



Identification of a novel *Hemoplasma* species from pigs in Zhejiang province, China

Yuan FU^{1,2)}, Tuanyuan SHI²⁾, Lihua XU²⁾, Wei WEI^{2,3)}, Fuzhuang LU²⁾, Xuejuan ZHANG²⁾, Xiufang YUAN²⁾, Junxing LI²⁾, Jinhui LV²⁾ and Weihuan FANG^{1)*}

¹⁾Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou, Zhejiang 310058, China

²⁾Institute of Animal Husbandry and Veterinary Medicine, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang 310021, China

³⁾College of Life Science and Technology, Southwest University for Nationalities, Chengdu, Sichuan, 610041, China

ABSTRACT. Hemoplasmas belong to *Mycoplasmataceae* (Mollicutes: Mycoplasmatales) and are able to infect a broad range of mammalian species. We investigated prevalence of hemotropic mycoplasma species in pig farms in the region of Zhejiang by a PCR scheme using universal primers targeting 16S rRNA and RNase P RNA gene (*rnpB*). Representative positive samples from different farms were selected for sequencing of 16S rRNA and the 219bp *rnpB* gene fragments for phylogenetic analysis. Sequencing analysis of PCR products from first samples identified a novel hemoplasma species present in several pig farms in the region with highest nucleotide identity of 92% to Candidatus *Mycoplasma turicensis*. A duplex PCR assay was then designed for differential detection of the novel hemoplasma from *Mycoplasma parvum*/*M. suis* in field samples. Of 324 blood samples from clinically healthy pigs, 26.5% was positive for this novel hemoplasma species and 50% positive for *M. suis*/*M. parvum*, indicating that the novel hemotropic mycoplasma species were of considerably high prevalence in Zhejiang province, China.

KEY WORDS: hemoplasma, *Mycoplasma suis*, *Mycoplasma parvum*, novel hemotropic *Mycoplasma hemosuis*

J. Vet. Med. Sci.

79(5): 864–870, 2017

doi: 10.1292/jvms.16-0545

Received: 4 November 2016

Accepted: 13 March 2017

Published online in J-STAGE:
3 April 2017

Hemoplasmas or hemotropic mycoplasmas belong to *Mycoplasmataceae* (Mollicutes: Mycoplasmatales) and are able to infect a broad range of mammalian species. The organisms lack cell wall and are not cultivable. They adhere to the surfaces of host erythrocytes and cause their deformity or even damage, leading to hemolytic anemia and icterus. Moreover, they can persist for years in latently infected animals without causing clinical symptoms [1–3, 6]. Early studies proposed the two hemoplasma species that could infect pigs as *Eperythrozoon suis* and *Eperythrozoon parvum* by morphological features [7]. Further analysis of the 16S rRNA and *rnpB* gene sequences reclassified these two species as swine hemoplasma species *Mycoplasma suis* and *Mycoplasma parvum* [7].

Here, we report a novel hemoplasma agent that caused latent infection in pigs in Zhejiang, China. The 16S rRNA-based phylogenetic analysis suggests that this agent is closely related to Candidatus *Mycoplasma turicensis* isolated from feline [12, 13]. Duplex PCR of pig blood samples revealed that about one fourth of the tested pigs were subclinically infected with the novel hemotropic mycoplasma species.

MATERIALS AND METHODS

Blood samples and bacterial strains

The first blood sample positive by Giemsa stained smear as a putative novel hemoplasma species was collected from a 20-day old three-breed cross (Landrace, Yorkshire and Duroc) growing piglet (ZJSX1101) with clinical symptoms of eperythrozoonosis in Shangyu county of Zhejiang province. A total of 324 blood samples were then obtained for PCR analysis from clinically healthy pigs of 13 different farms in Zhejiang, China from 2009 to 2013, including 86 two-breed cross (Landrace-Yorkshire) sows and 238 three-breed cross growing pigs at 30–120 days of age. Strains of *Hemophilus parasuis*, *Erysipelothrix rhusiopathiae*, *Mycoplasma hyosynoviae*, *Mycoplasma hyorhinis* and *Toxoplasma gondii* for PCR specificity tests were kindly provided by Dr. Y.C. Wang at the swine disease group, Zhejiang Academy of Agricultural Sciences, Hangzhou, China. The *M. wenyonii* strain was kindly provided by Dr. X. M. Song at Zhejiang Academy of Agricultural Sciences, Hangzhou, China, and that of *M. ovis* by Dr X. X. Wang at Henan Agricultural University, Zhengzhou, China.

