the culture was continued at 37°C under 5% CO_2. After 3 days, the inhibitory effect of the drug on *M. synoviae* in chicken embryos was assessed according to PCR identification. Results were expressed as number and percentage of *M. synoviae*-positive and *M. synoviae*-negative chickens.

Statistical analysis

All the derived data were evaluated for normal distribution before statistical analyses. All statistical analyses were performed using Fisher's exact test within SPSS 23.0 software. $P < 0.05$ and $P < 0.01$ were set as the threshold for statistical significance. The asterisks in the figures indicate significant differences ($^*P < 0.05$; $^{**}P < 0.01$).

Abbreviations

Amp: Ampicillin; CCU: Color change units; EDTA: Ethylene diamine tetraacetic acid; EAA: Eggshell apex abnormality; *M.*: *Mycoplasma*; MIC: Minimum inhibitory concentration; *P*: Pobability value; PCR: Polymerase chain reaction; QRDR: Quinolone resistance determining region; rpm: Revolutions per minute; SPF: Specific pathogen-free; TAE: Tris-acetic acid-EDTA; V: Volt; β-NAD: β-nicotinamide adenine dinucleotide trihydrate.

Acknowledgements

Not applicable.

Authors' contributions

ZTZ and SSH conceived and designed the project. CW and HPL performed the experiments; CW, HPL, and NJZ conducted the artificial infection test; ZTZ, CW, NJZ, RZ, ZLL, WTC, QRX, YCX, SSH, and RKY interpreted the data. CW wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Key Research and Development Project of Hubei Province (Grant No. 2021BBA252) and the Earmarked Fund for China Agriculture Research System (CARS-41).

Availability of data and materials

Datasets used and/or analyzed during the current study are available from the corresponding author (E-mail: ztzhou@mail.hzau.edu.cn) on reasonable request.

Declarations

Ethics approval and consent to participate

Experimental procedures were undertaken in accordance with the Animal Ethics Monitoring Committee and Animal Welfare Committee of Huazhong Agricultural University, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]College of Animal Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China. [2]Key Lab of Agricultural Microbiology of State, Huazhong Agricultural University, Wuhan 430070, Hubei, China. [3]Key Lab of Preventive Veterinary Medicine of Hubei Province, Huazhong Agricultural University, Wuhan 430070, Hubei, China. [4]JiangSu Ecolovo Group Ltd., Suqian 223833, Jiangsu, China.

Received: 16 September 2022 Accepted: 8 November 2022
Published online: 29 November 2022

References

Abd El-Hamid, M.I., N.F.S. Awad, Y.M. Hashem, M.A. Abdel-Rahman, A.M. Abdelaziz, I.A.A. Mohammed, and U.H. Abo-Sahma. 2019. *In vitro* evaluation of various antimicrobials against field *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates in Egypt. *Poultry Science* 98 (12): 6281–6288. https://doi.org/10.3382/ps/pez576.

Catania, S., D. Bilato, F. Gobbo, A. Granato, C. Terregino, L. Iob, and R.A. Nicholas. 2010. Treatment of eggshell abnormalities and reduced egg production caused by *Mycoplasma synoviae* infection. *Avian Diseases* 54 (2): 961–964. https://doi.org/10.1637/9121-110309-Case.1.

Catania, S., M. Bottinelli, A. Fincato, M. Gastaldelli, A. Barberio, F. Gobbo, and G. Vicenzoni. 2019. Evaluation of Minimum Inhibitory Concentrations for 154 *Mycoplasma synoviae* isolates from Italy collected during 2012-2017. *PLoS One* 14 (11): e0224903. https://doi.org/10.1371/journal.pone.0224903.

Dijkman, R., A. Feberwee, and W.J.M. Landman. 2017. Development, validation and field evaluation of a quantitative real-time PCR able to differentiate between field *Mycoplasma synoviae* and the MS-H live vaccine strain. *Avian Pathology* 46 (4): 403–415. https://doi.org/10.1080/03079457.2017.1296105.

Feberwee, A., J.J. De Wit, and W.J. Landman. 2009. Induction of eggshell apex abnormalities by *Mycoplasma synoviae*: field and experimental studies. *Avian Pathology* 38 (1): 77–85. https://doi.org/10.1080/03079450802662772.

Feberwee, A., R. Dijkman, D. Klinkenberg, and W.J. Landman. 2017. Quantification of the horizontal transmission of *Mycoplasma synoviae* in non-vaccinated and MS-H-vaccinated layers. *Avian Pathology* 46 (4): 346–358. https://doi.org/10.1080/03079457.2017.1282602.

Fiorentin, L., R.A. Soncini, J.L.A. da Costa, M.A. Mores, I.M. Trevisol, M. Toda, and N.D. Vieira. 2013. Apparent eradication of *Mycoplasma synoviae* in broiler breeders subjected to intensive antibiotic treatment directed to control *Escherichia coli*. *Avian Pathology* 32 (2): 213–216. https://doi.org/10.1080/0307945021000071641.

Gill, C.M., K. Abdelraouf, M. Oota, R. Nakamura, M. Kuroiwa, Y. Gahara, M. Takemura, Y. Yamano, and D.P. Nicolau. 2021. Discrepancy in sustained efficacy and resistance emergence under human-simulated exposure of cefiderocol against Stenotrophomonas maltophilia between *in vitro* chemostat and *in vivo* murine infection models. *Journal of Antimicrobial Chemotherapy* 76 (10): 2615–2621. https://doi.org/10.1093/jac/dkab221.

