

# First Molecular Detection of Anaplasma Marginale, Babesia Bovis and Babesia Bigemina in Different Breeds of Cattle and Rhipicephalus Microplus in the Northern Hainan, China

**Tianlin Bi**

Hainan University

**Biswajit Bhowmick**

Hainan University

**Liping An**

Hainan University

**Dejuan Liang**

Hainan University

**Kaixuan Wang**

Hainan University

**Xu Duan**

Hainan University

**Sijia Zeng**

Hainan University

**Jinhua Wang**

Hainan University

**Jianguo Zhao**

Hainan University

**Qian Han** (✉ [qianhan@hainanu.edu.cn](mailto:qianhan@hainanu.edu.cn))

Hainan University

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## Research

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# Abstract

## Background

*Anaplasma marginale*, *Babesia bovis* and *Babesia bigemina* cause huge economic losses to the world's cattle industry every year. At present, there are limited investigations on *A. marginale*, *B. bovis* and *B. bigemina* in Hainan. The purpose of this study was to investigate the infection of *A. marginale*, *B. bovis* and *B. bigemina* in different breeds of cattle (Leiqiong and Wagyu), buffaloes and *Rhipicephalus microplus* in the northern part of Hainan.

## Methods

In this study, 63 *R. microplus* and 151 samples of bovine blood were collected from cattle in the northern part of Hainan. *A. marginale*, *B. bovis* and *B. bigemina* were detected by PCR and nPCR.

## Results

The results showed that the infection rates of *A. marginale*, *B. bovis* and *B. bigemina* in 214 samples were 10.75% (23/214), 4.21% (9/214) and 9.35% (20/214), respectively. In Leiqiong cattle, the total infection rate of several pathogens was the highest, which was 73.33% (22/30), and there was cross-infection of several pathogens, the infection rate was 46.67% (14/30). Among them, *A. marginale* and *B. bigemina* had the highest cross-infection rate, which was 26.67% (8/30). No mixed infection was detected in *R. microplus*, buffalo and Wagyu. Among buffalo, the infection rates of *A. marginale*, *B. bovis* and *B. bigemina* were 2.17% (1/46), 6.52% (3/46) and 4.35% (2/46), respectively. *A. marginale* infection was only found in Wagyu, with an infection rate of 2.67% (2/75). *A. marginale* and *B. bigemina* infections were found in *R. microplus*, but no *B. bovis* infection was found.

## Conclusions

The results of this study provide a reference for the formulation of prevention and control strategies for *A. marginale*, *B. bigemina* and *B. bovis* in Hainan.

## Background

Hainan is the southernmost tropical island of China. It has a distinctly different climate and geographic location from the Chinese mainland. It has a warm climate, rich tropical flora and fauna resources, and a geographical environment isolated from the inland, suitable for the breeding of ticks and the transmission of tick-borne pathogens in animals. The most common breeds of Hainanese cattle are Leiqiong and Wagyu. Among them, Leiqiong cattle is a local cattle breed in Hainan, and Wagyu cattle were imported from Japan. Leiqiong cattle and buffalo are mainly raised by rural farmers. In Hainan, cattle and buffaloes are frequently heavily infested with multi-species of ticks, and they can transmit a variety of pathogens, including bacteria, viruses, protozoa and so on. Due to environmental and climate changes, the distribution range of ticks is expanding, and the health problems brought by ticks have also attracted global attention [1]. In China, the increase of emerging tick-borne pathogens has inspired scholars to study ticks and tick-borne pathogens in recent years [2]. According to the latest survey results, there are 124 tick species and 103 tick-borne pathogens in China, but the current distribution range of ticks in China has been significantly underestimated [3]. *R. microplus* is an obligate ectoparasite that frequently infects cattle and is considered to be one of the most harmful ectoparasites to domestic animals and wildlife [4, 5]. The cattle tick *R. microplus* is one of the most important disease vectors for livestock in tropical and sub-tropical areas [5, 6], and it is the most widespread tick species distributed in many provinces of China [7]. This tick transmits *A. marginale*, *B. bigemina*, *B. bovis*, *T. equi*, *E. corfeensis*, TBEV, *C. burnetii* and other pathogens [8, 9]. Hainan should strengthen the prevention and control of *R. microplus*, because Hainan is also a tropical region, and its geographical characteristics and climatic conditions are suitable for the breeding of *R. microplus* and tick-borne pathogen transmission. If the prevention and control of *R. microplus* is not strengthened, it is likely that *R. microplus* will breed in large numbers and harm the health of hosts. Affect the development of Hainan animal husbandry.

