# Molecular epidemiology and risk factors of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. infection in cattle in Chongqing, China

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Abstract

Tick-borne pathogens (TBPs) seriously affect cattle production and can be economically damaging. The epidemiology of these organisms in the Chongqing municipality of China is not well described. This study aimed to investigate the prevalence and risk factors of TBPs including *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. in cattle in Chongqing municipality. The results showed that 43.48% (150/345) of cattle were infected with at least one TBP, of which single infections were detected in 104 (30.14%), double infections in 34 cattle (9.86%) and triple infections in 12 (3.48%) of the cattle. The overall prevalence of *Anaplasma* spp., *Theileria* spp. and *B. bigemina* were 22.32%, 23.19% and 7.24%, respectively. Among these, the prevalence of *A. bovis*, *A. central*, *A. phagocytophilum*, *A. platys*, *A. marginale*, *T. sinensisi* and *T. orientalis* were 8.41%, 7.83%, 4.93%, 4.35%, 2.61%, 22.32% and 2.60%, respectively. We could not detect *B. bovis*, *T. annulata*, *T. luwenshuni* or *T. uilenbergi* in cattle. Cattle ≥1-year-old were more likely to be infected with *Theileria* spp. [adjusted odd ratio (AOR) = 2.70, 95% CI = 1.12–6.56] compared with younger cattle, while cattle ≥1-year-old had reduced susceptibility to *B. bigemina* (AOR = 0.14, 95% CI = 0.03–0.60). Cattle living at higher altitude (≥500 m) were more susceptible to *B. bigemina* (AOR = 6.97, 95% CI = 2.08–23.35) and *Theileria* spp. infection (AOR = 1.87, 95% CI = 1.06–3.32). The prevalence of *Theileria* spp. on farms with cats was significantly higher than that without cats (AOR = 2.56, 95% CI = 1.12–5.88). Infection with *A. bovis* and *A. central* were significantly associated with *A. phagocytophilum* infection. Furthermore, there were significant associations between *A. bovis* and *A. central* infection, *T. sinensisi* and *A. marginale* infection, and *B. bigemina* and *T. orientalis* infection. This study provides new data on the prevalence of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. in cattle in Chongqing, and for the first time we reveal a possible relationship between the afore-mentioned pathogens, which will help in formulating appropriate control strategies for these pathogens in this area.

Introduction

Tick-borne pathogens (TBPs) have always attracted the attention of researchers, not only for their damaging influence upon livestock production but also for their public health threat [1]. Among the tick-borne diseases, anaplasmosis, babesiosis and theileriosis are the most important and are distributed widely. These organisms affect cattle worldwide [2]. Till now, five *Anaplasma* pathogens (*A. marginale*, *A. bovis*, *A. centrale*, *A. phagocytophilum*, and *A. platys*) have been reported to cause bovine anaplasmosis, of which *A. phagocytophilum* has been shown to infect a variety of animals and humans [3–5]. Two mainly *Babesia* pathogens, *Babesia bovis* and *B. bigemina*, were found responsible for bovine babesiosis [6], and three species of *Theileria* including *T. annulata*, *T. sinensis*, and *T. orientalis* (also named *T. sergenti*) were the main causative agents of bovine theileriosis [7–10], and recently, *T. luwenshuni* has also been detected in blood samples from cattle and yaks [9].

Numerous studies have reported the infection and prevalence of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. in cattle across many countries [4,5,11–16]. In China, there have also been many studies [9,17–22]. However, these studies usually focus on single pathogen infections, and records on pathogen co-infections, the risk factors, and the mutual influence of each pathogen in cattle are absent. In addition, studies relating to the aforementioned pathogens in cattle in China have mainly been restricted to the northwest region, while the information is very limited for southwest China.

The total number of cattle approximated 300 million at the end of 2015 in Chongqing, and is one of the economic pillars of animal husbandry in this city. However, the prevalence of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. in cattle in this area is unclear. The objectives of this study were 1) to detect *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. in cattle in Chongqing, 2) to analyze the risk factors for infection of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp., and 3) to evaluate the associations of the aforementioned pathogens in cattle in Chongqing.

Materials and methods

Study area

Chongqing municipality is located in the southwest of China, between the northern latitudes of 28.10˚–32.13˚, and eastern longitudes of 105.11˚–110.11˚. Its altitude ranges between 73.1 m at the Yangtze River in Wushan and 2796.8 m at Liangshan peak in Wuxi. The climate tends to be subtropical, with a monsoon/humid climate and has an average annual temperature of 16–18˚C.

Blood sample collection and DNA extraction

Three hundred and forty five sodium citrate anticoagulated blood samples were collected from 10 ranches located in Tongnan, Rongchang, Jiangjing, Changshou, Liangping, Kaizhou, Yunyang, Wushan, Fuling, and Qianjiang, from May 2016 to April 2017. The ranches were selected based on the number of cattle (≥50) and convenience of sampling. The sampled animals were randomly selected from apparently healthy cattle, and the information including gender and age of cattle, as well as the altitude and the existence of cats in ranches were recorded. The blood samples were sent back to the laboratory within an ice box. Whole blood genome was extracted using a Wizard Genomic extraction kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. This study was approved by the Ethics Committee of Southwest. Consent was obtained from cattle owners before the collection of blood samples from their cattle by an experienced, practicing veterinarian.

