

Supplementary material

PCR details:

Babesia canis (used to amplify the 18S ribosomal DNA gene fragment of *B. canis*):

Primers:

Forward primer (BcW-A): 5'-CAT CTA AGG AAG GCA GCA GG-3'

Reverse primer (BcW-B): 5'-TTA ATG GAA ACG TCC TTG GC-3'

PCR mixture (50 µL):

10 µM both primers (1 µL of each primer),

10 mM dNTP Mix (2 µL),

10× Taq Buffer with KCl (5 µL),

2 IU of Taq DNA Polymerase (1 UI/1µL),

25 mM MgCl₂ (4 µL),

extracted template DNA (10 µL),

injection water (25 µL)

PCR:

Preamplification denaturation 94°C (2 min)

36 cycles:

Denaturation 94°C (30 s)

Annealing 53 °C (30 s)

Elongation 72 °C (30 s)

Final elongation 72 °C (5 min)

The size of the PCR products about 500 bp (electrophoresis in a 1.5% agarose gel)

Anaplasma phagocytophilum (used to amplify *epank1* gene fragment of *A. phagocytophilum*)

Primers:

Forward primer (LA6): 5'-GAG AGA TGC TTA TGG TAA GAC-3'

Reverse primer (LA1): 5'-CGT TCA GCC ATC ATT GTG AC-3'

PCR mixture (50 µL):

10 µM both primers (1 µL of each primer)

10 mM dNTP Mix (2 µL),

10× Taq Buffer with KCl (5 µL),

2 IU of Taq DNA Polymerase (1 UI/1µL),

25 mM MgCl₂ (4 µL),

extracted template DNA (10 µL),

injection water (25 µL)

PCR:

Preamplification denaturation 94°C (2 min)

2 cycles:

Denaturation 94°C (30 s)

Annealing 62 °C (30 s)

Elongation 72 °C (30 s)

2 cycles:

Denaturation 94°C (30 s)

Annealing 60 °C (30 s)

Elongation 72 °C (30 s)

2 cycles:

Denaturation 94°C (30 s)

Annealing 58 °C (30 s)

Elongation 72 °C (30 s)

2 cycles:

Denaturation 94°C (30 s)

Annealing 56 °C (30 s)
Elongation 72 °C (30 s)
28 cycles:
Denaturation 94°C (30 s)
Annealing 54 °C (30 s)
Elongation 72 °C (30 s)
Final elongation 72 °C (5 min)
The size of the PCR products about 440 bp (electrophoresis in a 1.5% agarose gel)

Figure S1. *Babesia canis* merozoites detected in microscopic blood smear examination

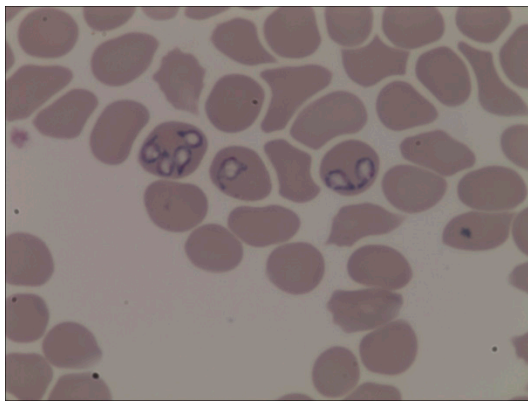
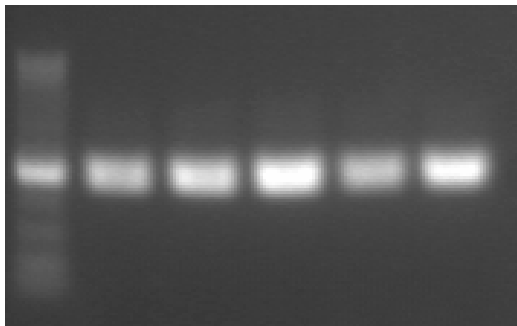


Figure S2. Results of *B. canis* PCR (Eurx Perfect™ 100-1000 bp DNA ladder), PCR product 500 bp.



Azotaemia (group A) in 22 out of 34 dogs (64.71%; Confidence interval: 46.49% - 80.25%)

Lack of azotaemia (group B) in 12 out of 34 dogs (35.29%; Confidence interval: 19.75% - 53.51%)

Pre-renal azotaemia (subgroup A1) in 13 out of 22 azotaemic dogs (59.09%; Confidence interval: 36.35% - 79.29%)

Renal azotaemia (subgroup A2) in 9 out of 22 azotaemic dogs (40.91%; Confidence interval: 20.71% - 63.64%).

Table S1. Correlations between renal indices in 22 azotaemic dogs infected with *B. canis* (group A)

	RFI	FE(Na ⁺)	UCr/SCr	SU/SCr
RFI		R = 0.991, p = 0.000*	R = -0.919, p = 0.000*	R = -0.318, p = 0.149
FE(Na ⁺)	R = 0.991, p = 0.000*		R = -0.924, p = 0.000*	R = -0.308, p = 0.163
UCr/SCr	R = -0.919, p = 0.000*	R = -0.924, p = 0.000*		R = 0.361, p = 0.098
SU/SCr	R = -0.318, p = 0.149	R = -0.308, p = 0.163	R = 0.361, p = 0.098	

*statistically significant result