*A. marginale* is a kind of *Anaplasma*, which can cause hemolytic anemia, miscarriage, yield loss and death in cattle [10]. According to statistics, in Brazil, the annual loss caused by parasites affecting cattle industry chain production is \$13.96 billion [11]. Among them is borderline *Anaplasmosis*, caused by gram-negative bacteria (*Rickettsia: Anaplasmosis*) [12], an obligate intracellular parasite that chronically infects cattle and wild animals. *A. marginale* can be transmitted biologically by ticks and mechanically by blood-sucking arthropods or blood-contaminated needles and surgical instruments. Approximately 20 tick species, mainly of the genera *Rhipicephalus* and *Dermacentor*, have been reported as vectors of anaplasmosis in bovine species [13]. *A. marginale* is a part of the bovine tick-borne pathogens (TBDs), together with *B. bigemina* and *B. bovis*, it has an economic impact through direct costs related to mortality and morbidity and indirect costs related to disease treatment and prevention [10, 14]. In China, *A. marginale* was first discovered in cattle in Hebei Province in 1987, more than 30 years ago [15]. However, so far, the results of investigation and research on *A. marginale* in China are still limited. In the northwest and northeast regions, *A. marginale* infection has not been detected in many animals, or the infection rate is extremely low.

*Babesiosis* is mainly caused by blood protozoa of the genus *Babesia*, which includes *B. bovis*, *B. divergens*, *B. ovata*, *B. major* and *B. bigemina*. Among these species, *B. bovis* and *B. bigemina* are the two most pathogenic species, transmitted mainly by cattle fever tick *R. microplus*, and this tick is widely distributed worldwide [16]. The most common clinical signs include fever, jaundice, hemoglobinuria and anaemia [17]. Babesiosis is widely distributed in China and has been detected in ticks, animals, and even humans in some areas. In recent years, it has been detected in giant pandas in China, horses

imported from Shanghai and bats in Gansu, and infected with different species of *Babesia*. Among them, the giant panda is a new species and *B. vesperuginis* was first found in bats in China [18, 19, 20]. A variety of *Babesia* infections were detected in ticks in Northeast China and Xinjiang Uygur Autonomous Region. These species were *B. bigemina*, *B. divergens*, *B. microti* and *B. venatorum* [21], *B. occultans*, *B. caballi*, *B. motasi*, *B. major* and *Babesia sp.* [22]. An investigation of *Babesia* infection in 10 patients bitten by ticks in Gansu revealed that these patients were infected with *Babesia* [23]. However, there are no studies regarding the use of molecular methods for the detection of *A. marginale*, *B. bovis* and *B. bigemina* in bovine species. Therefore, the present study was undertaken with the objective of understanding the epidemiology of these disease in the northern coastal region of Hainan and to contribute to more efficient surveillance programme and control of tick-borne diseases.

## Methods

### Study sites and collection of specimens

The study was conducted from May 2020 to September 2020 in the northern part of Hainan Province. A total of 151 bovine blood samples (cattle 30, buffalo 46, Wagyu 75) and 63 *R. microplus* ticks were analyzed. All procedures to gather samples from animals were approved by Hainan University Institutional Animal Care and Use Committee (HNUAUCC-2019-0000A). The sampling locations are shown in Figure 1. Blood samples were aseptically collected in vacutainer tubes containing EDTA and transported back to Vector Biology Laboratory with an ice bag. *R. microplus* were collected from the cattle in separate vials containing 70% ethanol, using fine-tipped tweezers. The samples were stored at -20° C until further use.

### DNA extraction and PCR amplification

Each tick was soaked in 70% ethanol and then rinsed with distilled water. This procedure was repeated three times. The ticks were homogenized using a tissue grinder machine with 3 mm stainless steel beads. Whole-genomic DNA from each tick was extracted using the Ezup Columnar Animal Genomic DNA Extraction Kit as per the manufacturer's instructions, and the extracted DNA was stored in a refrigerator at -20° C for future use. For the bovine blood samples, genomic DNA was extracted using the Ezup Column Blood Genomic DNA Extraction Kit according to the manufacturer's instructions. The extracted DNA was stored at -20° C for future use.