PCR detection of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp.

Anaplasma spp. (*A. bovis*, *A. central*, *A. marginale*, *A. phagocytophilum* and *A. platys*), *Babesia* spp.(*B. bovis* and *B. bigemina*) and *Theileria* spp. (*T. annulata*, *T. sinensis*, *T. orientalis*, *T. luwenshuni* and *T. uilenbergi*) infections were detected by PCR or nested PCR using the primers reported in previous studies [7,23–29], the detail of primers can be found in S1 Table. The primers were synthesized by Bioligo Biotechnology Co., Ltd (Shanghai, China). The PCRs were performed according to the amplification programs in Table 1, with a volume of 12.5 μL in the reaction system including: 6.25 μL Premix Taq (containing TaKaRa *Taq*, dNTP Mixture and *Taq* Buffer) (Takara Dalian, China), 0.5 μL of each forward and reverse primer (20 μmol/ L), 1 μL whole blood genome and 4.25 μL ddH$_2$O. The amplified PCR products were photographed after electrophoresis in 1% agarose gels. The PCR amplification product were randomly selected for sequencing to verify the reliability of test.

Risk factor analysis

Multivariable logistic regression was performed in SPSS for Windows (18.0 version, SPSS Inc., Chicago, IL, USA) to analyze factors associated with aforementioned infections. Adjusted odd ratios (AOR) and 95% confidence intervals (CI) were calculated. A *p*-value of <0.05 was considered statistical significant.

Results

Prevalence of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. infection

A total of 345 cattle in Chongqing were included in this study. Detailed information pertaining to infection is shown in Table 1 and Fig 1. The results showed that 43.48% (150/345) of cattle were infected with at least one TBP, of which single infections were detected in 104 (30.14%), double infections in 34 cattle (9.86%) and triple infections in 12 (3.48%) of the cattle. The overall prevalence of *Anaplasma* spp., *Theileria* spp., and *B. bigemina* in cattle were 22.32% (77/ 345), 23.19% (83/345), and 7.24% (25/345), respectively. Among the *Anaplasma* spp. detected, *A. bovis* (29/345, 8.41%) was the most prevalent species recorded, followed by *A. central* (27/ 345, 7.83%), *A. phagocytophilum* (17/345, 4.93%), and *A. platys* (15/345, 4.35%), while infection with *A. marginale* (9/345, 2.61%) was the lowest. Among the *Theileria* spp., *T. sinensisi*

Table 1. Overall prevalence of *Anaplasma* spp., *B. bigemina* and *Theileria* spp. infection in cattle in Chongqing of southwest China.

Counties	Prevalence of *Anaplasma* spp.(%)						Prevalence of *Theileria* spp.(%)			BB (%)
	Anaplasma	APH	AC	AB	AM	APL	*Theileria*	TS	TO	
Qianjiang	13.33(4/30)	6.67(2/30)	10.00(3/30)	6.67(2/30)	0(0/30)	0(0/30)	6.67(2/30)	0(0/30)	6.67(2/30)	0.00(0/30)
Changshou	16.00(7/50)	0(0/50)	2.00(1/50)	2.00(1/50)	2.00(1/50)	14.00(7/50)	2.00(1/50)	2.00(1/50)	0(0/50)	8.00(4/50)
Fuling	21.43(6/28)	3.57(1/28)	7.14(2/28)	10.71(3/28)	0(0/28)	0(0/28)	25.00(7/28)	21.43(6/28)	3.57(1/28)	39.29(11/28)
Liangping	25.00(50/20)	0(0/20)	0 (0/20)	10.00(2/20)	0(0/20)	15.00(3/20)	5.00(1/20)	0(0/20)	5.00(1/20)	0(0/20)
Rongchang	28.26(13/46)	13.04(6/46)	10.86(5/46)	15.21(7/46)	0(0/46)	4.35(2/46)	23.91(11/46)	21.73(10/46)	4.35(2/46)	2.17(1/46)
Wushan	28.13(9/32)	0(0/32)	6.25(2/32)	0(0/32)	25.00(8/32)	0(0/32)	66.66(23/32)	66.66(23/32)	0(0/32)	0(0/32)
Yunyang	21.31(13/61)	4.92(3/61)	14.75(9/61)	14.75(0/61)	0(0/61)	3.28(2/61)	18.03(14/61)	21.31(13/61)	4.92(3/61)	11.48(7/61)
Kaizhou	8.57(3/35)	2.86(1/35)	5.71(2/35)	0(0/35)	0(0/35)	0(0//35)	22.86(8/35)	22.86(8/35)	0(0/35)	5.17(2/35)
Tongnan	30.30(10/33)	12.12(4/33)	0(0/33)	27.27(9/33)	0(0/33)	0(0/33)	30.30(10/33)	30.30(10/33)	0(0/33)	0(0/33)
Jiangjin	60.00(6/10)	0(0/10)	30.00(3/10)	50.00(5/10)	0(0/10)	10.00(1/10)	60.00(6/10)	60.00(6/10)	0(0/10)	0(0/10)
Total	22.32(77/345)	4.93(17/345)	7.83(27/345)	8.41(29/345)	2.61(9/345)	4.35(15/345)	23.19(83/345)	22.32(77/345)	2.60(9/345)	7.24(25/345)

Note: APH: *A. phagocytophilum*; AC: *A. central*; AB: *A. bovis*; AM: *A. marginale*; APL: *A. platys*; TS: *T. sinensisi*; TO: *T. orientalis*; BB: *B. bigemina*.

and *T. orientalis* infections in cattle were 22.32% (77/345) and 2.60% (9/345), respectively. In addition, we could not detect *B. bovis*, *T. annulata*, *T. luwenshuni*, or *T. uilenbergi* in this study.

