Veterinary Parasitology 274 (2019) 108929

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Rhipicephalus (Boophilus) microplus ticks as reservoir and vector of ‘*Candidatus* Mycoplasma haemobos’ in China

Hongfei Shi[a,*], Lisha Duan[a], Fei Liu[a], Yun Hu[a], Zhaorong Shi[a], Xiaoying Chen[a], Huan Yang[a], Baolong Yan[b,*], Lunguang Yao[a,*]

[a] *Henan Provincial Engineering Laboratory of Insects Bio-reactor, China-UK-NYNU-RRes Joint Laboratory of Insect Biology, Nanyang Normal University, Nanyang, PR China*

[b] *Department of Parasitology, School of Basic Medical Sciences, Wenzhou Medical University, Wenzhou, PR China*

ARTICLE INFO

Keywords:
‘*Candidatus* Mycoplasma haemobos’
Transmission
Rhipicephalus (Boophilus) microplus
China

ABSTRACT

‘*Candidatus* Mycoplasma haemobos’ is an emerging pathogen in the genus *Mycoplasma*. The *Rhipicephalus (Boophilus) microplus* tick has been suspected to be the vector of ‘*C.* M. haemobos’. To determine the role of *R. (B.) microplus* in transmission of ‘*C.* M. haemobos’, we tested the competence of *R. (B.) microplus* larvae to acquire ‘*C.* M. haemobos’ from positive female ticks and to serve as a ‘*C.* M. haemobos’ vector in mice. Using PCR and sequencing, we also analyzed the epidemic strains of ‘*C.* M. haemobos’ among *R. (B.) microplus* ticks collected from goats and sheep in southern Henan Province, central China. Our results identified three epidemic strains of ‘*C.* M. haemobos’, and the positive female ticks naturally infected could pass ‘*C.* M. haemobos’ at egg and larval stages. Furthermore, ‘*C.* M. haemobos’ infected larvae could transmit the pathogens to mice during feeding, and the negative larvae could acquire ‘*C.* M. haemobos’ from infected mice. Our study shows that *R. (B.) microplus* ticks could serve as a vector and reservoir of ‘*C.* M. haemobos’.

1. Introduction

‘*Candidatus* Mycoplasma haemobos’ (‘*C.* M. haemobos’) is a pathogen that was first detected in cattle, with clinical signs including poor weight gain and severe anemia (Tagawa et al., 2008). The pathogen is an unculturable bacterium belonging to the genus *Mycoplasma*, based on the genetic analysis of its 16S rRNA. ‘*C.* M. haemobos’ infections have been reported in Asia, Europe, North America and South America (Ayling et al., 2012; Fujihara et al., 2011; Hornok et al., 2018; Johnson et al., 2016; Martinez-Ocampo et al., 2016; Santos et al., 2018; Shi et al., 2019). To date, natural infections have been reported in cattle, water buffalo, sheep, goat, red deer, fallow deer and roe deer, and such infections could cause lower milk yield, lower calf birth weight, fever, anorexia, depression and hematuria (Ade et al., 2018; Ayling et al., 2012; Girotto et al., 2012; Hornok et al., 2018; Santos et al., 2018; Sasaoka et al., 2013; Shi et al., 2019). In Guangxi Province in south China, ‘*C.* M. haemobos’ has been detected in cattle and buffalo, and in Henan province in central China, ‘*C.* M. haemobos’ has been detected in sheep and goats, although the two provinces are > 1000 km apart. The 16S rRNA genes of strains derived from the two regions are closely related (Shi et al., 2019; Su et al., 2010). ‘*C.* M. haemobos’ cases mainly occur in forests, hills, and grasslands where arthropods thrive.