The extracted DNA was used for the amplification of MSP4 gene of *A. marginale*, and the target fragment size was 870bp. All samples also were screened to test for the presence of *B. bovis* and *B. bigemina* using a nested PCR (n-PCR). For *B. bovis*, oBb- mit -F/R was used as the primer for the first PCR amplification, and iBb -mit-F/R was used as the primer for the second PCR amplification to amplify the *Cytb* gene of *B. bovis*, the target fragment size was 260/195bp. *B. bigemina* used oBbig- mit -F/R as the primers for the first PCR amplification and iBbig- mit- F/R as the primers for the second PCR amplification to amplify the *Cytb* gene of *B. bigemina* and the target fragment size was 394/250bp. All primers are listed in Table 1. A reaction system of 25 ul was used for PCR, including 1ul of DNA template, 1 ul of forward primers, 1ul of reverse primers, 12.5 ul of 2×Taq Plus Master Mix  $\square$ , 9.5 ul of ultra-pure water. 1ul of the product of the first PCR was used as the template for the second PCR for nested PCR reaction.

### DNA sequencing and phylogenetic analysis

All the PCR/nPCR amplification products of the positive samples were sent to Shanghai Sangon for sequencing and the segments for sequencing of *A. marginale*, *B. bovis* and *B. bigemina* were 870bp, 195bp and 250bp respectively. Sequence analyses were performed using the BLASTn search in GenBank database and the ClustalW method for sequence alignment (DNASStar, Madison, WI, USA). For each species, phylogenetic analyses were performed using sequences obtained in this study along with reference sequences obtained from NCBI. The evolutionary tree was constructed using Neighbor-Joining (NJ) algorithm based on the evolutionary distance matrix calculated by the Kimura two-parameter model of Mega X software. The auto-spreading value analysis was 1000 repetitions to evaluate the reliability of the evolutionary tree cluster.

### Nucleotide sequence accession numbers

The GenBank access numbers obtained in this study are as follows: MW939369-MW939374 for *A. marginale*, MW939375-MW939380 for *B. bigemina* and MW939381-MW939383 for *B. bovis*.

### Statistical analysis

Statistical analysis was conducted using a Chi-square test in Analytics Software (PASW) Statistics 25 (SPSS, Chicago, IL, USA). The p-values less than 0.05 is statistically significant.

## Results

### Analysis of infection rate

The total infection rate of three pathogens in 214 samples was 16.36% (35/214). The infection rates of *A. marginale*, *B. bovis* and *B. bigemina* were 10.75% (23/214), 4.21% (9/214) and 9.35% (20/214), respectively. In different kinds of samples, the pathogen infection rate was 73.33% in Leiqiong cattle and the infection rate in Wagyu was the lowest, which was 2.67% ( $P < 0.001$ ) (Table 2). Among the samples from different regions, *A. marginale*, *B. bovis* and *B. bigemina* were infected in cattle in Ding'an area and there were cross-infections, with a cross-infection rate of 47.62% (10/21). Among them, *A. marginale* and *B. bigemina* had the highest cross-infection rate, which was 23.81% (5/21) (Table 3). No mixed infections were found in *R.*

*microplus*, buffalo, and Wagyu. *A. marginale*, *B. bovis* and *B. bigemina* were found to be infected in buffaloes. The infection rates were 2.17% (1/46), 6.52% (3/46) and 4.35% (2/46), respectively. Only *A. marginale* infection exists in Wagyu and the infection rate is 2.67% (2/75). There were *A. marginale* and *B. bigemina* infections in *R. microplus*, but no *Babesia bovis* infection was found. In Xiuying and Dingan cattle, the infection rates of *A. marginale* were 100% (5/5) and 57.14% (12/21) and the infection rates of *B. bovis* were 20% (1/5) and 23.81% (5/21), the infection rates of *B. bigemina* were 80% (4/5) and 52.38% (11/21), which were higher than the pathogen infection rates in hosts in other regions (Table 3).