Risk factor analysis based on blood sample data

The prevalence of *Anaplasma* spp. (25.13%) and *Theileria* spp. (27.27%) in male cattle was significantly higher than that in females (*Anaplasma* spp.: AOR = 2.18, 95% CI = 1.05–4.52; *Theileria* spp.: AOR = 3.27, 95% CI = 1.47–7.25). Cattle ≥ 1-year were more likely to be infected with *Theileria* spp. than cattle aged below 1-year of age (25.00% vs. 20.99%), and the difference was statistically significant (AOR = 2.70, 95% CI = 1.12–6.56). In contrast, cattle ≥1-year had a lower risk of *B. bigemina* infection (AOR = 0.14, 95% CI = 0.03–0.60). Ranches at an altitude ≥500 m was found to be a risk factor for *B. bigemina* (AOR = 6.97, 95% CI = 2.08–23.35) and *Theileria* spp. infection (AOR = 1.87, 95% CI = 1.06–3.32). With exception of *Theileria* spp. (AOR = 2.56, 95% CI = 1.12–5.88), there were no significant associations between presence of cats and infection with *Anaplasma* spp., or *B. bigemina*, (Table 2).

Risk factor analysis based on pathogen co-infection

In order to evaluate the effect of specific pathogen infections and how they influence other pathogen infections within the same host, we considered each tested pathogen species as a potential risk factor in the analysis. The results of correlation analyses between each species of pathogen in infected cattle in Chongqing, are shown in Table 3. Infection with *A. bovis* and *A. central* were significantly associated with *A. phagocytophilum* infection, and *A.*
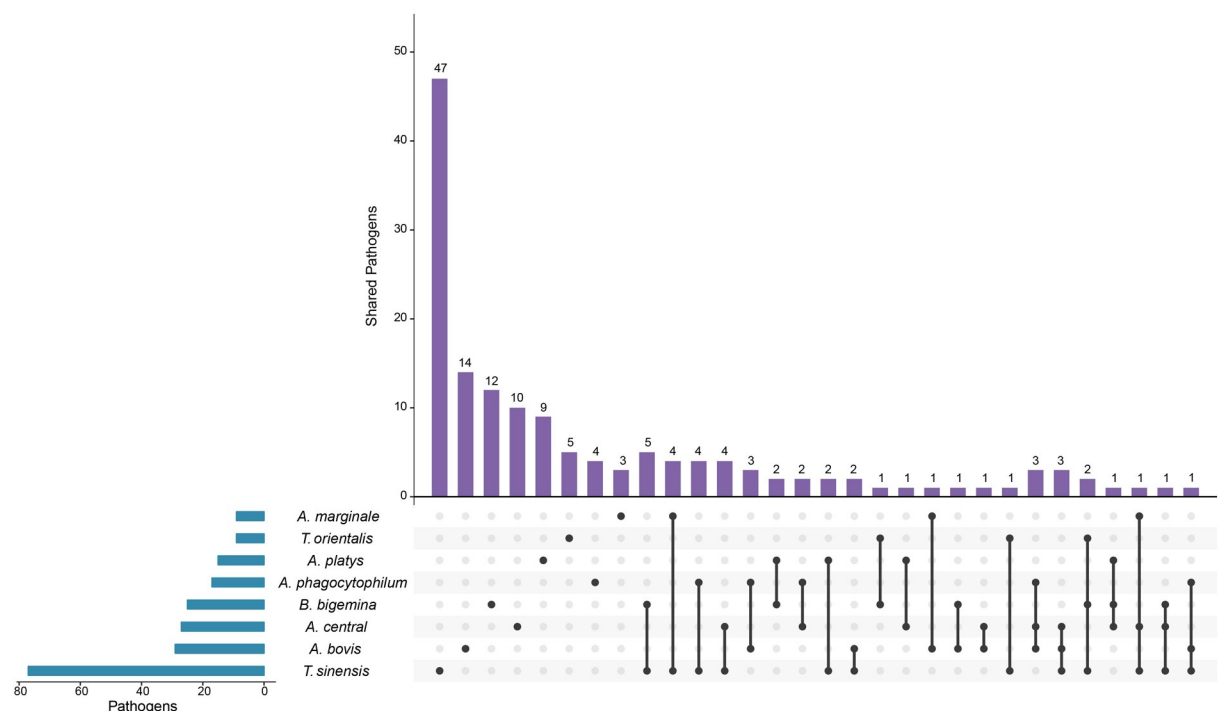
Fig 1. An UpSetR plot of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. infection in cattle from Chongqing of southwest China (n = 345). The blue horizontal coordinate columns represent the number of positive samples infected by pathogens. The purple vertical coordinate columns represent the number of positive samples infected by a single pathogen or multiple pathogens. The black dots represent the corresponding pathogens, and the dots connected by lines indicate co-infection of pathogens.

