SUMMARY

General aims:
The project aims to shed light on the presence of the protozoan parasite Blastocystis in domestic pigs, bred in the wild and semi-wild, excluding intensive breeding, and in wild pigs, or wild boars. *Blastocystis* sp. it is recognized as the most common microeukaryotic of the human intestinal tract (Stensvold, 2013). Its prevalence is much higher than that of other common single-celled intestinal parasites such as *Giardia*, *Entamoeba*, *Balantidium* and *Cryptosporidium*. It is frequently found in domestic animals, bred for zootechnical purposes, but also in wild animals.
The general purpose of this project is to improve the understanding of the epidemiology of *Blastocystis* sp. infection through the use of molecular biology methods. The sensitivity of DNA amplification and sequencing techniques and the ability to finely resolve the differences/affinities between human and animal isolates will allow to better define the zoonotic risk. In this way it will also be possible to study the main reservoirs of the parasite and the routes of infection which includes: production, slaughtering, handling, packaging and distribution of meat, up to the final consumer.
Methods:
The samples examined were collected in the vast internal area of the Central Apennines, corresponding to the Province of Rieti. In this area the presence of suidae (*Sus scrofa*) is widespread both domestic and wild bred for the production of food for self-consumption with traditional methods and in the wild affected by hunting
To verify the presence of the protozoan Blastocystis, swine stool samples were collected, wild or domestic, for a total of 158 samples. The samples were taken at the meeting points of the hunting teams for wild boars, while the feces of domestic pigs were collected at the time of slaughter.
DNA was extracted from the faecal materials and subjected to a polymerase chain reaction (PCR). Specific primers were used for the characterization of the subtypes of *Blastocystis* sp.: BL18SPPF1 and BL18SR2PP. PCR products positive in the presence of *Blastocystis* were purified and sequenced in-house or sent to BMR Genomics (Padua, Italy) for purification and in-service sequencing. The sequences obtained were analyzed and processed using specific software. The PCR products that showed positive results with double bands or double peaks in the chromatogram produced by the Sanger sequencing, were reprocessed according to a new protocol developed for this study and sequenced through Next Generation Sequencing (NGS) at BMR Genomics (Padua, Italy) with technology NGS on Illumina Miseq platform. The data obtained were analyzed following an optimized pipeline for this study.
To understand the evolutionary relationships and taxonomic status of the isolates, a phylogenetic analysis was conducted using Bayesian inference methods.

Results:
A total of 83 samples of faecal material were extracted, including 40 from domestic pigs and 43 from wild boars. Of these, 79 samples (42 wild boars and 37 pigs) were exploitable. 52 samples (26 wild boars - 26 domestic pigs) were positive in the presence of Blastocystis, 67.5% positive of the total in the samples analyzed (72.5% in domestic pigs and 62.8% in wild boars).
The Sanger sequencing of the material amplified from wild boars and domestic pigs confirmed the presence of *Blastocystis* assigned to 2 (ST5 and ST15) and 3 (ST1, ST3 and ST5) subtypes, respectively.
Eighteen samples were subjected to the NGS procedure. Among these, representative sequences of a single ST (3, 5 or 15) were found, as well as cases of multiple colonization. In particular, of the 6 cases observed in wild boars, 5 concern ST15 and ST5. The remaining case shows the presence of ST5 and ST3. The 4 cases of mixed colonization in domestic pigs always concerned ST 5, with ST15 (3 cases) or ST3 (1 case).
Discussion:

The presence of the protozoan parasite *Blastocystis* has been confirmed in this study for domestic pigs, as already found in other works (Wang et al., 2018; Wylezich et al., 2019), and in wild ones (first report).
The frequency of positives in domestic pigs is 70.2% in our dataset, a remarkably high percentage given the comparison with other published works (Wang et al., 2018). In domestic pigs only zoonotic subtypes have been detected. The most frequent subtype is ST5, which occurs in two distinct allelic forms. We can therefore assume a different origin of the parasite and different sources of infection, but which does not seem to be linked to the geographical origin. ST5 is considered zoonotic even if it is scarcely reported in humans and never identified in Italy. In domestic pigs, ST1 and ST3 have also been identified, with one specimen each, frequently isolated also in humans and therefore with marked zoonotic risk. We recall that in particular ST3 is the most common subtype in humans (Cian et al., 2017). All the pigs included in this study were raised in a domestic or domestic environment, never industrial, or in a semi-wild state.
Conclusion:
This study represents the first description of the subtypes of *Blastocystis* present in wild boars. The most common subtype is ST15, non-zoonotic and rare. To a lesser extent, ST5 was also found, with the same allelic forms found in domestic pigs, zoonotic. This reporting appears to indicate an overlap of infection between domestic and feral pigs, suggesting a common source of infection. According to the results of the study, wild boar would not pose a high risk to human health. Numerous cases have also been highlighted in wild boars that can be attributed to multiple infections, resolved using a new methodology perfected for this study.

Keywords: blastocystis, barcode, SSU rDNA, genetic subtypes, zoonoses, wild boar, NGS