*Correspondence to: Fang, W., Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou, Zhejiang 310058, China.
e-mail: whfang@zju.edu.cn

©2017 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Table 1. PCR primers used in this study

Species	Gene name	Primers	Sequence (5'-3')	Location	Amplification Length (bp)
<i>Haemoplasma</i>	16S rRNA	fHf1 rHf2	ACGCGTCGACAGATTTGATCCTGGCT CGCGGATCCGCTACCTTGTTACGACTT		1,445/1,490
<i>Haemoplasma</i>	<i>rnpB</i>	80F1 290R1	GAGGAAAGTCCRYGCTWGCAC TCCCYTACCRAAATTTTRGGTTTCT		219–232
Novel <i>Haemoplasma</i> species	16S rRNA	cmsf2 cmsr2	AAACTCTGATGGTACCTCCTGAATAAGTGA CCTTCGCTGGGGATGTCAAACCT	441–972	532
<i>Mycoplasma suis</i> / <i>M. parvum</i>	16S rRNA	msf2 msr2	TAAATTAAGGAGGCTGCCGMAAGGTG TACGCCAATAAATCCGGATAATGCTC	199–582	384

DNA extraction and PCR analysis

The hemoplasma DNA templates were extracted from 400 μ l of blood samples using the Blood DNA Mini Kit (Simgen Biotech. Co., Ltd., Hangzhou, China). The 16S rRNA universal primers fHf1 and rHf2 as well as *rnpB* universal primers 80F1 and 290R1 of the hemoplasma species were used for respective amplification of the 16S rRNA gene and the *rnpB* fragments of the putative novel strain and *M. suis* or *M. parvum* [4, 5] (Table 1). The 25 μ l reaction mixture contained 2.5 μ l of 10x PCR buffer containing MgCl₂, 100 μ M of each dNTP, 250 nM of each primer, 1U *Taq* DNA polymerase (TaKaRa Biotech. Co., Ltd., Dalian, China) and 5 μ l DNA template. The thermal program for 16s RNA consisted of 95°C for 5 min, 35 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 1.5 min, and final extension at 72°C for 10 min on the PTC-200 Thermo Cycler (MJ Research, Co., Ltd., Waltham, MA, U.S.A.). The amplification parameters for *rnpB* were 95°C for 10 min; 35 cycles of 95°C for 30 sec, 45°C for 30 sec, and 72°C for 30 sec and final extension at 72°C for 10 min.

Sequence analysis of the 16S rRNA and *rnpB* gene fragments

The amplified gene fragments were cloned into pMD18-T vector (TaKaRa Biotech) and transformed into *E. coli* DH5 α . Positive clones were sequenced using the M13 forward and reverse primers with the ABI Prism BigDye Terminator Cycle Sequencing kit on the ABI-PRISM3730 DNA Analyzer at Shanghai Sangon (Sangon Biotech Co., Ltd., Shanghai, China). Phylogenetic trees were conducted on the MEGA version 5.1 [8] using the neighbor-joining method. The data sets were resampled 1,000 times to generate bootstrap percentage values.

Development of a duplex PCR assay for detection of putative novel hemoplasma species and *M. suis* /*M. parvum* in blood samples

The primer pair cmsf2/cmsr2 were designed based on the 16S rRNA sequences of the hemoplasma species, and the primers msf2/msr2 were designed from the 16S rRNA sequences of *M. suis* and *M. parvum* (Table 1). The 25 μ l reaction mixture contained 2.5 μ l of 10x PCR buffer containing MgCl₂, 200 nM of each primer, 100 μ M of each dNTP, 1 U *Taq* DNA polymerase and 5 μ l template DNA. The cycling parameters were: 95°C for 5 min; 35 cycles of 95°C for 30 sec, 61.5°C for 30 sec and 72°C for 30 sec; and final extension at 72°C for 10 min. Specificity of the duplex PCR was tested against *M. ovis*, *M. wenyonii*, *H. parasuis*, *E. rhusiopathiae*, *M. hyosynoviae*, *M. hyorhinitis*, *T. gondii* and healthy pig blood DNA in individual PCRs. The PCR sensitivity was tested for the lowest concentration of detectable DNA templates on the pMD18-T backbone. The concentration of the recombinant pMD18-T containing the 16S rRNA fragment was adjusted to 100 ng/ μ l, as determined by spectrophotometry. Ten-fold serial dilutions (1×10^{-2} to 1×10^{-9} ng/ μ l) were then made for PCR using the primer pair msf2/msr2 or cmhf2/cmhr2, as previously described [4, 15].