To date, the natural mode of transmission of ‘*C.* M. haemobos’ remains unclear, but blood-sucking ticks are considered as candidate vectors for transmitting *Mycoplasma* pathogens (Lappin et al., 2006; Taroura et al., 2005). The tick *Dermacentor andersoni* has been shown to transmit *Mycoplasma wenyonii* in calves (Neimark and Kocan, 1997); however, *D. andersoni* has not been observed in China (Chen et al., 2010). Our previous research has shown that *Rhipicephalus (Boophilus) microplus* (*R. (B.) microplus*) samples from sheep, goats, and grasslands could carry ‘*C.* M. haemobos’ (Shi et al., 2019), but whether ‘*C.* M. haemobos’ can be transmitted transstadially or transovarially in *R. (B.) microplus* or whether *R. (B.) microplus* can transmit ‘*C.* M. haemobos’ to animals remains unclear. To address this issue, our study aimed to determine whether *R. (B.) microplus* is a possible vector by determining tick-borne transmission of ‘*C.* M. haemobos’ during the tick developmental stages and by experimental transmission of ‘*C.* M. haemobos’ to laboratory animals. Because the acquisition of specific pathogen-free (SPF) goats or sheep for experimental infection is not easy or economical, laboratory animals such as mice may be an ideal animal infection model, considering that mice can be infected by *M. wenyonii*, which is closely related to ‘*C.* M. haemobos’, and that *R. (B.) microplus* cycle is able to reproduce in mice (Jiang, 2014; Wang et al., 2011). Thus, BALB/c mice were selected as the animal model in this work.

---

[*] Corresponding authors.
E-mail addresses: 77956479@qq.com, kcn1@163.com (H. Shi), 1982ybllog@163.com (B. Yan), lunguangyao@163.com (L. Yao).

https://doi.org/10.1016/j.vetpar.2019.108929

2. Materials and methods

2.1. Animals and blood and tick sample collection

Blood samples of goats and sheep and *R. (B.) microplus* engorged female ticks from the body surfaces of animals were collected from backyard farms in the mountainous area of south Henan Province, central China, where '*C.* M. haemobos' epidemics had been confirmed (Shi et al., 2019). From May 2018 to June 2018 a total of 31 goats and 19 sheep without antibiotics or acaricide treatment were selected for this work. Blood and tick collections from each animal were performed simultaneously, and the blood samples were subjected to DNA extraction for PCR detection. All *R. (B.) microplus* engorged female ticks with lengths > 4.5 mm collected from each goat or sheep were measured and observed; then fully engorged female ticks from each goat or sheep were selected and separately placed on hollow polyethylene plates for analysis.

2.2. Blood PCR detection for R. (B.) microplus screening

To determine the infection status of the goats and sheep involved in this research, total DNA of all blood samples was extracted and measured as previously described (Shi et al., 2019). The partial DNA samples were used as templates to amplify the 16S rRNA fragment of '*C.* M. haemobos' using primers MHBforw and MHBrev as described elsewhere (Meli et al., 2010). According to the results of the PCR amplification, the '*C.* M. haemobos'-positive blood samples were further subjected to PCR testing to detect other pathogens, including *Mycoplasma ovis, 'Candidatus* Mycoplasma haemovis*'* ('*C.* M. haemovis'), *M. wenyonii*, *Babesia*, *Theileria, Anaplasma marginale, Pestivirus*, *Blue tongue virus*, and *Peste des petits ruminants virus*, which can cause similar clinical symptoms as '*C.* M. haemobos' infections, and the primers and programs were as previously described (Shi et al., 2019). The ticks derived from the goats and sheep that were singly infected by '*C.* M. haemobos' (group 1) and negative (group 2) for all detected pathogens were used in the next experiment, and the ticks derived from the goats and sheep that showed mixed infection with other pathogens were excluded from further analysis (Fig. 1).

2.3. Transovarial transmission of 'C. M. haemobos' in R. (B.) microplus females

Fully engorged female *R. (B.) microplus* ticks (groups 1 and 2) were rinsed in water and placed on hollow polyethylene plates; then the females were incubated at 27 °C ± 0.5 °C and 80%–90% relative humidity for egg production. After 10 days, the eggs were collected and the female ticks were subjected to DNA extraction, after which PCR amplification was performed to detect the pathogens as described in Section 2.2. Then, a cluster of half of the eggs from each female tick that was only positive for '*C.* M. haemobos' or negative for all pathogens were also subjected to DNA extraction and PCR detection. Both positive bands amplified from female ticks and eggs were recovered from the agarose gels using an EasyPure PCR purification kit (Transgen Biotech, China), and the purified products were directly sequenced. Lastly, all sequences were compared to the NCBI databases using a BLAST search for verification of the presence of '*C.* M. haemobos'. The remaining eggs from the same female tick with the '*C.* M. haemobos'-positive eggs (group 3) and the pathogen negative eggs (group 4) were applied to laboratory animals for the feeding experiments (Fig. 1).

