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SHORT COMMUNICATION

Prevalence and Phylogenetic Analysis of *Mycoplasma hyopneumoniae* in Native Pigs from Shanghai, China

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A B S T R A C T

Mycoplasma hyopneumoniae (*M. hyo*) causes significant losses to the economy of the pig industry in the world. Little information is available about the prevalence of *M. hyo* infection in Chinese native pigs. We report herein a high seroprevalence (61.5%) to *M. hyo* in Chinese native pigs (Meishan and Fengjing) from Shanghai, China. Meishan or Fengjing pigs had a significantly higher seroprevalence compared to Duroc × Landrace × Yorkshire pigs ($p<0.05$). Among Meishan and Fengjing groups, the peak prevalence was in breeding pigs (74.5%), followed by suckling piglets (71.4%), finishing pigs (69.6%) and nursery pigs (34.7%) and differences were statistically significant ($p<0.05$). PCR analysis of the nasal swabs showed that 5.1% (5/98) of the Meishan pigs (6.5%, 3/46) or Fengjing pigs (3.8%, 2/52) were positive for *M. hyo*. The nucleotide sequences of p46 genes of *M. hyo* from Meishan pigs or Fengjing pigs were highly conserved with 98.6-99.8% sequence homology with other reference strains. Our data confirm that *M. hyo* infection in native pigs was highly prevalent in Shanghai, and thus integrated strategies should be taken to control the disease.

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INTRODUCTION

Mycoplasma hyopneumoniae (*M. hyo*) is a highly infectious disease of swine industry characterized by excessive morbidity, little mortality, and a non-productive cough (Clavijo *et al*., 2021). *M. hyo* infection has a global pattern of distribution in all the subtropical and tropical regions, and they cause significant economic losses (Um *et al*., 2021). *M. hyo* genome codes for several immunodominant proteins, of which P46, is a species-specific 46-kDa surface antigen, involved in the mechanisms of bacterial pathogenesis and/or the immune responses of the host (Liu *et al*., 2023).

It has been reported that *M. hyo* is spread by both horizontal and vertical transmission (Grosse *et al*., 2009). Horizontal transmission in chronic cases is a main factor where transmission can occur in nursery and growing periods (Maes *et al*., 2018). But only a few studies focused on the etiology and transmission of the infectious agent of the disease (Betlach *et al*., 2021a). The gold standard method for *M. hyo* detection is culturing (Goodwin or KM2 medium) for bacteriological identification, but there are some complications in getting pure microbial growth (Thacker, 2004, Marois *et al*., 2007). Polymerase chain reaction (PCR) was a faster and more sensitive test (Takeuti *et al*., 2022) for detecting *M. hyo* (Marois *et al*., 2007).

M. hyo has a global prevalence of 30-80% and causes serious economic losses to the swine industry (Maes *et al*., 2018). Several studies have been conducted to show its prevalence (seropositive 14-65%) in pigs in different countries (Sibila *et al*., 2004; Grosse *et al*., 2009). In China, *M. hyo* infection in Duroc × Landrace × Yorkshire pigs has been found in a few provinces like Shanxi and Guangdong (He *et al*., 2011).

According to the clinical observation data show that native Pigs from China were more sensitive to *M. hyo* infection than Duroc × Landrace × Yorkshire pigs. However, there was little information about the seroprevalence of *M. hyo* infection in Chinese native pigs.

Therefore, the main objective of this present study was to find the seroprevalence of *M. hyo* infection in Chinese native pigs in Shanghai. The results obtained from this study can provide the basic data for the control and prevention of *M. hyo* infection in pigs in Shanghai, China.

MATERIALS AND METHODS

Sampling and preparation: The blood samples (n=738) were collected from Shanghai between January 2019 and December 2020, including Meishan pigs (n=256), Fengjing pigs (n=108) and Duroc×Landrace×Yorkshire pigs (n=374) (Table 1).

A total of 98 swabs were collected from Meishan pigs (n=46) or Fengjing pigs (n=52) with suspected *M. hyo* between January 2019 and December 2020. Nasal swab samples were obtained from pigs and stored at −80°C for further processing.

Serological assay: Antibodies to *M. hyo* were detected by using a commercial ELISA kit according to the procedure reported by the manufacturer (HerdCheck® M. hyo, IDEXX Co. Ltd., USA). The results were recorded at an optical density (OD) of 650 nm for each of the samples. The following formula was used for the estimation of S/P value, S/P = [OD_{650} of samples−NCX]/[PCX−NCX]. Serum with S/P value of <0.30 were considered as negative while the sample with S/P value of ≥0.30 and ≤0.40 were categorized as suspected, and the serum sample with S/P value of >0.40 were considered positive.