Table 2. Multivariate analysis of selected factors and their association with *Anaplasma* spp., *B. bigemina* and *Theileria* spp. infection in cattle in Chongqing of southwest China.

Factors	Positive/Examined	Prevalence (%)	AOR (95% CI)	*p*-value
***Anaplasma* spp.**				
Gender				
Male	47/187	25.13	2.18(1.05–4.52)	0.037
Female	30/158	18.99	Reference	
Age				
≥1 year	58/264	21.97	1.68(0.75- 3.75)	0.204
<1 year	19/81	23.46	Reference	
Altitude				
≥500 m	31/156	19.87	0.62(0.34–1.10)	0.104
<500 m	46/189	24.34	Reference	
Cats presence				
Yes	46/204	22.55	1.87(0.89- 3.93)	0.100
No	31/141	21.99	Reference	
B. bigemina				
Gender				
Male	14/187	7.49	0.450(0.10–1.92)	0.280
Female	11/158	6.69	Reference	
Age				
≥1 year	13/264	4.92	0.14(0.03–0.60)	0.009
<1 year	12/81	14.81	Reference	
Altitude				
≥500 m	20/156	12.82	6.97(2.08–23.35)	0.002
<500 m	5/189	2.65	Reference	
Cats presence				
Yes	22/204	10.78	1.40(0.28–7.03)	0.681
No	3/141	2.13	Reference	
***Theileria* spp.**				
Gender				
Male	51/187	27.27	3.27(1.47–7.25)	0.004
Female	32/158	21.52	Reference	
Age				
≥1 year	66/264	25.00	2.70(1.12–6.56)	0.027
<1 year	17/81	20.99	Reference	
Altitude				
≥500 m	52/156	33.33	1.87(1.06–3.32)	0.031
<500 m	31/189	16.40	Reference	
Cats presence				
Yes	55/204	26.96	2.56(1.12–5.88)	0.025
No	28/141	19.86	Reference	

Note: AOR = adjusted odds ratio.

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phagocytophilum was more likely to increase the risk of *A. central* infection (AOR = 3.80, 95% CI = 1.10–13.18, *p* = 0.035). However, *A. central* was less likely to impact upon infection with *A. phagocytophilum* (AOR = 3.50, 95% CI = 0.97–12.59, *p* = 0.055). Furthermore, there was a significant association between *A. bovis* and *A. central* infection, *T. sinensisi* and *A. marginale*

Table 3. Correlation analysis based on species of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. infection in cattle in Chongqing, southwest China.

Targets	Factors	No. examed	No. positive (%)	AOR	95% CI	*p*-value
APH						
	AB infected	29	7 (24.14)	6.84	2.20–21.20	0.001
	Non-*AB* infected	316	10 (3.16)	Reference		
	AC infected	27	5 (18.52)	3.50	0.97–12.59	0.055
	Non-*AC* infected	318	12 (3.77)	Reference		
AB						
	AC infected	27	7 (25.93)	3.56	1.21–10.40	0.020
	Non-*AC* infected	318	22 (6.92)	Reference		
	APH infected	17	7 (41.18)	7.09	2.29–21.97	0.001
	Non-*APH* infected	328	22(6.71)	Reference		
AM						
	TS infected	77	5 (6.49)	4.74	1.20–18.62	0.026
	Non- *TS* infected	268	4 (1.49)	Reference		
BB						
	TO infected	9	3 (33.33)	6.87	1.55- 30.47	0.011
	Non- *TO* infected	336	22 (6.55)	Reference		
AC						
	AB infected	29	7(24.14)	3.74	1.29–10.79	0.015
	Non- *AB* infected	316	20(5.33)	Reference		
	APH infected	17	5 (29.41)	3.80	1.10- 13.18	0.035
	Non- *APH* infected	328	22(6.71)	Reference		
TO						
	BB infected	25	3(12.00)	6.84	1.52–30.82	0.012
	Non- *BB* infected	320	6(1.88)	Reference		
TS						
	AM infected	9	5(55.56)	4.77	1.21- 18.64	0.025
	Non- *AM* infected	336	72(21.43)	Reference		

Note: AOR: adjusted odds ratio; APH: *A. phagocytophilum*; AC: *A. central*; AB: *A. bovis*; AM: *A. marginale*; APL: *A. platys*; TS: *T. sinensisi*; TO: *T. orientalis*; BB: *B. bigemina*.

infection, and *B. bigemina* and *T. orientalis* infection ($p<0.05$). There were no significant associations between other pathogens that we aimed to identify.