2.4. Feeding experiments using BALB/c mice

R. (B.) microplus is a one-host tick; therefore, the '*C.* M. haemobos' transmission and acquisition feeding experiments were performed at the larval stage by using SPF female BALB/c mice that were purchased from Beijing Vital River, China. For the experiments, immediately following anesthesia, the hair on the back of each mouse was clipped, and one feeding capsule was glued to the back; ticks were introduced into the capsule. Meanwhile, to prevent the capsule from being removed by the mouse during movement in the cage, a dense nylon cloth was used to cover the capsule. During the experiment, all mice were housed under controlled conditions in individually ventilated cages. All procedures were carried out in strict accordance with the Chinese Law for the Care and Use of Animals and were approved by the Guangdong Laboratory Animals Monitoring Institute (approval number: 18129). Every effort was made to minimize animal suffering during the study.

In the transmission feeding experiment, the eggs from group 3 were hatched, and 50 larvae from a single tick were placed in the capsule on each mouse. After feeding for 4–6 days, the engorged larvae were manually removed (0 days), and then blood from each mouse was collected from submental veins at 1, 7, 14 days after blood feeding. The engorged larval blood samples were subjected to '*C.* M. haemobos' detection by PCR and sequencing as described in Section 2.2. Then those positive mice were used as feeding animals for the next acquisition feeding experiment (Fig. 1).

In the acquisition feeding experiment, eggs from group 4 were hatched, and 50 larvae from a single tick were placed in the capsule on each '*C.* M. haemobos' positive mouse. After feeding for 4–6 days, the engorged larvae were manually removed, and then the engorged larvae samples were subjected to '*C.* M. haemobos' detection as described in Section 2.2 (Fig. 1).

For the control, *R. (B.) microplus* larvae from group 4 were fed on '*C.* M. haemobos' free mice, and blood and larval samples were collected and subjected to the same procedures as described above (Fig. 1).

3. Results

3.1. Blood samples

Among the 31 blood samples collected from goats, nine samples were positive for '*C.* M. haemobos' only; two samples were positive for *Babesia*; one sample was positive for *A. marginale*, and 19 samples were negative for all pathogens. Among the 19 blood samples collected from sheep, five samples were positive for '*C.* M. haemobos' only; one sample was positive for *Babesia*, and 13 samples were negative for all pathogens. All positive bands were recovered and sequenced, and the results showed that among the sequences from nine '*C.* M. haemobos'-positive goat blood samples, six sequences were the same as that of HN1811 strain (GenBank Accession number MH388474). The other three sequences were the same as the sequence of HN1823 strain (GenBank Accession number MH388475) in our previous research (Shi et al., 2019). All the sequences from five '*C.* M. haemobos'-positive sheep blood samples were similar to the sequence of the HN1814 strain (GenBank Accession number MH388477) described in our previous study (Shi et al., 2019).

3.2. R. (B.) microplus samples and transovarial transmission

For the engorged female ticks in group 1, 37 collected from goats tested positive for '*C.* M. haemobos' with the MH388474 sequence, and 15 were positive for the MH388475 sequences; 22 ticks collected from sheep with blood positive for '*C.* M. haemobos' were positive for the MH388477 sequence. After oviposition, all female ticks and each cluster of half eggs from each female tick were subjected to PCR, and the positive amplicons were sequenced. The results showed that in group 1 29/37 (78.4%), 11/15 (73.3%), and 18/22 (81.8%) ticks carried '*C.* M. haemobos' with the same sequence as that harbored by the hosts (Table 1). The '*C.* M. haemobos'-positive eggs were derived only from the positive ticks, and the sequences of '*C.* M. haemobos' in positive eggs were the same as those in the ticks. The positive rates of egg clusters from positive ticks in the three sequence types (MH388474, MH388475, MH388477) were 16/29 (55.2%), 5/11 (45.5%), and 11/