Occurrence of *M. suis*/*M. parvum* and the putative novel *Hemoplasma* species in pigs

To investigate the prevalence of hemoplasma infections in pigs in Zhejiang province, 324 blood samples from 13 pig farms were detected by the duplex PCR assay as described above. At least one PCR product from each verified hemoplasma positive pig farm was sequenced for phylogenetic analyses.

Nucleotide sequence accession numbers

The GenBank accession numbers of partial 16S rRNA gene and *rnpB* gene sequences derived from this study are JX489599 to JX489604, KC907396 and KC907397.

RESULTS

Molecular characterization of the putative novel *Hemoplasma* species

Initial microscopic examination showed that the blood smears obtained from the first infected pig ZJSX1101 had about 0.1% of red blood cells attached with hemoplasma-like organism with round and ellipse shapes (Fig. 1). The PCR products of the putative novel *Hemoplasma* species for both 16S rRNA and *rnpB* gene fragments were close to those of the *M. suis* and *M. parvum* (Fig. 2).

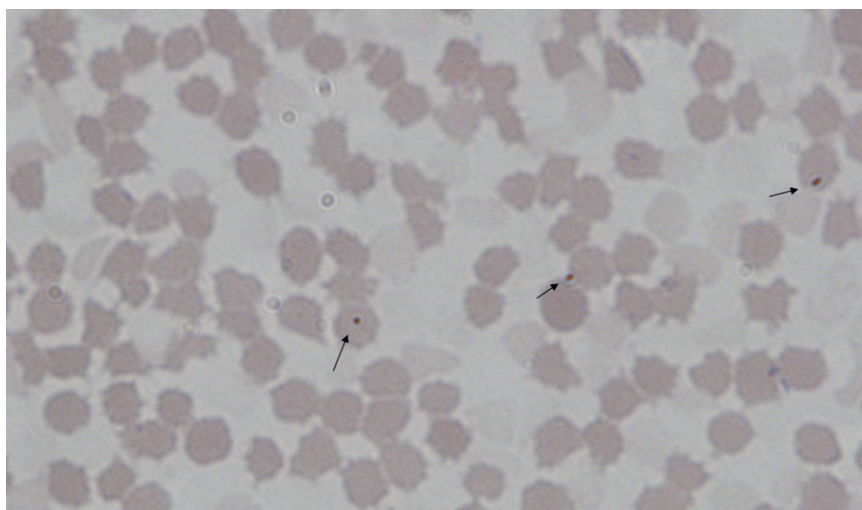


Fig. 1. Giemas stained smear of peripheral blood from a pig infected with a putative novel hemoplasma species. Approximately 0.1% of red blood cells were infected.

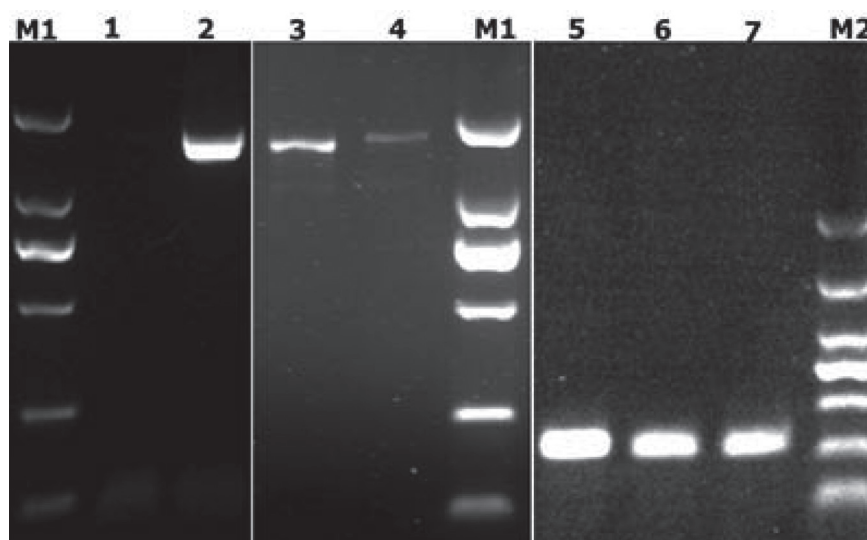


Fig. 2. Amplification of 16S rRNA and *rnpB* gene fragments from putative novel *Hemoplasma*, *M. suis* and *M. parvum* in pig blood samples. M1: DNA marker DL2000. Lanes: 1, negative control; 2, 16S rRNA of *M. suis*; 3, 16S rRNA of putative novel hemoplasma sp; 4, 16S rRNA of *M. parvum*; 5, *rnpB* gene of *M. suis*; 6, *rnpB* of putative novel hemoplasma sp.; 7, *rnpB* of *M. parvum*; M2 DNA Marker DL1000.