Discussion

For the first time, this systematic study investigated the epidemiology of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. infection in cattle in Chongqing, China. The infection rate of *Anaplasma* spp. in our study was lower than that reported in Algeria [5] and in Tunisia [16], but higher than that reported in northwest China [30]. The prevalence of *A. bovis* (8.41%) in cattle in Chongqing was higher than that of cattle reported in other locations, where the prevalence varied from 3.9% to 6.2% [5,16,18,30]. In contrast, the prevalence of *A. centrale* (7.83%) was lower than that of cattle in previous studies (range between 12.1%-39.4%) [5,11,16,31,32]. Compared to the high prevalence of *A. marginale* in cattle in Madagascar (89.7%), north-eastern Uganda (82.9%) [11], South Africa (57%) [31], Thailand (39.1%) [13] and in China (31.6%)[33], we demonstrated a relatively low infection rate of *A. marginale* (2.61%) in Chongqing. In addition, 4.93% of cattle tested positive for *A. phagocytophilum* in this study, which was similar to the positivity rate (5.3%) of this pathogen in white yaks [30]. *A. platys* infection

in cattle was first reported in Algeria [4], while Ben et al. reported a prevalence of *A*. *platys*-like species (3.5%, 13/367) in cattle in Tunisia [34]. In this study, we noted a prevalence of *A*. *platys* (4.35%, 15/345) in cattle for the first time in Chongqing.

The prevalence of *B*. *bigemina* in this study was similar to previous research by Liu et al [21], and is lower than that reported in other provinces of China [19–21,35], South Africa [36], and in Tanzania [37]. However, the prevalence in our study was higher than that recorded in the Philippines [38]. In this survey, only *T*. *sinensisi* and *T*. *orientalis* were detected, with the prevalence being lower than *T*. *sinensisi* and *T*. *orientalis* infection rates recorded elsewhere [13,14,17]. Similar to the previous report [17], we did not detected *B*. *bovis* infection in cattle. The reason may be that 1) *B*. *bovis* infection in tick is usually lower than *B*. *bigemina*, which result a lower transmission rates of *B*. *bovis*, and 2) *B*. *bovis*-infected red blood cells usually accumulate in the capillary bed and leading to low parasitemia in circulating blood [17]. For the reasons that *T*. *annualata*, transmitted by *Hyalomma anatolicum anatolicum*, is mainly distributed in Northern China [39], *T*. *luwenshuni* and *T*. *uilenbergi*, both transmitted by *Haemaphysalis qinghaiensis* and *H*. *longicornis*, usually infected sheep and goats in China [27], and there is no evidence of above ticks existence in Chongqing. It was not strange that we did not detected *T*. *annualata*, *T*. *luwenshuni*, or *T*. *uilenbergi* infection in cattle from Chongqing.

There were 117 described species in the Chinese tick, 38 of which carry multiple pathogens [40], and most of the ticks including *H*. *anatolicum*, *H*. *qinghaiensis*, *H*. *longicornis*, *H*. *bispinosa*, *Rhipicephalus (Boophilus) microplus*, *R*. *sanguineus*, *Dermacentor abaensis*, *D*. *silvarum* and *D*. *nuttalli* were founded in northwest, northeast or central of China [39,41–44], and these ticks are responsible for transmission of a large amount of TBPs. However, the only reported tick specie in Chongqing was *R*. *microplus* [45], which was recorded to be the vector of *A*. *phagocytophilumin*, *A*. *marginale*, *B*. *bigemina* and *B*. *bovis* in China [40,46,47]. The differences in the prevalence of some parasites in this study compared to that reported previously in other studies in China or other countries, might be associated with geographical difference and variation in tick species.

Risk factor analysis revealed a significant correlation of altitude and age with the prevalence of *B*. *bigemina* and *Theileria* spp., which supported a previous report that there was a trend in increased seropositivity for *B*. *bigemina* infection with age [37]. In addition, gender is a risk factor associated with prevalence of *Anaplasma* spp. and *Theileria* spp., in cattle, which showed that male cattle had higher risk for these two type of pathogens infection, and the presence of cats in farm had positive effect on *Theileria* spp. infection in cattle from Chongqing, and the reasons for these phenomenon are not clear.

This study first took a single infection as a risk factor in evaluating the impact on infection with other pathogens. We found that cattle infected with *A*. *bovis* or *A*. *central* were more likely to be infected with *A*. *phagocytophilum*, and there was also a strong association between *A*. *bovis* and *A*. *central* infection. In addition, a very close relationship was observed for co-infection with *T*. *sinensisi* and *A*. *marginale*, and *B*. *bigemina* and *T*. *orientalis*. *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. are all tick borne pathogens (TBPs), and some ticks can harbor mixed TBPs [40,47,48]. For the reasons that one species of TBP can be spread by different types of ticks, and equally that the same type of tick may also be the transmission vector for many species of TBPs, the significant correlation of the aforementioned pathogens might be attributed to the fact that infected cattle were bitten by ticks carrying different pathogens. From current data, it is not possible to estimate the chronological order of the aforementioned pathogen infections but there does appear to be significant relationships among some of these pathogens during infection of cattle. Parasite-parasite interaction may modify the impact of the pathogenic species and affect the performance and survival of host [49,50]. It is a pity that this study failed to evaluated the effect of above TBPs on health of cattle, since all the sampled

animals in this study were apparently healthy, and we did not track the outcome of these cattle and the causes of their death. Further research should be conducted to elucidate the type of ticks present in Chongqing and the proportion of ticks that carry TBPs. Furthermore, attempts should be made to confirm whether a pathogen significantly increases the incident infection of other pathogens and the effects on production performance of cattle.

Conclusions

The results of the present survey indicated that infection of cattle with *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. is widespread in Chongqing. We provide a possible relationship between afore-mentioned pathogenic infections, which will help in formulating appropriate control strategies for these pathogens in this area.