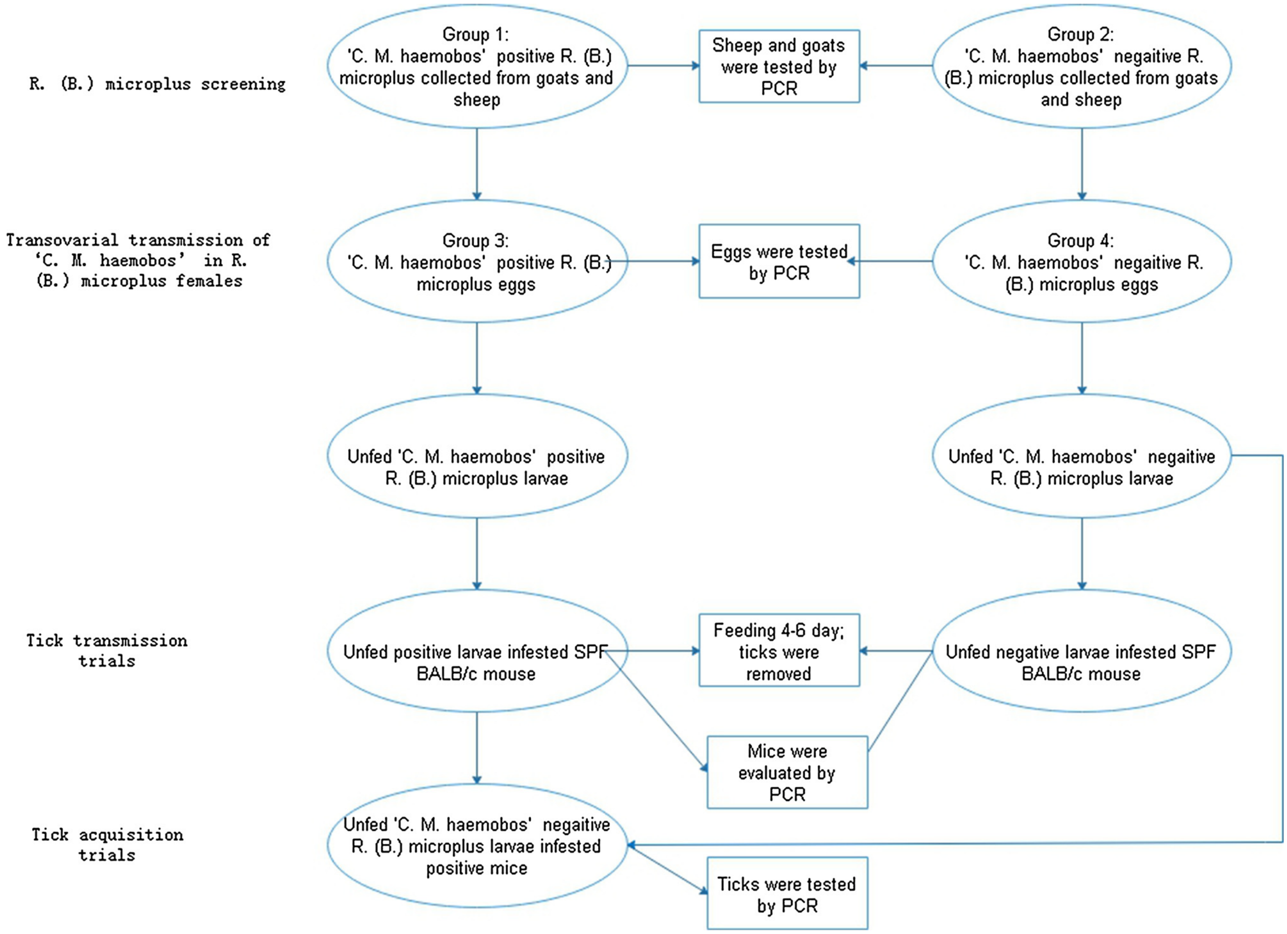

Fig. 1. Diagram illustrating experimental procedures of the present study.

18 (61.1%), respectively, as shown in Table 1. The engorged female ticks in group 2, including 28 and 17 ticks collected from 19 goats and 13 sheep, respectively, were both negative for '*C.* M. haemobos', and the ticks and egg clusters also tested negative for '*C.* M. haemobos' (Table 1).

3.3. Tick transmission of 'C. M. haemobos' to BALB/c mice

To investigate whether '*C.* M. haemobos' could be transmitted to BALB/c mice when ticks sucked blood, the larvae emerged from eggs in group 3 comprising 16, 5, and 11 clusters harboring '*C.* M. haemobos' with MH388474, MH388475, and MH388477 sequences, respectively, were allowed to feed on SPF BALB/c mice. Then at 1, 7 and 14 days after the feeding, blood samples from all mice were collected, subjected to PCR detection for '*C.* M. haemobos', and the positive amplicons were sequenced. Table 2 shows that at 1 day after feeding, two mice in the larvae (MH388474)-feeding group and one mouse in the larvae (MH388477)-feeding group tested positive for '*C.* M. haemobos', and no mice were positive in the larvae (MH388475)-feeding group. At 7 days after feeding, 56.3%, 54.5%, and 40.0% of the mice in these three groups were positive; then at 14 days after feeding, the positive rate of mice in the larvae (MH388474)-feeding group decreased to 50.0%. Meanwhile, the sequences of the positive amplicons showed 100% identity to those detected in the larvae. In the control experiments, 10 larvae clusters emerged from eggs in group 4 were used to feed on SPF mice, and no positive blood samples were detected after 1, 7, and 14 days (Table 2). The larvae that were removed manually from each mouse (0 day) were pooled and subjected to PCR detection. Table 2 shows that all pools in the experimental transmission groups were 100% positive for '*C.* M. haemobos', whereas all pools in the control group were negative. These results indicated that larvae of *R. (B.) microplus* ticks could transmit '*C.* M. haemobos' to mice by feeding.