DNA extraction: Nasal swabs were resuspended in 1mL of sterile phosphate-buffered saline (PBS) and vortexed vigorously. Genomic DNA from 400µL of each suspension was extracted using an extraction kit for DNA (Dragon Genomic DNA kit, Shanghai Nuolong Biotech Co., Ltd., Shanghai, China).

PCR protocol and Sequence analysis: The pairs of oligonucleotide primers (p46–forward 5′-ATGAAAAAAATGCTTAG-3′, p46–reverse 5′-TTAGGCATCAGGATTATCAAC-3′) were designed for *M. hyo* strain 168 to permit PCR amplification of entire p46 gene (1260bp in length). PCR was performed by using a total master mixture of 20µL with the following composition:10µL Taq PCR MasterMix (Shanghai Nuolong Biotech Co., Ltd., Shanghai, China), 0.5µL forward and reverse primers, 200nM of each primer (Sangon Biotech Co., Ltd. Shanghai, P.R. China), 2µL DNA template, and to make a total volume of 20µL ddH_2O was used. The PCR conditions for the thermocycler were applied according to the previous study (He *et al*., 2011). PCR products were imaged on 1% gel electrophoresis and Sanger sequencing was performed. DNA sequences were compared to assess the genetic similarity between *M. hyo* from Meishan pigs or Fengjing pigs. The phylogenetic tree was constructed based on the sequencing results of the p46 gene by neighbor-joining algorithm using the MEGA 5.0.

Statistical analysis: The Chi square test was used for the estimation of differences in the seroprevalence of *M. hyo* infection in pigs by using SPSS software, and *P* values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Out of 738 pig serum samples, 336 (45.5%) were found to be seropositive to *M. hyo*, and the prevalence rates among different breeds in Shanghai ranged from 29.9% (Duroc×Landrace×Yorkshire) to 62.9% (Meishan) (Table 1). In comparison to other studies from other areas of China, the present study shows that 33.7% is slightly lower than that in Guizhou (41.7%) and Guangdong (45.7%) and is a little higher than Shanxi (32.1%) (He *et al*., 2011). Differences of *M. hyo* seroprevalence could be due to differences in geographical distribution, sampling seasons, examined populations, husbandry practices, and detection methods.

Table 1: Seroprevalence of *M. hyo* infection in native pigs by ELISA in Shanghai, China

Factor	Category	No. sample	No. positive	Prevalence (%)
Breeds	Meishan(MS)	256	161	62.9%^{A}
	Fengjing(FJ)	108	63	58.3%^{A}
	Duroc×Landrace×Yorkshire	374	112	29.9%^{B}
	Total	738	336	45.5%
Age $^{\sharp}$	Suckling piglets (Day 0-Day 30)	63	45	71.4%^{A}
	Nursery pigs (Day 31-Day 70)	101	35	34.7%^{B}
	Finishing pigs (Day 71- Day 150)	102	71	69.6%^{A}
	Breeding pigs (Sows/replacement gilts)	98	73	74.5%^{A}
Gender$^{\sharp}$	Male	53	33	62.3%
	Female	311	191	61.4%
Season$^{\sharp}$	Spring	90	32	35.6%^{B}
	Summer	93	71	76.3%^{A}
	Autumn	89	63	70.8%^{A}
	Winter	92	58	63.0%
	Total	364	224	61.5%

Note: $^{\sharp}$The seroprevalence of *M. hyo* was analyzed for 364 native Pigs (256 Meishan Pigs and 108 Fengjing Pigs) from Shanghai. The different superscript letters A or B on the number on the column of Prevalence indicated that there was a significant difference between the two groups ($p<0.05$).

Our study showed that *M. hyo* prevalence was the highest in summer (76.3%), followed by autumn (70.8%), and was the lowest in spring (35.6%). These results revealed that *M. hyo* infection in pigs is prevalent all year round, with peaks in summer and autumn (Maes *et al*., 2018). This may be due to the high temperature and humidity in summer or autumn in Eastern China, which is conducive to the occurrence and spread of *M. hyo*.