Supporting information

S1 Table. Primers used for *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. detection in cattle.
(DOCX)

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References

1. Vayssier-Taussat M, Cosson JF, Degeilh B, Eloit M, Fontanet A, Moutailler S, et al. How a multidisciplinary 'One Health' approach can combat the tick-borne pathogen threat in Europe. Future Microbiol. 2015, 10(5):809–818. https://doi.org/10.2217/fmb.15.15 PMID: 26000651

2. Makala LH, Mangani P, Fujisaki K, Nagasawa H. The current status of major tick borne diseases in Zambia. Vet Res. 2003, 34(1):27–45. https://doi.org/10.1051/vetres:2002056 PMID: 12588682

3. Battilani M, De Arcangeli S, Balboni A, Dondi F. Genetic diversity and molecular epidemiology of *Anaplasma*. Infect Genet Evol. 2017, 49:195–211. https://doi.org/10.1016/j.meegid.2017.01.021 PMID: 28122249

4. Dahmani M, Davoust B, Benterki MS, Fenollar F, Raoult D, Mediannikov O. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. Comp Immunol Microbiol Infect Dis. 2015, 39:39–45. https://doi.org/10.1016/j.cimid.2015.02.002 PMID: 25748051

5. Rjeibi MR, Ayadi O, Rekik M, Gharbi M. Molecular survey and genetic characterization of *Anaplasma centrale*, *A. marginale* and *A. bovis* in cattle from Algeria. Transbound Emerg Dis. 2018, 65(2):456–464. https://doi.org/10.1111/tbed.12725 PMID: 29034616

6. Bock R, Jackson L, de Vos A, Jorgensen W. Babesiosis of cattle. Parasitology. 2004, 129 Suppl: S247–S269. PMID: 15938514

7. Liu A, Guan G, Liu Z, Liu J, Leblanc N, Li Y, et al. Detecting and differentiating *Theileria sergenti* and *Theileria sinensis* in cattle and yaks by PCR based on major piroplasm surface protein (MPSP). Exp Parasitol. 2010, 126(4):476–481. https://doi.org/10.1016/j.exppara.2010.05.024 PMID: 20685208

8. Luo J, Lu W. Cattle theileriosis in China. Tropical Animal Health & Production. 1997, 29(4):4S–7S.

9. Qin G, Li Y, Liu J, Liu Z, Yang J, Zhang L, et al. Molecular detection and characterization of *Theileria* infection in cattle and yaks from Tibet Plateau Region, China. Parasitol Res. 2016, 115(7):2647–2652. https://doi.org/10.1007/s00436-016-5011-8 PMID: 27000088

10. Bai Q, Liu GY, Yin H, Zhao Q, Liu DK, Ren JX, et al. *Theileria Sinensis* sp nov: A New Species of *Bovine Theileria*—Classical Taxonomic Studies. Acta Veterinaria et Zootechnica Sinica 2002, 33(1):73–77.

11. Byaruhanga C, Collins NE, Knobel DL, Khumalo Z, Chaisi ME, Oosthuizen MC. Molecular detection and phylogenetic analysis of *Anaplasma marginale* and *Anaplasma centrale* amongst transhumant cattle in north-eastern Uganda. Ticks Tick Borne Dis. 2018, 9(3): 580–588. https://doi.org/10.1016/j.ttbdis.2018.01.012 PMID: 29422446

12. Han DG, Ryu JH, Chae JB, Kim DW, Kwon CH, Choi KS. First report of *Anaplasma phagocytophilum* infection in Holstein cattle in the Republic of Korea. Acta Trop. 2018, 183:110–113. https://doi.org/10.1016/j.actatropica.2018.04.014 PMID: 29654732

13. Jirapattharasate C, Adjou MP, Cao S, Iguchi A, Liu M, Wang G, et al. Molecular detection and genetic diversity of bovine *Babesia* spp., *Theileria orientalis*, and *Anaplasma marginale* in beef cattle in Thailand. Parasitol Res. 2017, 116(2):751–762. https://doi.org/10.1007/s00436-016-5345-2 PMID: 28028631

14. Zhou M, Cao S, Sevinc F, Sevinc M, Ceylan O, Moumouni P, et al. Molecular detection and genetic identification of *Babesia bigemina*, *Theileria annulata*, *Theileria orientalis* and *Anaplasma marginale* in Turkey. Ticks Tick Borne Dis. 2016, 7(1):126–134. https://doi.org/10.1016/j.ttbdis.2015.09.008 PMID: 26492823

15. Adjou MP, Aboge GO, Terkawi MA, Masatani T, Cao S, Kamyingkird K, et al. Molecular detection and characterization of *Babesia bovis*, *Babesia bigemina*, *Theileria* species and *Anaplasma marginale* isolated from cattle in Kenya. Parasit Vectors. 2015, 8:496. https://doi.org/10.1186/s13071-015-1106-9 PMID: 26420543

16. Belkahia H, Ben SM, Alberti A, Abdi K, Issaoui Z, Hattab D, et al. First molecular survey and novel genetic variants' identification of *Anaplasma marginale*, *A. centrale* and *A. bovis* in cattle from Tunisia. Infect Genet Evol. 2015, 34:361–371. https://doi.org/10.1016/j.meegid.2015.06.017 PMID: 26117444