3.4. 'C. M. haemobos' infection of ticks by acquisition feeding

Each '*C.* M. haemobos'-positive mouse listed in Table 2 was used as a feeding animal for 50 negative larvae, and then engorged larvae were detected for '*C.* M. haemobos' by PCR. As the volume of a single larva was too small, five larval ticks were grouped together as a pool for DNA extraction and PCR analysis. In eight mice that tested positive for '*C.* M.

Table 1
Rate of '*C.* M. haemobos' in eggs among *R. (B.) microplus* female ticks after oviposition.

	Group 1			Group 2	
Host	Goat		Sheep	Goat	Sheep
'*C.* M. haemobos' sequence type of blood (sample No.)	MH388474 (6)	MH388475 (3)	MH388477 (5)	−(19)	−(13)
No. of PCR-positive ticks/ No. of ticks (%)	29/37 (78.4)	11/15 (73.3)	18/22 (81.8)	0/28 (0)	0/17 (0)
No. of PCR-positive egg clusters/ No. of PCR-positive ticks (%)	16/29 (55.2)	5/11(45.5)	11/18 (61.1)	0	0

Table 2
Rate of detection of '*C.* M. haemobos' DNA in blood samples collected from mice after tick feeding.

Larvae feeding group	No. of positive mice/No. of mice sucked by larvae (%)			No. of positive larvae pools /No. of larvae pools (%)
	1 day	7 days	14 days	0 days
Larvae (MH388474)	2/16 (12.5)	9/16 (56.3)	8/16 (50.0)	16/16 (100%)
Larvae (MH388475)	0/5 (0)	2/5 (40.0)	2/5 (40.0)	5/5 (100%)
Larvae (MH388477)	1/11 (9.1)	6/11 (54.5)	6/11 (54.5)	11/11 (100%)
Larvae (negative)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)

haemobos' with MH388474, 25 of 80 pools (31.3%) were positive; in two mice that tested positive for '*C.* M. haemobos' with MH388475, 5 in 20 pools (25.0%) were positive; and in six mice that tested positive for '*C.* M. haemobos' with MH388477, 13 in 60 pools (23.3%) were positive. These results indicated that larvae could acquire '*C.* M. haemobos' from infected mice

4. Discussion

To date, the natural mode of transmission of '*C.* M. haemobos' remains unclear, although it has been suggested that horn flies (*Haematobia irritans*), stable flies (*Stomoxys calcitrans*), and horse flies (*Tabanus bovines* and *T. bromius*) may be involved (Hornok et al., 2011). In our previous study, we showed that *R. (B.) microplus* harbors '*C.* M. haemobos' (Shi et al., 2019). The detection of '*C.* M. haemobos' DNA either in ticks or any other vector does not mean that they have vector competence to transmit hemoplasmas; this may be due to the feeding process that arthropods can acquire '*C.* M. haemobos' from the blood of infected animals, and it is possible that arthropods do not maintain the pathogen after molting and transmit it during blood sucking. Further investigation to validate the role of the candidate vector is scare, and in our previous study (Shi et al., 2019) we showed that the prevalence rates of '*C.* M. haemobos'-positive *R. (B.) microplus* collected from infected goats and sheep were 57% and 53%, respectively. In addition, even in ticks collected from grassland where the infected animals grazed, the prevalence rate was up to 48%. These high prevalence rates in ticks suggests that ticks alone may be sufficient to maintain '*C.* M. haemobos' in nature and could be a vector and reservoir host of '*C.* M. haemobos'.