### Phylogenetic analysis

In this study, a total of 23 *A. marginale* sequences, 9 *B. bovis* sequences and 20 *B. bigemina* sequences were analyzed. The representative 6 *A. marginale* sequences, 6 *B. bigemina* sequences and 3 *B. bovis* sequences from different regions and different hosts were obtained from NCBI database and construct the phylogenetic tree analysis. The phylogenetic tree results show that the six *A. marginale* sequences (MW939369-MW939374) obtained are clustered on a clade and are similar to those obtained from cattle from India (MH373246, KX989510, MG676459), Brazil (MH172467) and Cuba (MK809386) between 99.61-100%, the similarity with the sequence obtained from the Henan (MH191396) yak in China is 99.61% (Figure 2). The six *B. bigemina* sequences (MW939375-MW939380) obtained form two independent clades, one of which is in an independent clade, and the other five are clustered on one clade with the Japanese (AB499085) and Chinese (KX698109) sequences. The similarities between the two sequences are 88.18% and 85.45% respectively. These sequences are in different branches from the sequence of Zhejiang, China (GQ214234) and there are evolutionary differences. The three *B. bovis* sequences (MW939381-MW939383) obtained were clustered on a clade and the similarities with the Japanese (AB499088) and the US (EU075182) sequences were all 99.24% (Figure 3).

### Discussion

In China, there are limited reports on the research on *A. marginale*, which can be seen from the existing data that *A. marginale* is distributed in many regions of China. Bovine anaplasmosis caused by *A. marginale* is widely distributed in many provinces of China [24, 25]. It was first reported from cattle as early as 1987 in Lushi County, Henan Province. In the present study, *A. marginale* was detected in *R. microplus*, Leiqiong, buffaloes and Wagyu. Among Leiqiong cattle, the infection rate of *A. marginale* was the highest, 56.67% (17/30), which was higher than the previous report that the infection rate of *A. marginale* in 10 provinces in China was 31.60%. This is the first time that *A. marginale* infection has been detected in *R. microplus*, buffaloes and wagyu in Hainan. In the previous study, *Anaplasma spp.* was detected in cattle in Haikou and the infection rate was 100% [24, 25]. Similar results were observed in Leiqiong cattle from Hainan, in which the infection rate of *A. marginale* infection rate was 100%, and this infection was also detected in *R. microplus*. This was consistent with previous research results, which suggested that there was a high rate of *A. marginale* infection in Hainan. The situation of *A. marginale* infection in Hainan was severe, *A. marginale* infection and transmission occurred in different species of cattle and *R. microplus*.

*B. bigemina* is widely distributed in China and is infected in domestic animals in many areas. Previous seroepidemiological studies have shown that *B. bigemina* has an infection rate of 6.40%-47.27% in cattle from 17 provinces in China [26]. In this study, *B. bigemina* was infected in Leiqiong cattle, buffaloes and *R. microplus* and the highest infection rate was 53.33% (16/30) in cattle, which was lower than 62.2% (23 / 37) in Hainan cows in previous study [16]. In this study, the infection rate of *B. bigemina* in Xiuying cattle samples was 80% (4/5) than that of Dingan cattle was 52.38% (11/21), while the infection rate of *B. bigemina* in Wuwei cattle was only 0.6% [27], the infection rate of *B. bigemina* among cattle in Chongqing area was 7.24% [28], these results were lower than *B. bigemina* infection rate of cattle in northern Hainan. In addition, *B. bigemina* was also detected in Gansu yak and *Hy. asiaticum* in Northwest China, with infection rates of 17.76% and 9.4% (3/32), respectively [27, 29]. The results showed that the infection rate of *B. bigemina* in cattle in northern Hainan was significantly higher than that in other areas of China.

Previous reports and the results of this study showed that there was a high infection rate of *B. bovis* in Hainan. In this study, *B. bovis* infection was found in Leiqiong cattle and buffaloes and the infection rates were 20.00% (6/30) and 6.52% (3/46), respectively. The infection rate of *B. bovis* in cattle was higher than that of Xiuying cattle 10.2% (6/59) [30], lower than that dairy cattle 43.2% (16/37) [16]. Previous studies showed that the infection rate of *B. bovis* was 8.9% (51/575) in 575 blood samples from ten provinces in China [30], that 646 samples from cattle, cows and yaks in 14 provinces was 20.7% [16]. These data show that *B. bovis* in Hainan is higher than that in other areas in China. In addition, *B. bovis* and *B. bigemina* were also detected in Dingan Buffalo, the infection rates were 6.52% (3/46) and 4.35% (2/46) respectively, which were higher than those in other areas of China. The infection rates of *B. bovis*, *B. bigemina* in Hubei buffalo were 1.0%, 0.7% and 3.9% respectively [31]. However, there was no cross infection of *B. bovis* and *B. bigemina* in this study. In this study, *B. bovis* was not found in *R. microplus*, only *B. bigemina* was found. Similar to the results of Tengchong in Yunnan, *B. microti*, *B. orientalis*, *B. bigemina* were detected in ticks in Tengchong, but *B. bovis* was not detected [32].