17. Li S, Liu J, Liu A, Li Y, Wang S, Wang S, et al. Molecular investigation of piroplasma infection in white yaks (*Bos grunniens*) in Gansu province, China. Acta Trop. 2017, 171:220–225. https://doi.org/10.1016/j.actatropica.2017.04.009 PMID: 28427963

18. Yang J, Li Y, Liu Z, Liu J, Niu Q, Ren Q, et al. Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. Parasit Vectors. 2015, 8:108. https://doi.org/10.1186/s13071-015-0727-3 PMID: 25889906

19. Li Y, Liu P, Wang C, Chen G, Kang M, Liu D, et al. Serologic Evidence for *Babesia bigemina* Infection in Wild Yak (*Bos mutus*) in Qinghai Province, China. J Wildl Dis. 2015, 51(4):872–875. https://doi.org/10.7589/2014-03-076 PMID: 26267460

20. Niu Q, Liu Z, Yu P, Yang J, Abdallah MO, Guan G, et al. Genetic characterization and molecular survey of *Babesia bovis*, *Babesia bigemina* and B*abesia ovata* in cattle, dairy cattle and yaks in China. Parasit Vectors. 2015, 8:518. https://doi.org/10.1186/s13071-015-1110-0 PMID: 26452623

21. Liu J, Guan G, Liu A, Li Y, Yin H, Luo J. A PCR method targeting internal transcribed spacers: the simultaneous detection of *Babesia bigemina* and *Babesia bovis* in cattle. Acta Parasitol. 2014, 59(1):132–138. https://doi.org/10.2478/s11686-014-0222-6 PMID: 24570060

22. Liu A, Guan G, Du P, Gou H, Zhang J, Liu Z, et al. Rapid identification and differentiation of *Theileria sergenti* and *Theileria sinensis* using a loop-mediated isothermal amplification (LAMP) assay. Vet Parasitol. 2013, 191(1–2):15–22. https://doi.org/10.1016/j.vetpar.2012.08.006 PMID: 22947247

23. D'Oliveira C, Van DWM, Habela MA, Jacquiet P, Jongejan F. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. J Clin Microbiol. 1995, 33(10):2665. PMID: 8567902

24. Barlough JE, Madigan JE, DeRock E, Bigornia L. Nested polymerase chain reaction for detection of *Ehrlichia equi* genomic DNA in horses and ticks (*Ixodes pacificu*s). Vet Parasitol. 1996, 63(3–4):319–329. PMID: 8966998

25. Inokuma H, Ohno K, Onishi T, Raoult D, Brouqui P. Detection of ehrlichial infection by PCR in dogs from Yamaguchi and Okinawa Prefectures, Japan. J Vet Med Sci. 2001, 63(7):815–817.

26. Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki A, et al. Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and

ticks on two major islands in Japan. Appl Environ Microbiol. 2006, 72(2):1102–1109. https://doi.org/10.1128/AEM.72.2.1102-1109.2006 PMID: 16461655

27. Yin H, Liu Z, Guan G, Liu A, Ma M, Ren Q, et al. Detection and differentiation of *Theileria luwenshuni* and *T. uilenbergi* infection in small ruminants by PCR. *Transboundary & Emerging Diseases* 2008, 55 (5-6):233–237.

28. Terkawi MA, Huyen NX, Shinuo C, Inpankaew T, Maklon K, Aboulaila M, et al. Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffaloes in the northeast region of Thailand. Vet Parasitol. 2011, 178(3–4):201–207.

29. Zhang L, Wang Y, Cai D, He G, Cheng Z, Liu J, et al. Detection of *Anaplasma marginale* in Hyalomma asiaticum ticks by PCR assay. Parasitol Res. 2013, 112(7):2697–2702. https://doi.org/10.1007/s00436-013-3437-9 PMID: 23636309

30. Yang J, Liu Z, Niu Q, Liu J, Guan G, Xie J, et al. First molecular survey and identification of *Anaplasma* spp. in white yaks (Bos grunniens) in China. Parasitology. 2016, 143(6):686–691. https://doi.org/10.1017/S003118201600041X PMID: 27003378

31. Hove P, Chaisi ME, Brayton KA, Ganesan H, Catanese HN, Mtshali MS, et al. Co-infections with multiple genotypes of *Anaplasma marginale* in cattle indicate pathogen diversity. Parasit Vectors. 2018, 11 (1):5. https://doi.org/10.1186/s13071-017-2595-5 PMID: 29298712

32. Sisson D, Hufschmid J, Jolles A, Beechler B, Jabbar A. Molecular characterisation of *Anaplasma* species from African buffalo (Syncerus caffer) in Kruger National Park, South Africa. Ticks Tick Borne Dis. 2017, 8(3):400–406. https://doi.org/10.1016/j.ttbdis.2017.01.003 PMID: 28169172

33. Yang J, Han R, Liu Z, Niu Q, Guan G, Liu G, et al. Insight into the genetic diversity of *Anaplasma marginale* in cattle from ten provinces of China. Parasit Vectors. 2017, 10(1):565. https://doi.org/10.1186/s13071-017-2485-x PMID: 29132409

