

Appendix Table S1. The PCR equipment was a Mastercycler X50s, Eppendorf, Germany.

1. PCR amplification to detect piroplasms based on the *18S rRNA*, *EMA-1* and *rap-1* gene

For all fragments, the reactions were performed in a final volume of 25 µL containing 12.5 µL of Premix *Taq* DNA polymerase (TakaRa, China), 1.0 µM of each primer and 1.0 µl of DNA template. If there was a second PCR, the reaction mixture was the same as the first PCR, except that the template was replaced by 1 µL of the first PCR product. The PCR cycling conditions were as shown in the following table.

2. Characteristics of amplified fragments and corresponding primer sequences

Primer name	Gene	Primer sequence (5'-3')	Fragment	Cycling conditions of PCR assays	Reference
RLB(out)		RLB-F2 (GACACAGGGAGGTAGTGACAAG)		95 °C for 3 min, followed by 35 cycles at 95 °C for 60 s , 52 °C for 50 s and 72 °C for 1.5 min, with a final extension at 72 °C for 5 min.	[1]
		RLB-R2 (CTAAGAATTTACCTCTGACAGT)			
RLB(in)	<i>18S rRNA</i>	RLB-F2 (GACACAGGGAGGTAGTGACAAG)	400bp	The second PCR 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 5 min.	[1]
		RLB-FINT (GACAAGAAATAACAATACRGGGC)			
EMA-1	<i>EMA-1</i>	EMA-1F (GCATCCATTGCCATTTTCGAG)	750bp	96°C for 10 min, followed by 40 cycles at 94°C for 60 s, 60.5°C for 60 s, and 72°C for 60 s, with a final extension at 72°C for 10 min.	[2]
		EMA-1R (TGCGCCATAGACGGAGAAGC)			
rap-1	<i>rap-1</i>	rap-1F(CCAACCGCTGACCCTTC)	568bp	95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 7 min.	[3]
		rap-1R (CTTCAGCTTCATGTACCACTTCTT)			

- [1] Liu, J., Yang, J., Guan, G., Liu, A., Wang, B., Luo, J., Yin, H., 2016. Molecular detection and identification of piroplasms in sika deer (*Cervus nippon*) from Jilin Province, China. *Parasit Vectors* 9, 156. <https://doi.org/10.1186/s13071-016-1435-3>
- [2] Alhassan, A., Pumidonming, W., Okamura, M., Hirata, H., Battsetseg, B., Fujisaki, K., Yokoyama, N., Igarashi, I., 2005. Development of a single-round and multiplex PCR method for the simultaneous detection of *Babesia caballi* and *Babesia equi* in horse blood. *Vet Parasitol* 129, 43–49. <https://doi.org/10.1016/j.vetpar.2004.12.018>
- [3] Otgonsuren, D., Amgalanbaatar, T., Narantsatsral, S., Enkhtaivan, B., Munkhgerel, D., Zoljargal, M., Davkharbayar, B., Myagmarsuren, P., Battur, B., Battsetseg, B., Sivakumar, T., Yokoyama, N., 2024. Epidemiology and genetic diversity of *Theileria equi* and *Babesia caballi* in Mongolian horses. *Infect Genet Evol* 119, 105571. <https://doi.org/10.1016/j.meegid.2024.105571>