The results showed that there were infections of *A. marginale*, *B. bigemina* and *B. bovis* in different species of cattle and *R. microplus* in northern Hainan, the infection rate was high and the infection situation was complex. *A. marginale* and *B. bigemina* were found in cattle ticks in this study. It is the first time that these two pathogens were detected in *R. microplus* in Hainan. Previous studies on the infection of several pathogens in Hainan cattle more, this study also studied the infection of several pathogens in *R. microplus*, Buffalo and Wagyu, which is also the innovation of this study. This study has proved that several pathogens are widespread in *R. microplus* and different kinds of cattle in northern Hainan, the infection situation in cattle is relatively complex with high infection rate. Combined with the previous research reports and the results of this study, the infection situation of several pathogens in Hainan is more serious and complex than other areas in China.

There are more complicated pathogen infections in northern Hainan than other areas in China, which may be caused by the differences of geographical environment and climate. Hainan is the southernmost tropical island in China. It has a climate and geographical location that is obviously different from inland China. The warm climate, abundant tropical animal and plant resources and isolated geographical environment are suitable for tick breeding and tick-borne pathogen transmission. Combined with previous studies, it is found that there are obvious differences in the distribution and infection degree of *A. marginale* and *Anaplasma spp.* in China and these differences may be caused by the differences of region and climate. The climate distribution in China is obvious. Hainan, the southernmost part of China, has a tropical marine monsoon climate. The three northeastern provinces in the northernmost part of China have a temperate monsoon climate and a temperate continental climate. The south is hot and rainy, while the northeast is cold and dry. In China, from south to north, the tropical maritime monsoon climate has gradually changed into temperate monsoon climate and temperate continental climate. In previous studies, it was found that the infection rate of *A. marginale* was 34.05 % (102/300) in cattle in Hunan, Guangdong, Guangxi, Hainan, China's mid-south temperate monsoon climate, subtropical monsoon climate and tropical monsoon climate. The infection rate of *A. marginale* in Yunnan, Guizhou, Sichuan and Chongqing, which are located in the subtropical monsoon climate and plateau mountain climate of southwest China, is 36.6 % (67/183). In Liaoning and Inner Mongolia in Northeast China, the infection rate of *A. marginale* was only 9.5 % (7/74) [24]. However, these areas have obvious climate change characteristics, and the temperature gradually rises, changing into subtropical and tropical monsoon climate. The results of another survey also show that the infection rate of *Anaplasma* in tropical and subtropical regions of China, such as Hainan, Yunnan, Fujian and Jiangsu, is significantly higher than that in Heilongjiang, Inner Mongolia and northern China [25]. These research results show that geographical location and climate differences will lead to the distribution of *A. marginale* and different infection rates.