34. Ben SM, Belkahia H, El MN, Saidani M, Alberti A, Zobba R, et al. *Anaplasma platys*-like strains in ruminants from Tunisia. Infect Genet Evol. 2017, 49:226–233. https://doi.org/10.1016/j.meegid.2017.01.023 PMID: 28130168

35. Qin SY, Wang JL, Ning HR, Tan QD, Yin MY, Zhang XX, et al. First report of *Babesia bigemina* infection in white yaks in China. Acta Trop. 2015, 145:52–54. https://doi.org/10.1016/j.actatropica.2015.02.005 PMID: 25700713

36. Mtshali PS, Tsotetsi AM, Thekisoe MM, Mtshali MS. Nested PCR detection and phylogenetic analysis of *Babesia bovis* and *Babesia bigemina* in cattle from Periurban localities in Gauteng Province, South Africa. J Vet Med Sci. 2014, 76(1):145–150. https://doi.org/10.1292/jvms.13-0197 PMID: 24065081

37. Swai ES, Karimuribo ED, French NP, Fitzpatrick JL, Bryant MJ, Kambarage DM, et al. Seroprevalence of *Babesia bigemina* in smallholder dairy cattle in Tanzania and associated risk factors. J S Afr Vet Assoc. 2007, 78(1):15–20. PMID: 17665760

38. Yu L, Terkawi MA, Cruzflores MJ, Claveria FG, Aboge GO, Yamagishi J, et al. Epidemiological survey of *Babesia bovis* and *Babesia bigemina* infections of cattle in Philippines. J Vet Med Sci. 2013, 75 (7):995–998. PMID: 23470278

39. Luo JX, Yin H, Guan GQ, Ma ML, Sun CQ, Lu BY, et al. Experiments on Transmission of *Babesia* U sp. and *Theleria annulata* with *Hyalomma anatolicum anatolicum*. Acta Veterinaria et Zootechnica S inica. 2005, 36(11): 1197–1201. (in Chinese).

40. Yu Z, Wang H, Wang T, Sun W, Yang X, Liu J. Tick-borne pathogens and the vector potential of ticks in China. Parasit Vectors. 2015, 8:24. https://doi.org/10.1186/s13071-014-0628-x PMID: 25586007

41. Han R, Yang J, Niu Q, Liu Z, Chen Z, Kan W, et al. Molecular prevalence of spotted fever group *rickettsiae* in ticks from Qinghai Province, northwestern China. Infect Genet Evol. 2018, 57: 1–7. https://doi.org/10.1016/j.meegid.2017.10.025 PMID: 29107656

42. Yu P, Liu Z, Niu Q, Yang J, Abdallah MO, Chen Z, et al. Molecular evidence of tick-borne pathogens in *Hyalomma anatolicum* ticks infesting cattle in Xinjiang Uygur Autonomous Region, Northwestern China. Exp Appl Acarol. 2017, 73: 269–281. https://doi.org/10.1007/s10493-017-0162-6 PMID: 28875270

43. Niu Q, Liu Z, Yang J, Gao S, Pan Y, Guan G, et al. Genetic characterization and molecular survey of *Babesia* sp. Xinjiang infection in small ruminants and ixodid ticks in China. Infect Genet Evol 49: 330–335. https://doi.org/10.1016/j.meegid.2017.01.025 PMID: 28131866

44. Chen Z, Liu Q, Liu JQ, Xu BL, Lv S, Xia S, et al. Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. Parasit Vectors. 2014, 7: 237. https://doi.org/10.1186/1756-3305-7-237 PMID: 24886497

45. Tang M, Nie K, Wu Q, Jin A, Wen C, Sun T, et al.Investigation of livestock and poultry parasites in Chongqing. Chinese Journal of Veterinary Parasitology.2003, 11(1): 25–30. (in Chinese).

46. Tian Z, Du J, Yang J, Liu A, Liu X, Liu G, et al. A PCR-RFLP Assay targeting RPS8 gene for the discrimination between bovine *Babesia* and *Theileria* species in China. Parasit Vectors. 2015, 8: 475. https://doi.org/10.1186/s13071-015-1085-x PMID: 26382041

47. Zhang L, Liu H, Xu B, Lu Q, Li L, Chang L, et al. *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China. Am J Trop Med Hyg. 2012, 87(1):185–189. https://doi.org/10.4269/ajtmh.2012.12-0005 PMID: 22764312

48. Palomar AM, Portillo A, Santibanez P, Mazuelas D, Roncero L, Garcia-Alvarez L, et al. Detection of tick-borne *Anaplasma bovis*, *Anaplasma phagocytophilum* and *Anaplasma centrale* in Spain. Med Vet Entomo. 2015, 29(3):349–353.

49. Thumbi SM, Bronsvoort BM, Poole EJ, Kiara H, Toye PG, Mbole-Kariuki MN, et al. Parasite co-infections and their impact on survival of indigenous cattle. PLoS One. 2014, 9: e76324. https://doi.org/10.1371/journal.pone.0076324 PMID: 24586220

50. Craig BH, Tempest LJ, Pilkington JG, Pemberton JM. Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep. Parasitology. 2008. 135: 433–441. https://doi.org/10.1017/S0031182008004137 PMID: 18215336