Hannan, P. 2000. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary *mycoplasma* species. *Veterinary Research* 31 (4): 373–395. https://doi.org/10.1051/vetres:2000100.

Karita, S., K. Nakayama, M. Goto, K. Sakka, W. Kim, and S. Ogawa. 2003. A novel cel▪ lulolytic, anaerobic, and thermophilic bacterium, Moorella sp. strain F21. *Journal of the Agricultural Chemical Society of Japan* 67 (1): 183–185. https://doi.org/10.1271/bbb.67.183.

Kleven, S.H. 2008. Control of avian *mycoplasma* infections in commercial poultry. *Avian Diseases* 52 (3): 367–374. https://doi.org/10.1637/8323-041808-Review.1.

Koutoulis, K.S., G. Kefalas, N. Mouttotou, K.D. Gussem, and D. Theodosiou. 2013. Efficacy of tylosin tartrate on Mycoplasma infections and Eggshell Apex

Abnormalities in layer hens under field conditions. *American Journal of Animal and Veterinary Sciences* 8 (4): 246–252. https://doi.org/10.3844/ajavsp.2013.246.252.
Kreizinger, Z., D. Grózner, K.M. Sulyok, K. Nilsson, V. Hrivnák, D. Benčina, and M. Gyuranecz. 2017. Antibiotic susceptibility profiles of *Mycoplasma synoviae* strains originating from Central and Eastern Europe. *BMC Veterinary Research* 13 (1): 1–10. https://doi.org/10.1186/s12917-017-1266-2.
Landman, W.J.M., and A. Feberwee. 2012. Longitudinal field study on the occurrence of *Mycoplasma synoviae* in Dutch turkey flocks with lameness and experimental induction of the condition. *Avian Pathology* 41 (2): 141–149. https://doi.org/10.1080/03079457.2011.652595.
Le Carrou, J., A.K. Reinhardt, I. Kempf, and A.V. Gautier-Bouchardon. 2006. Persistence of *Mycoplasma synoviae* in hens after two enrofloxacin treatments and detection of mutations in the parC gene. *Veterinary Research* 37 (1): 145–154. https://doi.org/10.1051/vetres:2005046.
Lockaby, S.B., F.J. Hoerr, L.H. Lauerman, and S.H. Kleven. 1998. Pathogenicity of *Mycoplasma synoviae* in Broiler Chickens. *Veterinary Pathology* 35 (3): 178–190. https://doi.org/10.1177/030098589803500303.
Lysnyansky, I., I. Gerchman, I. Mikula, F. Gobbo, S. Catania, and S. Levisohn. 2013. Molecular characterization of acquired enrofloxacin resistance in *Mycoplasma synoviae* field isolates. *Antimicrobial Agents and Chemotherapy* 57 (7): 3072–3077. https://doi.org/10.1128/AAC.00203-13.
Marois, C., F. Doufur-Gesbert, and I. Kempf. 2000. Detection of *Mycoplasma synoviae* in poultry environment samples by culture and polymerase chain reaction. *Veterinary Microbiology* 73 (4): 311–318. https://doi.org/10.1016/s0378-1135(00)00178-4.
Mouton, J.W. 2018. Soup with or without meatballs: impact of nutritional factors on the MIC, kill-rates and growth-rates. *European Journal of Pharmaceutical Sciences* 125: 23–27. https://doi.org/10.1016/j.ejps.2018.09.008.
Raviv, Z., N. Ferguson-Noel, V. Laibinis, R. Wooten, and S.H. Kleven. 2007. Role of *Mycoplasma synoviae* in commercial layer *Escherichia coli* peritonitis syndrome. *Avian Diseases* 51 (3): 685–690. https://doi.org/10.1637/0005-2086(2007)51[685:ROMSIC]2.0.CO;2.
Shahid, M.A., M.S. Marenda, P.F. Markham, and A.H. Noormohammadi. 2018. Complementation of the *Mycoplasma synoviae* MS-H vaccine strain with wild-type obg influencing its growth characteristics. *PLoS One* 13 (3): e0194528. https://doi.org/10.1371/journal.pone.0194528.
Sui, C., H. Cui, J. Ji, X. Xu, Y. Kan, L. Yao, and Q. Xie. 2022. Epidemiological investigations and locally determined genotype diversity of *Mycoplasma synoviae* in Central China from 2017 to 2019. *Poultry Science* 101 (1): 101522. https://doi.org/10.1016/j.psj.2021.101522.
Sun, S.K., X. Lin, F. Chen, D.A. Wang, J.P. Lu, J.P. Qin, and T.R. Luo. 2017. Epidemiological investigation of *Mycoplasma Synoviae* in native chicken breeds in China. *BMC Veterinary Research* 13 (1): 1–9. https://doi.org/10.1186/s12917-017-1029-0.
Xue, J., M.Y. Xu, Z.J. Ma, and G.Z. Zhang. 2017. Serological investigation of *Mycoplasma synoviae* infection in China from 2010 to 2015. *Poultry Science* 96 (9): 3109–3112. https://doi.org/10.3382/ps/pex134.
Yoder, H.W., Jr. 1970. Preincubation heat treatment of chicken hatching eggs to inactivate *mycoplasma*. *Avian Diseases*: 75–86. https://doi.org/10.2307/1588558.
Zhang, X.R., M.J. Guo, D. Chen, C.C. Zhang, Y.Z. Cao, and Y.T. Wu. 2022. Antibiotic resistance of *Mycoplasma Synoviae* strains isolated in China from 2016 to 2019. *BMC Veterinary Research* 18 (1): 1–8. https://doi.org/10.1186/s12917-021-03104-4.

Publisher's Note