This is also proved in the results of other people's studies. The types of anamorphs and the distribution of infection rates in China reflect a characteristic of gradually increasing from north to south. The climate in Northeast China is cold, and *Anaplasma* infection has a single type or even no infection [33, 34]. The climate in the northeast is cold, with a temperate monsoon climate and a temperate continental climate, which may not be suitable for the spread of invisible bodies. The climate in Xinjiang, China is also a temperate continental climate and the climate characteristics of Northeast China are very similar. The results of the investigation of anaplasma in Xinjiang are similar to those in Northeast China. In the Xinjiang Uygur Autonomous Region, 5822 ticks collected from cattle, goats, sheep, camels and horses were tested for tick-borne pathogens. These ticks consist of 12 species, but the results show that only *A. ovis* infections exist among these ticks and no other types of *Anaplasma* infections have been detected [35]. However, up to now, 50 species of ticks have been found in Xinjiang, which is a province with developed animal husbandry in China. However, no other types of *Anaplasma* infection have been found in these areas. In Shandong, China, 3145 *Ha. longhorned* were tested by *Anaplasma* and only *A. bovis*, *A. phagocytophilum* and *A. capra* were found to be infected. The infection rates were 1.55%, 0.10% and 0.03%. No other *Anaplasma* was found infection [36]. In these areas, *Anaplasma* infection is not very serious and *Anaplasma* infection in some areas is single or there is even no *Anaplasma* infection. In the southern region, after climate change and temperature rise, the infection degree and types of *Anaplasma* increased significantly. Located in the subtropical monsoon humid climate area of Chongqing in southwest China, *A. bovis*, *A. central*, *A. phagocytophilum*, *A. platys* and *A. marginale* were detected in cattle, the infection rates were 8.41%, 7.83%, and 4.93%, 4.35% and 2.61% [28]. The reasons for these results may be due to changes and differences in geographical and climatic conditions.

Tropical countries abroad have also shown complicated infections of *A. marginale*, *B. bigemina* and *B. bovis* similar to Hainan. Overseas investigations and studies have shown that the infection rates of *A. marginale*, *B. bigemina* and *B. bovis* in Santa Catarina cattle in Brazil are 27%, 16% and 29%, respectively [37]. The infection rates of *A. marginale*, *B. bigemina* and *B. bovis* in Thai cattle were 39.1%, 12.5% and 11.1%, respectively [38]. In Cuban buffaloes, *A. marginale*, *B. bigemina* and *B. bovis* have high cross-infections. Among them, *B. bovis* and *B. bigemina*, *B. bigemina* and *A. marginale*, *B. bovis* and *A. marginale* have cross-infection rates of 20%, 24 % and 26%[39]. These areas have climate characteristics very similar to Hainan. Therefore, combined with domestic and foreign research reports, the spread of *A. marginale*, *B. bigemina* and *B. bovis* may be related to climate and temperature.

## Conclusion

In this study, the infection of several pathogens was detected in *R. microplus*, Leiqiong cattle, buffalo and Wagyu. It was found that the infection of several pathogens in Leiqiong cattle was more serious and complicated, there were cross-infections. Buffalo, Wagyu and *R. microplus* also have at least one pathogenic infection. This is the first survey of *A. marginale*, *B. bigemina* and *B. bovis* infections in *R. microplus*, buffalo and Wagyu in Hainan. *A. marginale*, *B. bigemina* have been detected in *R. microplus*, *A. marginale*, *B. bigemina* and *B. bovis* have been detected in buffaloes. *A. marginale* was detected in Wagyu. The high infection rates of *A. marginale*, *B. bigemina* and *B. bovis* in the survey areas in northern Hainan may be due to differences in geographic location and climatic conditions. The spread of *A. marginale*, *B. bigemina* and *B. bovis* may be related to temperature, spread more easily in tropical and subtropical regions. The results of this research provide a reference for the formulation of prevention and control strategies for Hainan *A. marginale*, *B. bigemina* and *B. bovis*.

## Abbreviations

PCR: polymerase chain reaction; nPCR: nested PCR; EDTA: Ethylene diamine tetraacetic acid; CYTb: cytochrome b gene.

## Declarations

### Acknowledgement

Not applicable.

#### Authors' contributions

QH and JZ designed this study and critically revised the manuscript. TB participated in its design, coordination and drafted the manuscript. LA, DL, KW, JW and JZ participated in sample collection. XD, SZ and BB performed the experiments, data analysis, and drafted the manuscript. All authors read and approved the final manuscript.

#### Funding

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#### Availability of data and materials

The GenBank access numbers obtained in this study are as follows: MW939369-MW939374 for *A. marginale*, MW939375-MW939380 for *B. bigemina* and MW939381-MW939383 for *B. bovis*.

#### Ethics approval and consent to participate

All procedures to gather samples from animals were approved by Hainan University Institutional Animal Care and Use Committee (HNUAUCC-2019-0000A).