Sequencing analysis revealed that the 16S rRNA PCR amplicon was 1,445 bp for the putative novel *Hemoplasma* species, while that of *M. suis* and *M. parvum* was 1,490 bp. The 1,445 bp 16S rRNA fragment of the putative hemoplasma species (accession no. JX489601) had 92% (1,295/1,401) nucleotide identity to Candidatus *Mycoplasma turicensis* and 81% (1,212/1,495) to *M. wenyonii* (Table 2). The 1,490bp fragment of the sample collected from a pig farm in Jiaxing, Zhejiang province (accession no. JX489599) had 99% (1,464/1,469) identity to *M. parvum* and 95% (1,402/1,470) to *M. suis*, while the 1,490 bp fragment of the sample collected from a pig farm in Ningbo, Zhejiang province (accession no. KC907396), had 99% (1,464/1,469) identity to *M. suis* and 95% (1,405/1,470) to *M. parvum*. These results suggest that pigs from these two farms had different infections, one with *M. parvum* and the other with *M. suis*.

The *rnpB* amplicon from the putative novel hemoplasma was 219 bp (accession no. JX489602), while *M. suis* and *M. parvum* had amplicons of 232 bp (accession no. KC907397) and 228 bp (accession no. JX489601), respectively. The sequenced *rnpB* fragment of the novel hemoplasma species was 79% similar to Candidatus *M. hemohominis* (*Homo sapiens*, Plymouth, GU562825) and 71% to *M. suis* (KC907397) (Table 2).

The putative novel porcine hemoplasma was phylogenetically closer to Candidatus *M. turicensis*, clustered together with *M. hemocanis* and *M. hemofelis*, and belonged to the hemofelis group by their 16S rRNA (Fig. 3). They apparently branched away

Table 2. Identity of the novel *Haemoplasma* species and other *Haemoplasma* species based on 16S rRNA and *rnpB* gene

16s rRNA			<i>rnpB</i>		
Species	Source/GenBank	Identity (%)	Species	Source/GenBank	Identity (%)
Candidatus <i>Mycoplasma turicensis</i>	feline/ DQ464423	92.0	Candidatus <i>M. haemohominis</i>	<i>Homo sapiens</i> / GU562825	81.0
<i>M. coccoides</i>	murine/AY171918	91.0	Candidatus <i>M. turicensis</i>	feline/ EF212003	76.0
Candidatus <i>Mycoplasma haemohominis</i>	<i>Homo sapiens</i> / GU562823	91.0	<i>M. parvum</i>	pig/JX489602	74.0
<i>Mycoplasma</i> sp.	buffalo/EF424082	89.8	<i>M. coccoides</i>	murine/EU078619	73.0
<i>M. muris</i>	murine/U82963	89.6	<i>M. suis</i>	pig/KC907397	71.0
<i>M. haemocanis</i>	canis/AY529641	88.4			
<i>M. haemofelis</i>	feline/EU145745	88.2			
<i>M. parvum</i>	pig/JX489599	82.0			
<i>M. suis</i>	pig/KC907396	82.0			
<i>M. wenyonii</i>	bovine/AY946266	81.0			