As '*C.* M. haemobos' is an unculturable bacterium, we screened for positive and negative ticks in the present study, and the three '*C.* M. haemobos' representative strains (MH388474, MH388475, and MH388477) involved in this study were also detected in previous work (Shi et al., 2019). We demonstrated that *R. (B.) microplus* harboring these three strains can hatch positive eggs (Table 1). Further investigation using feeding experiments with larvae emerged from positive eggs indicated that the larvae can transmit '*C.* M. haemobos' into the mice. These results revealed that in endemic areas, *R. (B.) microplus* ticks serve as a reservoir for '*C.* M. haemobos', which further indicates that adult *R. (B.) microplus* female ticks could transmit '*C.* M. haemobos' via the transovarial route. Transovarial transmission of '*C.* M. haemobos' by *R. (B.) microplus* represents an effective mechanism for pathogen dissemination, but as a host, *R. (B.) microplus* would suffer negative effects from infection by *Babesia* (Davey, 1981; Mangold et al., 1993). Whether this strategy which adopted by '*C.* M. haemobos' is harmful for the invertebrate host is unknown, and further studies are thus warranted. In the mountainous area of south Henan Province, *R. (B.) microplus* have been identified as predominant vectors, and the ticks could live through the winter in the larval stage (Shang et al., 2014; Wu and Ma, 2013; Yu et al., 2016; Zhao et al., 2015). In addition, in the present study, our results demonstrated that the larvae emerged from positive eggs could carry and transmit '*C.* M. haemobos', which suggests that the number of '*C.* M. haemobos' infections would be likely to rise when the weather was warmer and the larvae begin to engorge, this hypothesis has been verified in our previous investigation that the seasonal characteristic of this disease coincides with the reproductive cycle of the *R. (B.) microplus* (Shi et al., 2019). The *R. (B.) microplus* ticks have been also shown to be a vector in transovarial transmission of *Babesia* spp. in cattle (Oliveira et al., 2005), and *Babesia* spp. has also been detected in ticks in this research, it is urgent to investigate the epidemic status of '*C.* M. haemobos' and *Babesia* spp. in *R. (B.) microplus* from cattle, since *Babesia* spp. had been detected in cattle in south Henan Province (Ma, 2006).

In feeding experiments with BALB/c mice, we demonstrated that *R. (B.) microplus* ticks transmitted three strains of '*C.* M. haemobos' to mice during feeding, and that the larvae acquired three strains of '*C.* M. haemobos' during feeding on positive mice. In terms of the exact route of tick transmission, several possible routes have been detected, including tick saliva, excretions of coxal glands, and feces (Rehacek and Brezina, 1968). Among the tick species feeding on the sheep or goats, *Dermacentor marginatus* has been shown to be a vector for transmission of Q-fever via dried tick feces (Hartelt et al., 2008; Hellenbrand et al., 2005). The exact route that *R. (B.) microplus* adopted for transmission between ticks and mice was not investigated in the present study, in view of the fact that the bloodsucking duration in mice was short and that the engorged larvae were removed immediately. Thus, routes other than feces contamination were more likely, and further study is needed to confirm this hypothesis. Currently, although some bloodsucking arthropods are considered as candidate vectors for transmitting '*C.* M. haemobos' (Hornok et al., 2011), the experiments on transmission of the pathogens by these arthropods have not been conducted. In this study we first demonstrated that the *R. (B.) microplus* could transmit '*C.* M. haemobos' not only transovarially but also transstadially. One limitation of our work is that the transmission capability of ticks was evaluated in a laboratory animal model, and further research involving natural hosts should be conducted.

5. Conclusions

The '*C.* M. haemobos' can be passed transovarially during the development the *R. (B.) microplus* tick. The positive larval ticks can transmit '*C.* M. haemobos' to BALB/c mice during feeding, while negative larval ticks can acquire '*C.* M. haemobos' in experimentally infected mice.

Compliance with ethical standards

All procedures were carried out in strict accordance with Chinese Law for the Care and Use of Animals and approved by the Guangdong Laboratory Animals Monitoring Institute (approval number: 18129).

Funding

The National Natural Science Foundation of China (Grant no. 31371381 and 31902263), the Henan Provincial Scientific and technological research project (Grant nos. 182102110084 and 162102110046) and Nanyang Normal University (CN) (Grant no. 15081) supported this study.

Declaration of Competing Interest

The authors declare that no competing financial interests and no conflicts of interest exist.

Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

References

Ade, J., Niethammer, F., Schade, B., Schilling, T., Hoelzle, K., Hoelzle, L.E., 2018. Quantitative analysis of Mycoplasma wenyonii and "Candidatus Mycoplasma haemobos" infections in cattle using novel gapN-based realtime PCR assays. Vet. Microbiol. 220, 1–6.