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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## Tables

**Table 1 Pathogen primers and PCR/nPCR amplification conditions**

Pathogen	Target gene	Primer sequence	Fragment length	References
<i>A. marginale</i>	msp4	MSP45 GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	870	[40]
		MSP43 CCGGATCCTTAGCTGAACAGGAATCTTGC		
<i>B. bovis</i>	CYTb	oBb- mit -F TGAACAAAGCAGGTATCATAGG	260	[41]
		oBb- mit -R CCAAGGAGATTGTGATAATTCA		
		iBb -mit-F TCCACGATCTGTGATACGTCA	195	
		iBb- mit -R CAAATCCTTTGCAAACCTCAA		
<i>B. bigemina</i>	CYTb	oBbig- mit -F TCCAACACCAAATCCTCCTA	394	[41]
		oBbig- mit -R CGTGGGTTTCGTTTTTGTAT		
		iBbig- mit- F AAGAGATACCATATCAGGGAACCA	250	
		iBbig- mit- R TTGGGCACTTCGTTATTTCC		

**Table 2 The prevalence of *A. marginale*, *B. bigemina* and *B. bovis* among different species of cattle and *R. microplus* in northern Hainan, China**

Host	Number of inspections	Pathogen infection rate in different samples (%)			
		<i>A. marginale</i>	<i>B. bovis</i>	<i>B. bigemina</i>	Total
Leiqiong	30	56.67(17/30)	20.00(6/30)	53.33(16/30)	73.33(22/30)
Buffalo	46	2.17(1/46)	6.52(3/46)	4.35(2/46)	13.04(6/46)
Wagyu	75	2.67(2/75)	0	0	2.67(2/75)
<i>R. microplus</i>	63	4.76(3/63)	0	3.17(2/63)	7.94(5/63)
Total	214	10.75(23/214)	4.21(9/214)	9.3(20/214)	16.36 (35/214)

**Table 3 The prevalence of *A. marginale*, *B. bigemina* and *B. bovis* in samples from different regions in northern Hainan, China**

Sampling location	Host	Number of inspections	Pathogen infection rate in different samples (%)						
			<i>A. mar</i>	<i>B. bovis</i>	<i>B. big</i>	<i>A. mar+B. bovis</i>	<i>A. mar+B. big</i>	<i>B. bovis+B. big</i>	<i>A. mar+B. bovis+B. big</i>
Xiuying	<i>R. microplus</i>	13	7.69(1/13)	0	7.69(1/13)	0	0	0	0
	Leiqiong	5	100(5/5)	20(1/5)	80(4/5)	0	60(3/5)	0	20(1/5)
Longhua	<i>R. microplus</i>	10	0	0	0	0	0	0	0
Danzhou	<i>R. microplus</i>	5	0	0	0	0	0	0	0
	Leiqiong	4	0	0	25(1/4)	0	0	0	0
Dingan	<i>R. microplus</i>	12	0	0	8.3(1/12)	0	0	0	0
	Leiqiong	21	57.14(12/21)	23.81(5/21)	52.38(11/21)	4.8(1/21)	23.81(5/21)	9.52(2/21)	9.52(2/21)
	buffalo	46	2.17(1/46)	6.52(3/46)	4.35(2/46)	0	0	0	0
Chengmai	Wagyu	75	2.67(2/75)	0	0	0	0	0	0
Wenchang	<i>R. microplus</i>	23	8.7(2/23)	0	0	0	0	0	0
Total		214	10.75(23/214)	4.21(9/214)	9.35(20/214)	0.47(1/214)	3.74(8/214)	0.93(2/214)	1.40(3/214)