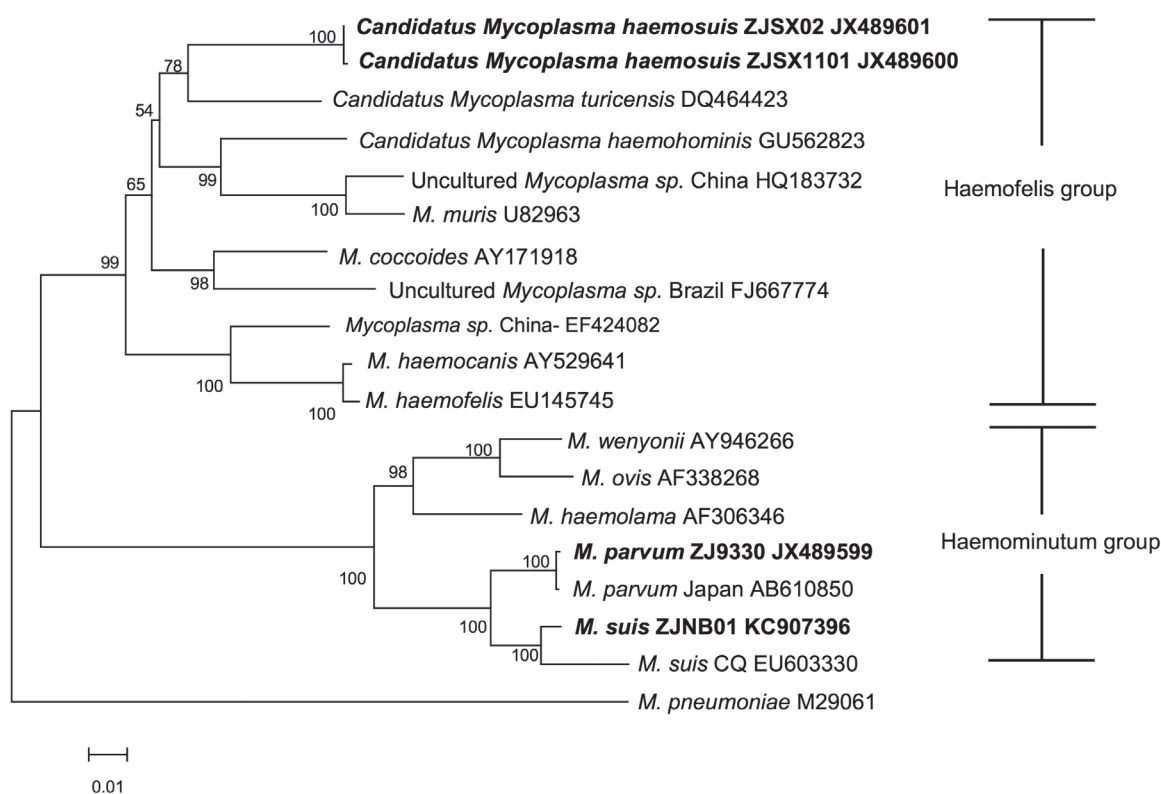


Fig. 3. Phylogenetic tree displaying the relationship of the novel *Hemoplasma* strains to other relevant species based on nearly complete 16S rRNA sequences by the neighbor-joining method. The numbers at the nodes were generated from 1,000 bootstrap resamplings. Values of <50 are not shown. Evolutionary distances are to the scale shown. GenBank accession numbers are shown. The species names in bold were from this study.

from the hemominutum group including *M. suis* and *M. parvum* as previously described [5, 9]. Phylogenetic analysis of the *rnpB* gene sequences also showed results similar to the 16S rRNA (Fig. 4).

Differential detection of *M. parvum*/*M. suis* and putative novel *Hemoplasma* species by duplex PCR

The duplex PCR assay was specific for detection of the novel porcine *Hemoplasma* species (532 bp), *M. parvum* and *M. suis* (384 bp) (Fig. 5). There were no amplicons from other tested bacterial species (Fig. S1). The lower detection limit was between 280 and 290 copies of the recombinant plasmids containing the 16S rRNA gene inserts (Fig. S2).

