Preliminary investigations on the sequence heterogeneity of the 18S rRNA gene of *Theileria equi* and *Babesia caballi* strains collected from a horse population in Central Italy

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Introduction

Equine piroplasmosis (EP) is a tick-borne disease caused by protozoa Babesia caballi and Theileria equi. Horses, donkeys, mules and zebras are susceptible to piroplasmosis. EP is endemic in many tropical and subtropical countries. In Italy, circulation of both protozoa is well documented. Phylogenetic analysis of the V4 hypervariable region of the 18S rRNA gene divided the two parasites in three genetic groups (1).

Aim of the present study: As these studies are limited to parasites detected in South Africa, a study was carried out to identify the genetic groups circulating in Italy.

Material and methods

- **Population study:** EDTA blood samples were collected during 2013/2014 from 78 symptomatic (32) and asymptomatic (46) horses of three Regions in Central Italy (Fig. 1).
- **Sample selection:** those presenting low Ct values in Real Time PCRs with or without an ELISA negative result. 71 samples were positive for T. equi, 6 for B. caballi and 1 for both parasites.
- **Phylogenetic analysis target:** hypervariable V4 region (1) of the 18S rRNA genera *T. equi* (~430bp) and *B. caballi* (~390bp) using the Nested PCR (nPCR) protocol (1).
- **Sequencing:** amplicons were purified using the QIAGEN kit (GmbH, Hilden, Germany) and sequenced using the primers of the second step of the nPCR with the BigDye Terminator Cycle Sequencing Ready Reaction kit, version 3.1 •(AB, Foster City, CA, USA) in an automated sequencer(3500 Genetic Analyzer,AB, Foster City, CA, USA).
- **Sequences analysis and phylogenetic trees:** Sequences analysis was performed using the Basic Local Alignment Search Tool (BLAST) and compared to those registered in NCBI GenBank (http://www.ncbi.nlm.nih.gov). Sequences showing a minimum 98% query coverage and a 98% identity were considered for the evaluation of genetic distances and sequence identities. Phylogenetic trees were constructed using GENeious v.8.1.7.

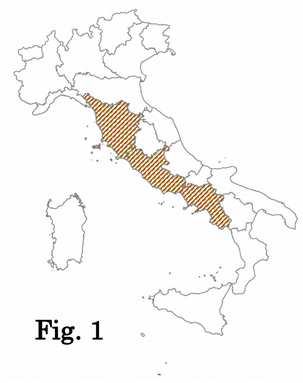


Fig. 1

Results

The sequences obtained could be divided in three equidistant groups (98% homology and query coverage within each group). All three genetic groups previously described were present in the three Italian Regions: Tuscany, Latium and Campania (Figs 2, 3, 4 and 5).

Fig. 2: representation of the 3 genetic groups for *T. equi*

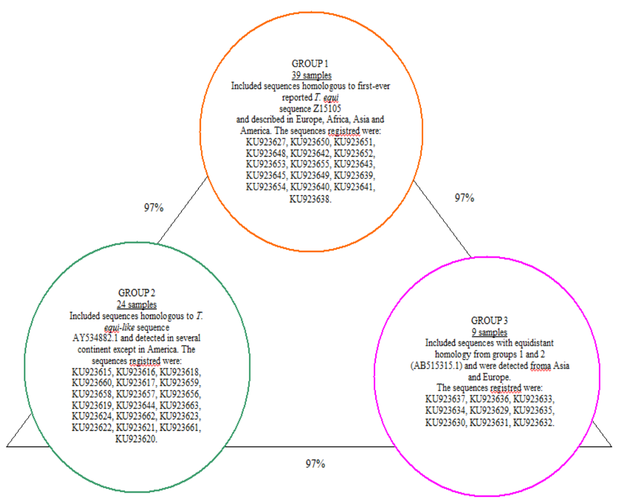


Fig. 3: phylogenetic tree for *T. equi*

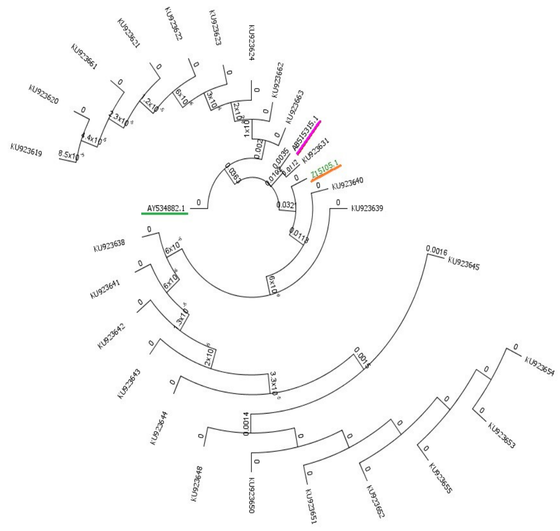


Fig. 4: representation of the 3 genetic groups for *B. caballi*

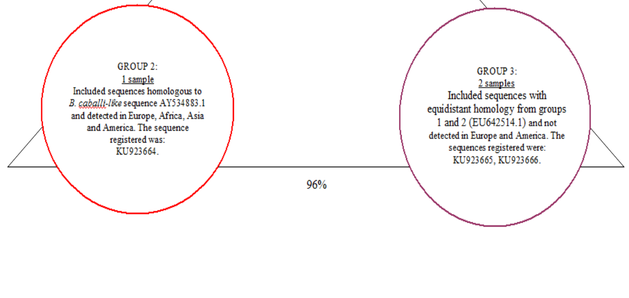
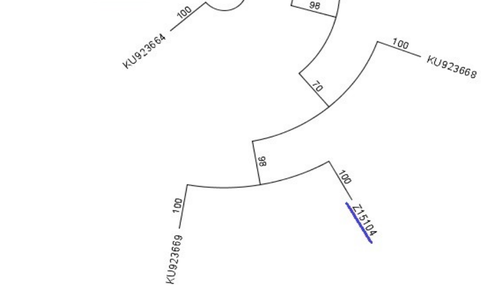


Fig. 5: phylogenetic tree for *B. caballi*



Correlation of phylogenesis with clinical status *T. equi*

Among the 27 PCR positive/ELISA negative horses, 23 (85,1%) had group 1 sequences,

- Of the 29 sequences obtained from symptomatic animals, 24 belonged to group 1 (Z test P<0.0001) (Tab. 1).

Tab. 1: Z test

T. EQUI	GROUP 1	NON-GROUP 1	TOTAL
SYMPTOMATIC	24	5	**29**
ASYMPTOMATIC	15	28	**43**
TOTAL	**39**	**33**	**72**

Considerations on *B. caballi* sequences

- As the number of positive samples was limited, no statistical analysis was carried out,

- Of the 7 horses only 2 were ELISA positive and sequences belonging to group 2 were found only among asymptomatic horses.

Discussion

- The study detected the presence in Italy of the three genetic groups for both parasites as already described in South Africa (2). This is the first evidence of group 3 for both parasites in Europe.
- Clustering is apparently independent from the geographic distribution, as all groups are present throghout the study area.-
- The wide distribution of the groups should be releated to the continuos long distance movements to wich horses are subject.
- The biological significance of the correlation between genetic group one and the clinical and serological pattern requires further investigations in terms of the virulence of the parasites as already proposed by Nagore et al..

References

1.Nagore, D. et al. Detection and identification of equine *Theileria* and *Babesia* species by reverse blotting: epidemiological survey and phylogenetic analysis. *Vet. Parasitol. 2004; 123:41-54.*

2.Bhoora, R. et al. Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. *Vet. Parasitol. 2009; 159:112-120.*