SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: FA1408
STSM title: Prevalence study of *Sarcocystis* spp. infection in meat cattle in Portugal and Tunisia
STSM start and end date: 19/02/2018 to 23/02/2018
Grantee name: Jacinto Gomes

PURPOSE OF THE STSM/

This STSM aims to reinforce the cooperation between two research groups already working on *Sarcocystis* spp. in order to establish a common protocol for molecular surveys of the zoonotic parasites in cattle meat but also in meat products. Due to a lack of data from most European Union countries, the impact on human health is unclear in this region as well as the situation in animal populations. It can be assumed that the zoonotic *Sarcocystis* species are infecting many livestock populations including cattle. Limitations of reporting are related to the commonly used detection method, usually visual inspection at the slaughterhouse, which does not allow differentiation between the zoonotic species and the non-zoonotic ones. Consequently a harmonised scheme for monitoring *Sarcocystis* that includes specific and sensitive methods is necessary. Despite the possible impact on human health, studies concerning the prevalence of *Sarcocystis* spp. in Portugal and Tunisia are scarce. In Tunisia, there is only one study on *Sarcocystis hominis* in cattle, where the infection prevalence in beef cattle was determined to be 26% (39/150). In Portugal there is also only one study focused in detecting zoonotic *Sarcocystis* in wild boars and there is no information on cattle *Sarcocystis*. The main objective of this mission is to discuss the implementation of a digestion method for meat and processed meat products for further DNA purification and possibly for recovery of *Sarcocystis* cysts to be used for genotyping and the comparison of different molecular assays for detection and identification of cattle *Sarcocystis*.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The laboratory work began with an introduction of the reference detection method for *Trichinella* larvae according to the EU regulation 2075/2015 that describes specific rules on official controls for this parasite in meat. The magnetic stirrer method for pooled sample digestion (reference method) was previously used for the digestion of wild boars muscles for *Sarcocystis* at INIAV. The introduction included the fundament

of the technique and the applicability for other parasites, the materials necessary (reagents and consumables) and the equipment used. After the introduction, the practical work began with the preparation of muscles samples from cattle and the digestion technique. This practical session was conducted with the participation of Safa Amairia, a PhD Student from ENMVST working on *Sarcocystis* in beef and meat products. During the procedures, several critical control points were assessed and a session for discussing troubleshooting of the digestion applicable to meat parasites including *Sarcocystis* detection but also for *Sarcocysts* isolation. Part of the focus was on sample preparation for DNA extraction and purification. A second part of the practical work was on the preparation of samples from processed meat products, particularly Merguez, a typical sausage from Tunisia. This product has some specificities that difficult the purification of nucleic acids, including the fat content and the spices used. Purification and DNA amplification with primers for eukaryotic organisms was carried out for quality control. Samples from Portugal (DNA extracted from beef were included) and the PCR amplification of *Sarcocystis* genus was performed. Included on this STSM, it was organized a workshop for PhD students and Laboratory of Parasitology staff with the title "Isolement des Parasites de la Viande" and the following presentations:

- Situation épidémiologique de *Sarcocystis* spp.en Tunisie (Safa Amairia, PhD student)
- Etude de l'infection du sanglier par *Sarcocystis* au Portugal (Jacinto Gomes)
- Recherche de parasites dans la viande par la technique de digestion (Jacinto Gomes)

The workshop included also a laboratory session (2h) and s discussion session.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The main result was the optimization of the digestion technique for beef and meat processed products with the purpose of obtaining higher yield and purer DNA to be used for the detection of *Sarcocystis*. Some difficulties were overcome whit the digestion of Merguez which is a spicy mutton or beef-based fresh sausage from North African cuisine. This sausage is made with uncooked lamb, beef, or a mixture stuffed into a lamb-intestine casing and heavily spiced with cumin and harissa but also other spices such as fennel and garlic. Since Merguez is usually eaten grilled there is the possibility of being undercooked and thus, to constitute a risk for *Sarcocystis* infection. The difficulty of DNA purification and PCR amplification was due possibly to high quantity of inhibitor substances, high fat content. However, a troubleshooting session with PhD students and researchers allowed the discussion of several approaches for improvement of both techniques. We believe this STSM will support the achievement of both teams on the scientific objectives of Working Group 2 (Analytical and Diagnostic Methods), such as the transfer of methods and techniques but also to establish more information on the epidemiology of this FBP.

FUTURE COLLABORATIONS (if applicable)

Both groups are starting self-funded projects on this subject and a closer cooperation will allow a more competitive joint proposal for further funding. Currently there are a PhD student in Tunisia working on

Sarcocystis in beef and meat products and a proposal for a PhD grant in Portugal. There is also the possibility of hosting MSc and PhD students from Tunisia in Portugal (INIAV) to study several foodborne parasites, including *Trichinella*, Toxoplasma and *Sarcocystis*. The collaboration on *Sarcocystis* spp. is expected to result at least in one or more publications in peer-reviewed journals, for instance with the comparison of samples and phylogenetic studies.