Supplementary File 1 (PCR protocols):

For ***Bartonella* spp.** detection, citrate synthase gene gltA fragment was targeted (~800 bp), using 443F (5'-GCT ATG TCT GCA TTC TAT CA-3') (Birtles and Raoult, 1996) and BhCS.1137n primers (5'-AAT GCA AAA AGA ACA GTA AAC A-3') (Norman et al., 1995).

Mixture: 25 μl reaction mixture which contained 12.5 μl 2× Green Master Mix (Rovalab GmBH, Teltow, Germany), 6.5 μl water, 1 μl of each primer (0.01 mM final concentration) and 4 μl aliquot of isolated DNA.

1. Table: PCR conditions for *Bartonella* detection

Primers 443F and BhCS.1137 (Norman et al., 1995)	Temperature	Time	Cycles
Initial denaturation	94 °C	5 minutes	
Denaturation	94 °C	30 seconds	35 cycles
Annealing	48.8 °C	30 seconds	
Extension	72 °C	1 minute	
Final extension	72 °C	5 minutes	

For ***Polychromophilus* spp**. detection, a cytochrome b fragment (704 bp) was amplified using the PLAS1 (5'-GAG AAT TAT GGA GTG GAT GGT G-3') and PLAS2 (5'-GTG GTA ATT GAC ATC CWA TCC-3') primers for the first PCR round. For the second round we used PLAS3 (5'-GGT GTT TYA GAT AYA TGC AYG C-3') and PLAS4 (5'-CATC CWA TCC ATA RTA WAG CAT AG-3') primers (Duval et al., 2007).

Nested PCR Mixture:

First PCR round: 25 μl reaction mixture which contained 0.05 μl Qiagen Taq Polymerase, 0.25 μl dNTP, 0.75 μl of each primers, 5 μl PCR buffer, 1 μl MgCL2, 14.2 μl H2O and 3 μl aliquot of isolated DNA.

Second PCR round: 25 µl reaction mixture which contained, 1 µl of PCR product from first round, 0.05 µl Qiagen Taq Polymerase, 0.25 µl dNTP, 0.75 µl of each primers, 5 µl PCR buffer, 1 µl MgCL2 and 16.2 µl H2O.

2. Table: PCR conditions for *Polychromophilus* detection

Primers PLAS1, PLAS2, PLAS3, PLAS4 (Duval et al., 2007).	Temperature	Time	Cycles
Initial denaturation	94 °C	5 minutes	
Denaturation	94 °C	30 seconds	25 cycles (35 cycles in second run)
Annealing	55 °C	30 seconds	
Extension	72 °C	45 seconds	
Final extension	72 °C	10 minutes	

For ***Trypanosoma* spp**. 18S small-subunit rRNA gene fragment (642 bp) was amplified using the TRYF (5'-CAG AAA CGA AAC ACG GGA G-3') and TRYR (5'-CCT ACT GGG CAG CTT GGA-3') primers for the first PCR round and the SSUF (5'-TGG GAT AAC AAA GGA GCA-3') and SSUR (5'-CTG AGA CTG TAA CCT CAA AGC-3') primers for the second round (Noyes et al., 1999).

Nested PCR Mixture:

First PCR round: 25 µl reaction mixture which contained 0.04 µl Qiagen Taq Polymerase, 0.25 µl dNTP, 0.75 µl of each primers, 5 µl PCR buffer, 0.5 µl MgCL2, 14.71 µl H2O, and 3 µl aliquot of isolated DNA.

Second PCR round: 25 µl reaction mixture which contained 1 µl of PCR product from first round, 0.04 µl Qiagen Taq Polymerase, 0.25 µl dNTP, 0.75 µl of each primers, 5 µl PCR buffer, 0.5 µl MgCL2 and 16.71 µl H2O.

3. Table: PCR conditions for *Trypanosoma* detection

Primers TRYF, TRYR, SSUF, SSUR (Noyes et al., 1999).	Temperature	Time	Cycles
Initial denaturation	94 °C	5 minutes	
Denaturation	94 °C	30 seconds	25 cycles (35 cycles in second run)
Annealing	55 °C	30 seconds	
Extension	72 °C	45 seconds	
Final extension	72 °C	10 minutes	

All PCR products were visualized on 1.5% agarose gel.

References

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