

Supplementary Information

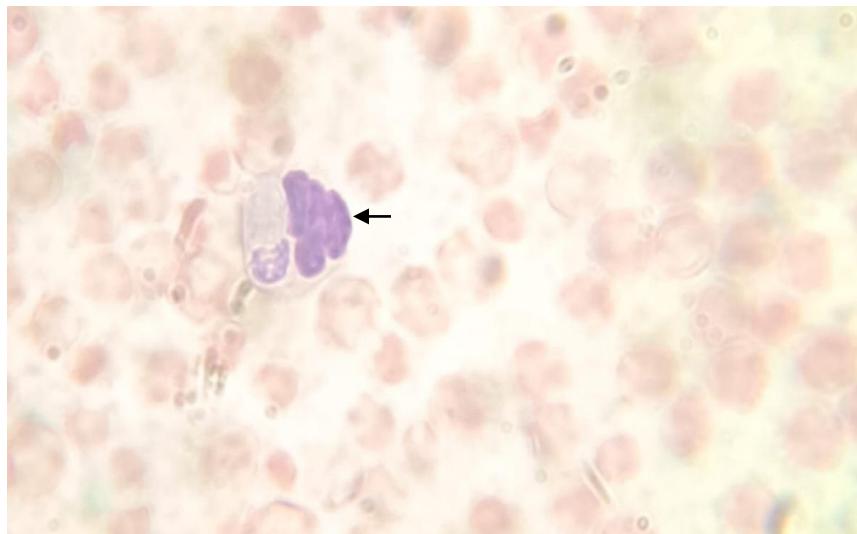


Fig 1: capsular gamont of *H. canis* inside neutrophil detected by microscopy in dog blood smear.

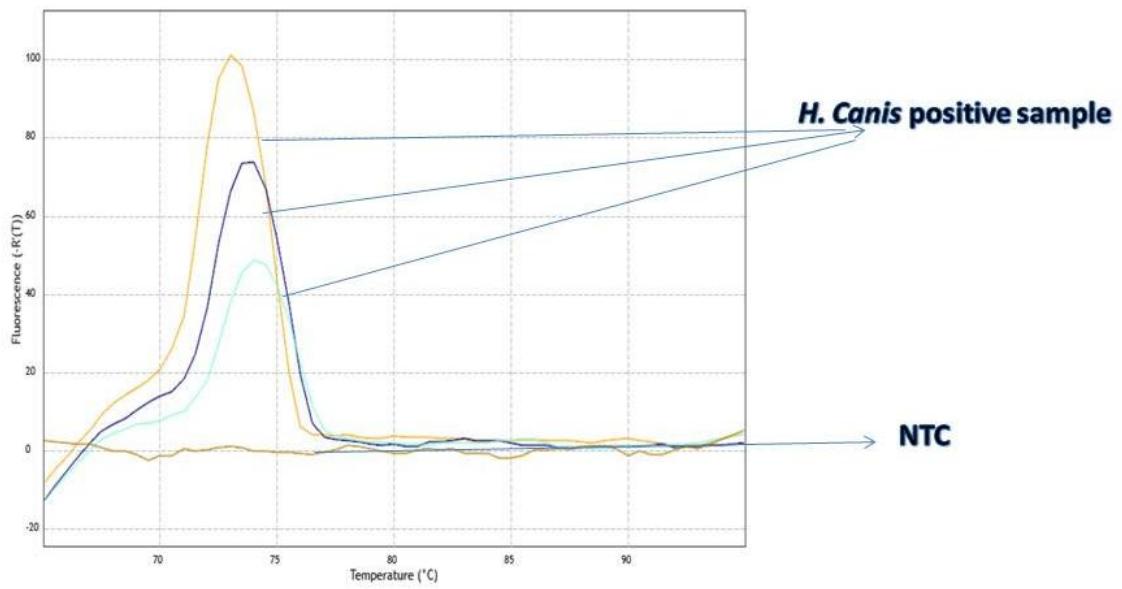


Fig 2. Detection of *Hepatozoon canis* by melt curve analysis of SYBR Green based real-time PCR assay

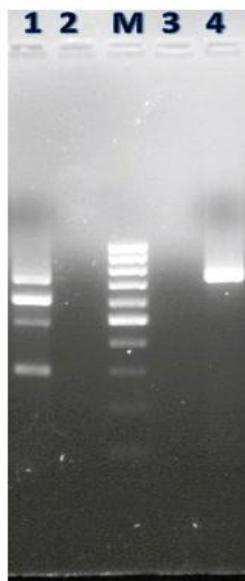


Fig 3A

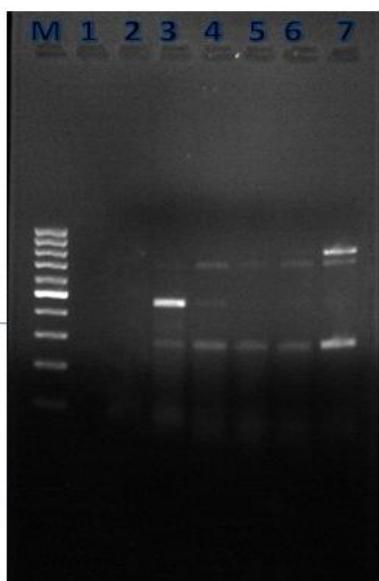


Fig 3B



Fig 3C

Fig 3. Multiplex PCR assays of dog blood sample for the detection of co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys*, 489 bp of *Babesia gibsoni*, 602bp of *Babesia vogeli* and 817 bp of *Ehrlichia canis* (M: Gene Ruler 100 bp DNA ladder (Thermo Scientific)

Fig 3A: L1- co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys*, 489 bp of *Babesia gibsoni* , 602bp of *Babesia vogeli*; L2- NTC, L3- negative sample, L4- PCR product of 737 bp of *Hepatozoon canis*.

Fig 3B. L1, L2: negative sample, L3,L4 – co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys* and 489 bp of *Babesia gibsoni* s; L5,L6 co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys*, L7- co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys* and 817 bp of *Ehrlichia canis*.

Fig 3C. L1, 2, 3 – co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys*; L4- co-infection of Fragments of 737 bp of *Hepatozoon canis* with 292 bp of *Anaplasma platys* and 817 bp of *Ehrlichia canis*.

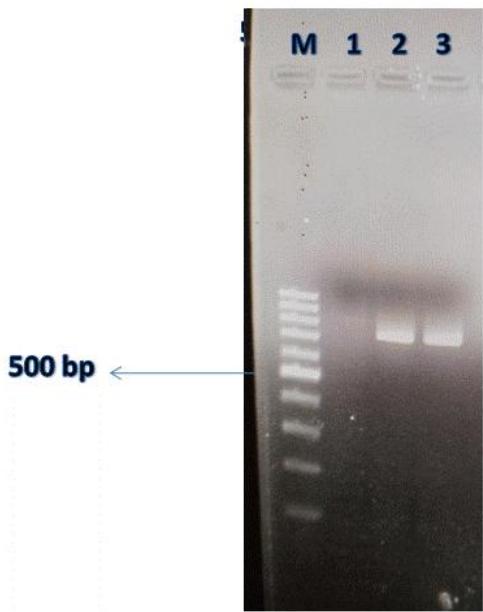


Fig 4. Singleplex PCR assays for the detection of Fragments of 737 bp of *Hepatozoon canis* in ticks