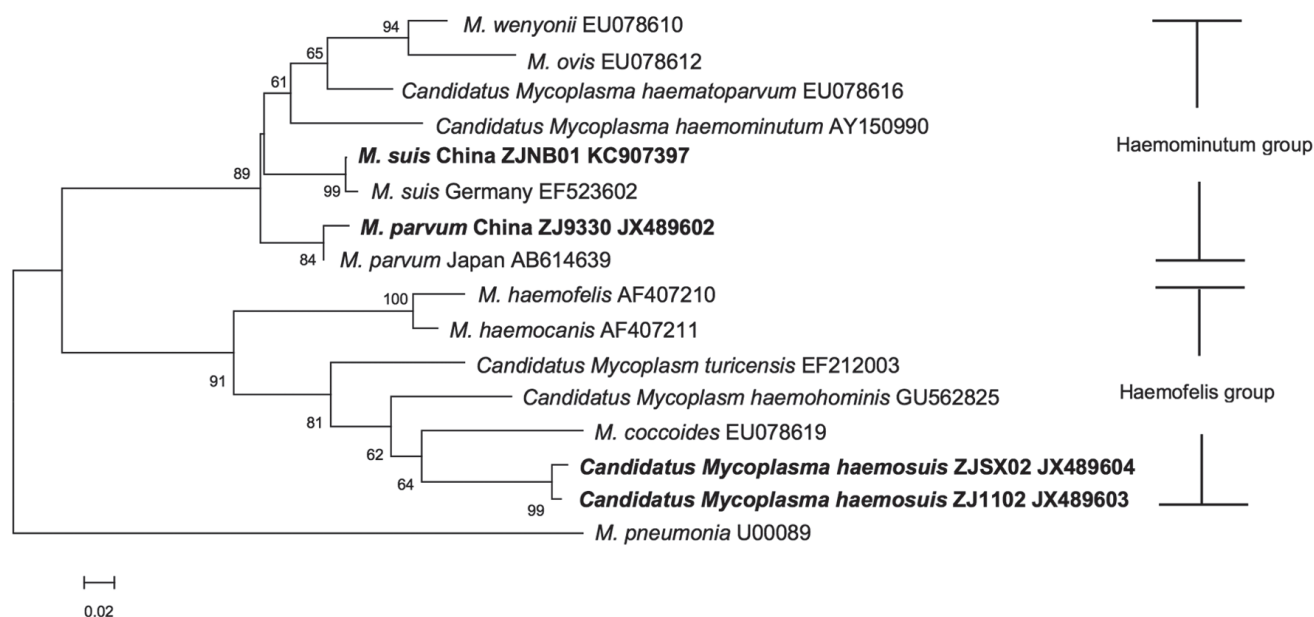


Fig. 4. Phylogenetic tree displaying the relationship of the novel *Hemoplasma* strains to other relevant species based on the *rnpB* fragments by the neighbor-joining method. The numbers at the nodes were generated from 1,000 bootstrap resamplings. Values of <50 are not shown. Evolutionary distances are to the scale shown. GenBank accession numbers are shown. The species names in bold were from this study.

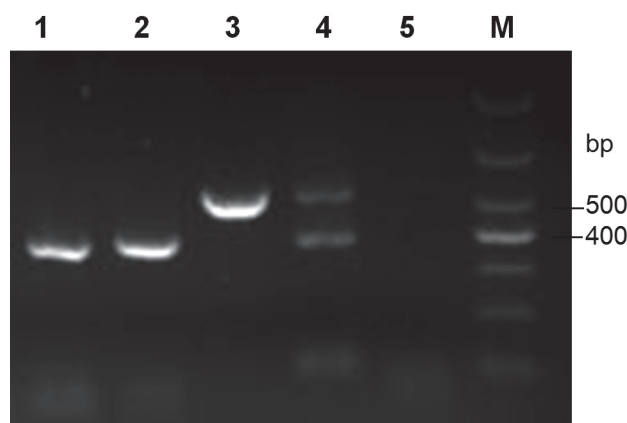


Fig. 5. Differential detection of the novel *Haemoplasma* species from *M. suis*/*M. parvum* by duplex PCR. M: DNA marker DL1000. Lanes: 1, 384 bp product from blood infected by *M. suis*; 2, 384 bp product from blood infected by *M. parvum*; 3, 532 bp product from blood infected by new hemoplasma; 4, positive controls 384 bp and 532 bp of 16S rRNA inserts in pMD18-T; 5, negative control.

Occurrence of *M. suis*, *M. parvum* and the putative novel *Hemoplasma* species in pigs

To investigate the prevalence of the putative novel *Hemoplasma* species and *M. suis*/*M. parvum* in Zhejiang, blood samples from 13 pig farms were detected by PCR. Only one pig farm was negative for hemoplasma. Twelve farms had different levels of infection with hemoplasma species. Of the 324 samples tested, prevalence of the putative novel *Hemoplasma* species was 26.5% on average, while that of *M. suis*/*M. parvum* was 50%. Sows had higher infection rate with hemoplasma (36%) than growing pigs (24.1%). For *M. suis*/*M. parvum*, the infection rate was much higher in sows (70.9%) than in growing pigs (42.6%). The rate of mixed infections of putative novel *Hemoplasma* species and *M. suis*/*M. parvum* was 17.3% (Table 3).