Ayling, R.D., Bisgaard-Frantzen, S., Adler, A., Blowey, R.W., Barlow, A.M., Millar, M.F., van der Burgt, G.M., 2012. Detection of' Candidatus Mycoplasma haemobos', Mycoplasma wenyonii and Anaplasma phagocytophilum from cattle in England. Vet. Rec. 170, 543.

Chen, Z., Yang, X., Bu, F., Yang, X., Yang, X., Liu, J., 2010. Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of China. Exp. Appl. Acarol. 51, 393–404.

Davey, R., 1981. Effects of Babesia bovis on the ovipositional success of the southern cattle tick, Boophilus microplus. Ann. Entomol. Soc. Am. 74, 331–333.

Fujihara, Y., Sasaoka, F., Suzuki, J., Watanabe, Y., Fujihara, M., Ooshita, K., Ano, H., Harasawa, R., 2011. Prevalence of hemoplasma infection among cattle in the western part of Japan. J. Vet. Med. Sci. 73, 1653–1655.

Girotto, A., Zangirolamo, A.F., Bogado, A.L., Souza, A.S., da Silva, G.C., Garcia, J.L., Vilas Boas, L.A., Biondo, A.W., Vidotto, O., 2012. Molecular detection and occurrence of' Candidatus Mycoplasma haemobos' in dairy cattle of Southern Brazil. Rev. Bras. Parasitol. Vet. 21, 342–344.

Hartelt, K., Pluta, S., Oehme, R., Kimmig, P., 2008. Spread of ticks and tick-borne diseases in Germany due to global warming. Parasitol. Res. 103 (Suppl 1), S109–S116.

Hellenbrand, W., Schöneberg, I., Pfaff, G., Kramer, M., Steng, G., Reintjes, R., Breuer, T., 2005. The relevance of Coxiella burnetii infections in animals for Q fever in humans - measures for prevention and control. Tierärztliche Praxis. Ausgabe G, Grosstiere/ Nutztiere 33, 5–11.

Hornok, S., Micsutka, A., Meli, M.L., Lutz, H., Hofmann-Lehmann, R., 2011. Molecular investigation of transplacental and vector-borne transmission of bovine haemoplasmas. Vet. Microbiol. 152, 411–414.

Hornok, S., Sugar, L., Fernandez de Mera, I.G., de la Fuente, J., Horvath, G., Kovacs, T., Micsutka, A., Gonczi, E., Flaisz, B., Takacs, N., Farkas, R., Meli, M.L., Hofmann-Lehmann, R., 2018. Tick- and fly-borne bacteria in ungulates: the prevalence of Anaplasma phagocytophilum, haemoplasmas and rickettsiae in water buffalo and deer species in Central Europe, Hungary. BMC Vet. Res. 14, 98.

Jiang, M., 2014. The Study of Infection and Transmission of Babesia microti in Mice by Three Tick Species (in Chinese). Master. Chinese Center for Disease Control and Prevention 68 pp.

Johnson, K.A., do Nascimento, N.C., Bauer, A.E., Weng, H.Y., Hammac, G.K., Messick, J.B., 2016. Detection of hemoplasma infection of goats by use of a quantitative polymerase chain reaction assay and risk factor analysis for infection. Am. J. Vet. Res. 77, 882–889.

Lappin, M.R., Griffin, B., Brunt, J., Riley, A., Burney, D., Hawley, J., Brewer, M.M., Jensen, W.A., 2006. Prevalence of Bartonella species, haemoplasma species, Ehrlichia species, Anaplasma phagocytophilum, and Neorickettsia risticii DNA in the blood of cats and their fleas in the United States. J. Feline Med. Surg. 8, 85–90.

Ma, C., 2006. Epidemiology and Control of Piroplasmosis in Dairy in Nanyang City (in Chinese). Master. Nanjing Agricultural University 36 pp.

Mangold, A.J., Aguirre, D.H., Cafrune, M.M., de Echaide, S.T., Guglielmone, A.A., 1993. Evaluation of the infectivity of a vaccinal and a pathogenic Babesia bovis strain from Argentina to Boophilus microplus. Vet. Parasitol. 51, 143–148.

Martinez-Ocampo, F., Rodriguez-Camarillo, S.D., Amaro-Estrada, I., Quiroz-Castaneda, R.E., 2016. Draft genome sequence of "Candidatus Mycoplasma haemobos," a hemotropic Mycoplasma identified in cattle in Mexico. Genome Announc. 4.