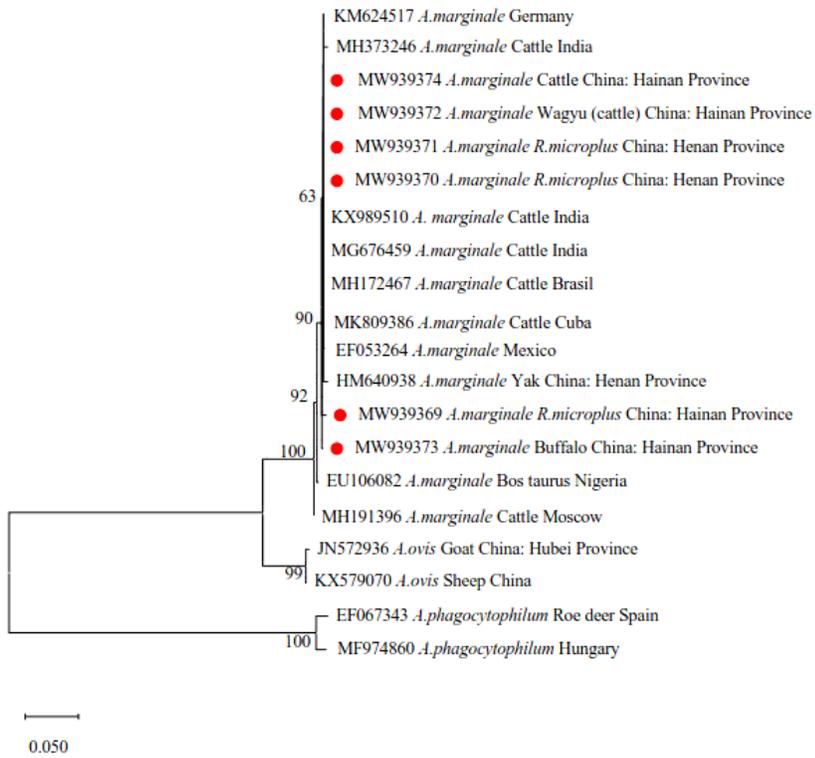
A. mar = A. marginale, B. big= B. bigemina

## Figures



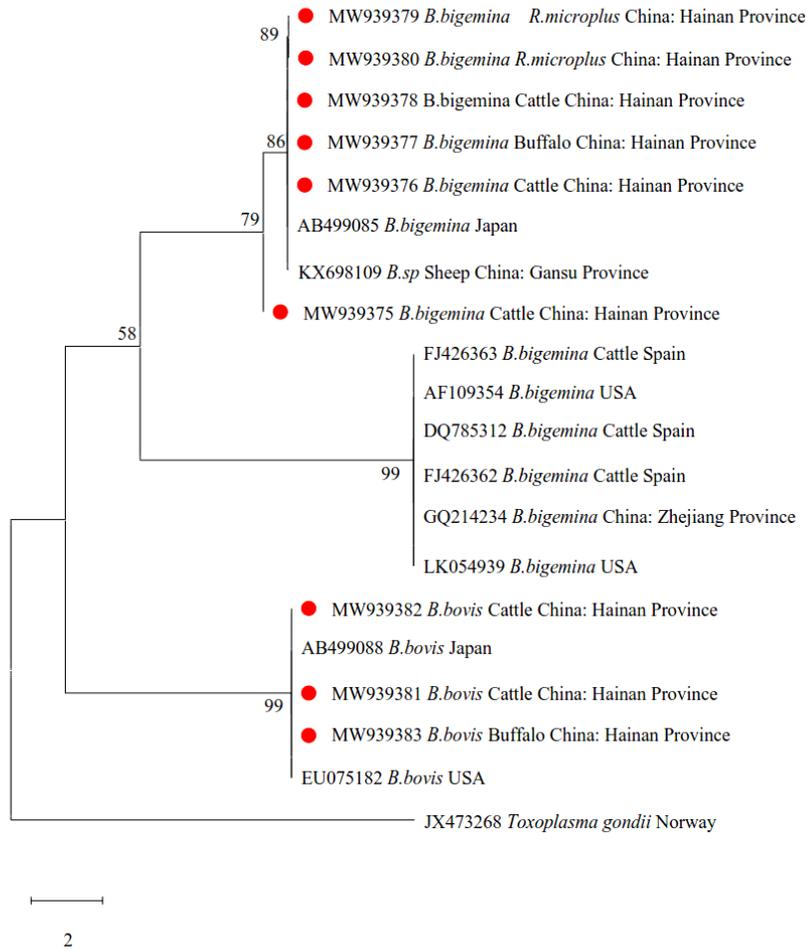
**Figure 1**

The sample collection map in the northern part of Hainan, China, the sampling location is indicated by black dots. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



**Figure 2**

Evolution tree analysis of *A. marginale* MSP4 gene from different breeds of Cattle and *R. microplus* in the northern part of Hainan. Sequences obtained in this study were represented by red dots.



**Figure 3**  
 Evolution tree analysis of *B. bigemina* and *B. bovis* Cytb gene sequences from different breeds of Cattle and *R. microplus* in the northern part of Hainan. Sequences obtained in this study were represented by red dots.

### Supplementary Files

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