Of the 37 sequences representing 12 verified hemoplasma positive pig farms, 21 belonged to putative novel *Hemoplasma* species in samples from 12 farms, 13 *M. parvum* from 11 farms and 3 *M. suis* from 2 farms. One sample had mixed infection with all three *Hemoplasma* species, and one had dual infection with putative novel *Hemoplasma* and *M. parvum*. The 384 bp PCR fragments of *M. suis*/*M. parvum* had an overlapping region of about 120 bp with the 532 bp PCR fragments of the novel *Hemoplasma*. Phylogenetic analysis of these short overlapping sequences shows that all putative novel *Hemoplasma* strains fell into the same group but different from *M. suis* and *M. parvum*. There appeared to be divergence also within the *M. suis* and *M. parvum* isolates from different farms in Zhejiang province.

Table 3. Frequency distribution of *Haemoplasma* species in the examined swine farms assessed using PCR assay

Organism(s)	Positive No/No. pigs tested (%)		
	Sows	Growing pigs	All pigs
No. of pigs tested	86	238	324
<i>M. suis/M. parvum</i>	61 (70.9)	101 (42.4)	162 (50)
<i>M. suis/M. parvum</i> only	38 (44.2)	68 (28.6)	106 (32.7)
Novel <i>Haemoplasma</i> species	31 (36)	55 (23.1)	86 (26.5)
Novel <i>Haemoplasma</i> species only	8 (9.3)	22 (9.2)	30 (9.3)
Mixed infections with <i>M. suis/M. parvum</i> and novel <i>Haemoplasma</i> species	23 (26.7)	33 (13.9)	56 (17.3)
Total	69 (80.2)	123 (51.7)	192 (59.3)

DISCUSSION

The present study is the first report of a novel *Hemoplasma* species naturally infecting pigs in China. Sequence analysis of the 16S rRNA gene indicates that the novel *Hemoplasma* has close relationship with *Candidatus Mycoplasma turicensis*, a hemoplasma species isolated from feline [12, 14]. Phylogenetic tree confirmed that the new *Hemoplasma* species is different from *M. suis* and *M. parvum*.

We developed a duplex PCR method for differential detection of the novel *Hemoplasma* species and *M. suis/M. parvum* infections in pigs. Because there is high similarity of the 16S rRNA gene sequences between *M. suis* and *M. parvum* [11], it is difficult to design primers to differentiate *M. suis* from *M. parvum* by PCR. With the duplex PCR method, we found that infection with the novel *Hemoplasma* species in pigs from surveyed farms in Zhejiang was 26.5%, about half of that with *M. suis/M. parvum*. There were co-infections of *M. suis/M. parvum* and the novel *Hemoplasma* species that may contribute to pathogenicity in a synergistic manner. Sequencing results of 37 PCR positive samples from 12 pig farms show that *M. parvum* and the novel *Hemoplasma* species seemed to be prevalent in Zhejiang province. Sows had higher infection, possibly because of persistent exposure with increasing age [10].

In summary, we have identified the novel hemotropic mycoplasma species as a potential pathogen to pigs. The duplex PCR method could differentiate the novel *Hemoplasma* species from *M. suis/M. parvum* in blood samples. Although the overall carrier status was high with majority (nearly 60%) of surveyed pigs carrying hemoplasma species, absence of apparent clinical manifestations suggests their low pathogenicity. They might be opportunistic pathogens. Their pathogenic potentials await further investigation, though it might be difficult because of their non-culturable nature [1].

CONFLICT OF INTEREST. There is no conflict of interest.

ACKNOWLEDGMENTS. This work was supported by the Scientific and Technological Innovation Team of Zhejiang Province (2010R50027-26) and the Innovation Project of Zhejiang Academy of Agricultural Sciences, Hangzhou, China.