PCR analysis of the nasal swabs showed that 5.1% (5/98) of the Meishan pigs (6.5%,3/46) or Fengjing pigs (3.8%,2/52) were positive for *M. hyo infection*. The length of the p46 gene of *M. hyo*. is 1260 bp, encoding 419 amino acids (46kD) (Liu *et al*., 2023). *M. hyo* from Meishan pigs or Fengjing pigs were named as SHMS or SHFJ, respectively. The phylogenetic tree was constructed based on nucleotide sequences of p46 gene of *M. hyo* by using MEGA5.0 software (Fig. 1). The results demonstrated that the nucleotide sequences of p46 genes of SHMS or SHFJ shared 99.7% sequence homology. When compared with GenBank DNA sequences, there were 98.6 - 98.9% sequence homology with strain 168 (accession CP002274, virulent strain), 99.5-99.6% with strain J (accession D16682，International reference strain) and 98.6-99.8% with other strains from China. The nucleotide sequences of p46 genes of *M. hyo* from Meishan pigs or Fengjing pigs have highly conserved homology with other reference strains.

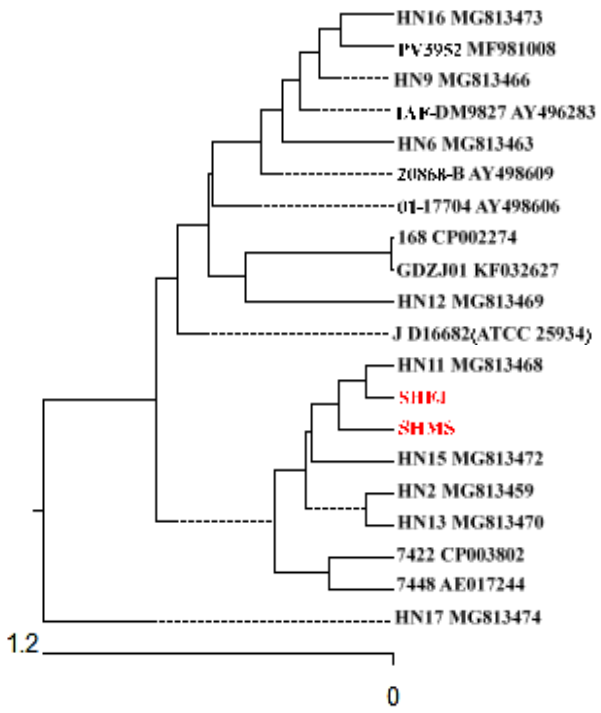

Fig. 1: Phylogenetic tree based on the p46 gene of *M. hyo* found in native Pigs from Shanghai, China. *M. hyo* strains SHMS or SHFJ were from Meishan pigs or Fengjing pigs.

We report herein a high seroprevalence (61.5%) to *M. hyo* in Chinese native pigs (Meishan and Fengjing) from Shanghai, China. Meishan or Fengjing pigs had a significantly higher seroprevalence compared to Duroc × Landrace × Yorkshire pigs (P <0.05). Among Meishan and Fengjing groups, seasonal seroprevalence of *M. hyo* infection was higher in summer (76.3%) and autumn (70.0%) than in spring (35.6%), and the differences were found to be statistically significant (P<0.05) (Table 1). Moreover, the peak prevalence of *M. hyo* was found in breeding pigs (Sows/replacement gilts) (74.5%), followed by suckling piglets (71.4%), while both have more prevalence than nursery pigs (34.7%), finishing pigs (69.6%), and the differences among them were found to be statistically significant (P<0.05) (Table 1).

Higher seroprevalence was found in the breeding pigs (sows/replacement gilts) than nursery pigs and finishing pigs (P<0.05), but there was no significant difference between the breeding pigs and suckling piglets (P>0.05). The results were consistent with a previous work by He *et al*. (2011), who demonstrated that *M. hyo* prevalence in sows and piglets was apparently higher, compared with fattening pigs and growing pigs (Luehrs *et al*., 2017).

At present, the management of *M. hyo* in Chinese native pigs (Meishan pig and Fengjing pig) includes optimizing management practices using antimicrobials (tulathromycin), vaccination programs, and housing conditions (Maes *et al*., 2021; Betlach *et al*., 2021b). Vaccination is the most common strategy for controlling *M. hyo*, vaccinating gilts once a year and piglets twice a year (Liu *et al*., 2023) and providing better housing conditions can prevent the occurrence of infections. The results of this study provide a basis for the prevention and control of *Mycoplasma pneumoniae* in Chinese native pigs (Meishan and Fengjing). These findings provide a basis for future work in the prevention and control of *M. hyo* in Chinese native pigs (Meishan and Fengjing).

In conclusion, this study indicated that *M. hyo* infection in native pigs was relatively serious and widespread in Shanghai. Therefore, prompt actions and integrated strategies should be adopted to control *M. hyo* infection in Chinese native pigs.

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Authors contributions: DZ and JZ formalized the experimental layout and conducted the experiment, ZG, AW and JZ analyzed the data and managed the manuscript. JW and MNA helped in the final drafting of the manuscript.

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