Figure S1. Alignment of *ITS-1* gene showing identity of *Trypanosoma brucei* species obtained in this study with *T. brucei* [Suzena - AF306775]; *T. evansi* [Isolate 006 - HQ593640] and *T. evansi* [Strain B2 - FJ712715].

Figure S2. Alignment of *18S rRNA* gene showing identity of *Babesia bigemina* from the current study with *Babesia bigemina* [strain MT26 – KU206297] and [strain MT25 – KU206296].

Figure S3. Alignment of *18S rRNA* gene sequence showing the identity of *Theileria parva* obtained in this study with *Theileria parva* [HQ684067] and [KM211712].

Figure S4. Alignment of two *ITS-1* genesequences from *Trypanosoma evansi* species obtained from Zambian tabanid flies with *Trypanosoma evansi* [isolate – HQ593640] and [buffalo strain – FJ7127151]

Figure S5. PCR using TCS primers for the detection of *T. congolense* Savannah from tabanid flies collected from South Africa. M is a molecular marker, –ve is the negative control, +ve is the *T. congolense* IL 3000 positive control. L1 to L11 are samples that tested positive for *T. congolense* Savannah.

Figure S6. PCR using TBR primers for the detection of *Trypanozoon* species collected from South Africa and Zambia. M is a molecular marker, -ve is no template control, +ve is *T. b. brucei* (GUTat 3.1) positive control. L1 is a positive sample from South Africa and L2 to L7 are positive samples from Zambia.