REFERENCES

- Hoelzle, L. E. 2008. Haemotropic mycoplasmas: recent advances in *Mycoplasma suis*. *Vet. Microbiol.* **130**: 215–226. [Medline] [CrossRef]
- Meli, M. L., Willi, B., Dreher, U. M., Cattori, V., Knubben-Schweizer, G., Nuss, K., Braun, U., Lutz, H. and Hofmann-Lehmann, R. 2010. Identification, molecular characterization, and occurrence of two bovine hemoplasma species in Swiss cattle and development of real-time TaqMan quantitative PCR assays for diagnosis of bovine hemoplasma infections. *J. Clin. Microbiol.* **48**: 3563–3568. [Medline] [CrossRef]
- Messick, J. B. 2004. Hemotropic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. *Vet. Clin. Pathol.* **33**: 2–13. [Medline] [CrossRef]
- Messick, J. B., Cooper, S. K. and Huntley, M. 1999. Development and evaluation of a polymerase chain reaction assay using the 16S rRNA gene for detection of *Eperythrozoon suis* infection. *J. Vet. Diagn. Invest.* **11**: 229–236. [Medline] [CrossRef]
- Peters, I. R., Helps, C. R., McAuliffe, L., Neimark, H., Lappin, M. R., Gruffydd-Jones, T. J., Day, M. J., Hoelzle, L. E., Willi, B., Meli, M., Hofmann-Lehmann, R. and Tasker, S. 2008. RNase P RNA gene (rnpB) phylogeny of Hemoplasmas and other *Mycoplasma* species. *J. Clin. Microbiol.* **46**: 1873–1877. [Medline] [CrossRef]
- Splitter, E. J. 1950. *Eperythrozoon suis*, the etiologic agent of ictero-anemia or an anaplasmosis-like disease in swine. *Am. J. Vet. Res.* **11**: 324–330. [Medline]
- Splitter, E. J. 1950. *Eperythrozoon suis* n. sp. and *Eperythrozoon parvum* n. sp., 2 new blood parasites of swine. *Science* **111**: 513–514. [Medline] [CrossRef]
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739. [Medline] [CrossRef]
- Tasker, S., Peters, I. R., Mumford, A. D., Day, M. J., Gruffydd-Jones, T. J., Day, S., Pretorius, A. M., Birtles, R. J., Helps, C. R. and Neimark, H. 2010. Investigation of human haemotropic *Mycoplasma* infections using a novel generic hemoplasma qPCR assay on blood samples and blood smears. *J. Med. Microbiol.* **59**: 1285–1292. [Medline] [CrossRef]
- Tasker, S., Binns, S. H., Day, M. J., Gruffydd-Jones, T. J., Harbour, D. A., Helps, C. R., Jensen, W. A., Olver, C. S. and Lappin, M. R. 2003. Use of a PCR assay to assess the prevalence and risk factors for *Mycoplasma haemofelis* and ‘*Candidatus Mycoplasma haemominutum*’ in cats in the

- United Kingdom. *Vet. Rec.* **152**: 193–198. [[Medline](#)] [[CrossRef](#)]
11. Watanabe, Y., Fujihara, M., Obara, H., Nagai, K. and Harasawa, R. 2011. Two genetic clusters in swine hemoplasmas revealed by analyses of the 16S rRNA and RNase P RNA genes. *J. Vet. Med. Sci.* **73**: 1657–1661. [[Medline](#)] [[CrossRef](#)]
 12. Willi, B., Boretti, F. S., Cattori, V., Tasker, S., Meli, M. L., Reusch, C., Lutz, H. and Hofmann-Lehmann, R. 2005. Identification, molecular characterization, and experimental transmission of a new hemoplasma isolate from a cat with hemolytic anemia in Switzerland. *J. Clin. Microbiol.* **43**: 2581–2585. [[Medline](#)] [[CrossRef](#)]
 13. Willi, B., Tasker, S., Boretti, F. S., Doherr, M. G., Cattori, V., Meli, M. L., Lobetti, R. G., Malik, R., Reusch, C. E., Lutz, H. and Hofmann-Lehmann, R. 2006. Phylogenetic analysis of “Candidatus *Mycoplasma turicensis*” isolates from pet cats in the United Kingdom, Australia, and South Africa, with analysis of risk factors for infection. *J. Clin. Microbiol.* **44**: 4430–4435. [[Medline](#)] [[CrossRef](#)]
 14. Willi, B., Museux, K., Novacco, M., Schraner, E. M., Wild, P., Groebel, K., Ziegler, U., Wolf-Jäckel, G. A., Kessler, Y., Geret, C., Tasker, S., Lutz, H. and Hofmann-Lehmann, R. 2011. First morphological characterization of ‘Candidatus *Mycoplasma turicensis*’ using electron microscopy. *Vet. Microbiol.* **149**: 367–373. [[Medline](#)] [[CrossRef](#)]
 15. Zachar, V., Thomas, R. A. and Goustin, A. S. 1993. Absolute quantification of target DNA: a simple competitive PCR for efficient analysis of multiple samples. *Nucleic Acids Res.* **21**: 2017–2018. [[Medline](#)] [[CrossRef](#)]