Meli, M.L., Willi, B., Dreher, U.M., Cattori, V., Knubben-Schweizer, G., Nuss, K., Braun, U., Lutz, H., 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.

Neimark, H., Kocan, K.M., 1997. The cell wall-less rickettsia eperythrozoon wenyonii is a Mycoplasma. FEMS Microbiol. Lett. 156, 287–291.

Oliveira, M.C., Oliveira-Sequeira, T.C., Araujo Jr., J.P., Amarante, A.F., Oliveira, H.N., 2005. Babesia spp. infection in Boophilus microplus engorged females and eggs in Sao Paulo State. Rev. Bras. Parasitol. Vet. 130, 61–67.

Rehacek, J., Brezina, R., 1968. Detection of Coxiella burneti in saliva of experimentally infected ticks, Hyalomma dromedarii Koch. Bull. World Health Organ. 39, 974–977.

Santos, N.J.R., Brito, D.R.B., Abate, H.L., Paixao, S.F., Soares, E.D.S., Vieira, T., Garcia, J.L., Vieira, R.F.C., Vidotto, O., 2018. Hemotropic mycoplasmas infection in water buffaloes (Bubalus bubalis) from northeastern Brazil. Comp. Immunol. Microbiol. Infect. Dis. 56, 27–29.

Sasaoka, F., Suzuki, J., Watanabe, Y., Fujihara, M., Nagai, K., Hirata, T., Harasawa, R., 2013. Two genotypes among' Candidatus Mycoplasma haemobos' strains based on the 16S-23S rRNA intergenic spacer sequences. J. Vet. Med. Sci. 75, 361–364.

Shang, Z., Zhang, C., Zhang, Z., Shunan, C., Yang, Z., Amp, A.S., School, T., 2014. Epidemiological characteristics of Canine Babesiosis and main vector of infection in west of Henan (in Chinese, with English abstract). J. Henan Univ. Sci. Tech.: Nat. Sci. 35, 73–76.

Shi, H., Hu, Y., Leng, C., Shi, H., Jiao, Z., Chen, X., Peng, Y., Yang, H., Kan, Y., Yao, L., 2019. Molecular investigation of "Candidatus Mycoplasma haemobos" in goats and sheep in central China. Transbound. Emerg. Dis. 66, 22–27.

Su, Q.L., Song, H.Q., Lin, R.Q., Yuan, Z.G., Yang, J.F., Zhao, G.H., Huang, W.Y., Zhu, X.Q., 2010. The detection of "Candidatus Mycoplasma haemobos" in cattle and buffalo in China. Trop. Anim. Health Prod. 42, 1805–1808.

Tagawa, M., Matsumoto, K., Inokuma, H., 2008. Molecular detection of Mycoplasma wenyonii and' Candidatus Mycoplasma haemobos' in cattle in Hokkaido, Japan. Vet. Microbiol. 132, 177–180.

Taroura, S., Shimada, Y., Sakata, Y., Miyama, T., Hiraoka, H., Watanabe, M., Itamoto, K., Okuda, M., Inokuma, H., 2005. Detection of DNA of' Candidatus Mycoplasma haemominutum' and Spiroplasma sp. in unfed ticks collected from vegetation in Japan. J. Vet. Med. Sci. 67, 1277–1279.

Wang, P., Zhao, Y.L., Zhang, F.M., 2011. Establishment of animal models for Eperythrozoon wenyoni infection (in Chinese, with English abstract). Anim. Husbandry Vet. Med. 43, 22–26.

Wu, S., Ma, J., 2013. Prevention and control of tick-borne diseases (in Chinese, with English abstract). J. Henan Inst. Sci. Tech. 41, 54–56.

Yu, P.F., Niu, Q.L., Liu, Z.J., Yang, J.F., Chen, Z., Guan, G.Q., Liu, G.Y., Luo, J.X., Yin, H., 2016. Molecular epidemiological surveillance to assess emergence and re-emergence of tick-borne infections in tick samples from China evaluated by nested PCRs. Acta Trop. 158, 181–188.

Zhao, Q., Gao, L.J., Tang, Z.Q., Zhang, Y.Q., Guo, X.S., Liu, J.Q., 2015. Investigation of species, temporal and spatial distribution of ticks in Henan province, China (in Chinese, with English abstract). Zhongguo Mei Jie Sheng Wu Xue Ji Kong Zhi Za Zhi 26, 75–77.