

MOLECULAR, PHYLOGENETIC AND ULTRASTRUCTURAL CHARACTERIZATION OF A NEW SARCOCYSTIS SPECIES IN ALPACAS FROM PERÚ. Giuliana Medrano (1), Armando Hung (1), Doris Melo (2), Elizabeth Neira (2), Nancy Rojas (2). (1) Lab Biología Molecular, Facultad de Veterinaria y Zootecnia, Universidad Peruana Cayetano Heredia, Lima-Perú; (2) Instituto de Patología, Hospital Loayza, Lima-Perú. Email: 26705@upch.edu.pe

Introduction: Sarcocystiosis is an important disease affecting almost the 100% of South American camelids in Peru (3). Traditionally, *Sarcocystis* has been identified according to biological characteristics like biochemistry, physiology, morphology and life cycle in the past, but limitations occur with the use of these criteria to infer phylogenetic relationships or identification of a new species. So far, species *S. aucheniae* as a causal agent of macroscopic cyst in squeletical muscles are the species molecularly identified in alpacas and llamas. The molecular identification of the species causing microscopic cyst in cardiac muscles remains to be elucidated.

Objective: The aim of the present work was to determine the molecular and ultrastructural characterization of the species producing micro- and macroscopic cysts in alpacas in Peru by phylogenetic analysis based in the ssu rRNA gene and by ultrastructure analysis.

Materials and methods: Partial sequences of the small subunit ribosomal RNA (ssu rRNA) gene of *Sarcocystis* forming micro- and macroscopic cysts were obtained by polymerase chain reaction and sequencing techniques (2). For phylogenetic analysis with BioEdit software (1), the nucleotide sequences obtained were aligned with homologous sequences of four *Sarcocystis* species and other eukaryotes including *Toxoplasma* and *Neospora*. For ultrastructure analysis, very small pieces of the heavily infected cardiac and skeletal muscle were fixed in 3% glutaraldehyde and post-fixed in 1% osmium tetroxide (4, 5). Semi- and ultrathin sections were dipped in uranyl acetate and lead citrate before their examination in an EM-301 transmission electron microscope with a magnification range from x2,200 – x8,000.

Results: Nucleotide divergence of the aligned sequences showed that *Sarcocystis* isolated from microscopic cysts belongs to a new different species designed as *S. lamacanis*. The partial sequence obtained in this study was registered in the GenBank with accession number AY840990. The sequences obtained from macroscopic cyst isolated in Peru had a 100% of similarity with *S. aucheniae*. The phylogenetic relationship between *S. lamacanis* and *S. aucheniae* is supported by the genetic distances observed among their ssu rRNA gene sequences. In fact, the genetic distance between the ssu rRNA genes of the *S. lamacanis* and *S. aucheniae* (0.1220) is smaller than those between *S. lamacanis* and the others species analyzed (0.1334-0.1718). Ultrastructure analysis performed in this study confirmed these findings. Ultrastructural details of the primary cyst wall (PW) are considered as the most valuable data to observe difference between species (6). The present ultrastructure study revealed that the primary cyst wall of microscopic cyst differ from those observed in macroscopic cyst. Microscopic cysts (Mi) were obtained from cardiac muscle of alpacas highly infected (Fig. 1A). Microscopic cyst showed short and wide protrusions (PT) in their base and the free end was more narrow and rounded (Fig. 1B). Magnified part of the primary cyst wall of microscopic cyst shows projections with internal fiber-like structures (FS) (Fig.1C). Macroscopic cysts were obtained from skeletal muscle (Fig. 1D). These cysts have a primary cyst wall with branched projections. A magnified part of the primary cyst wall of macroscopic cyst shows tangential cuts of branched protrusions (Fig. 1E-F). Protrusions are cover by two layers: one is in contact with muscle tissue and is a weak osmophilic and has a striated appearance, the other layer is very osmophilic without the striated figure (Fig. 1F).

Conclusion: The results obtained by molecular, phylogenetic and ultrastructural analysis in this study, confirm that microscopic cysts are produced by the new species *S. lamacanis* and macroscopic cyst are produced by *S. aucheniae* in alpacas infected in Peru.

References

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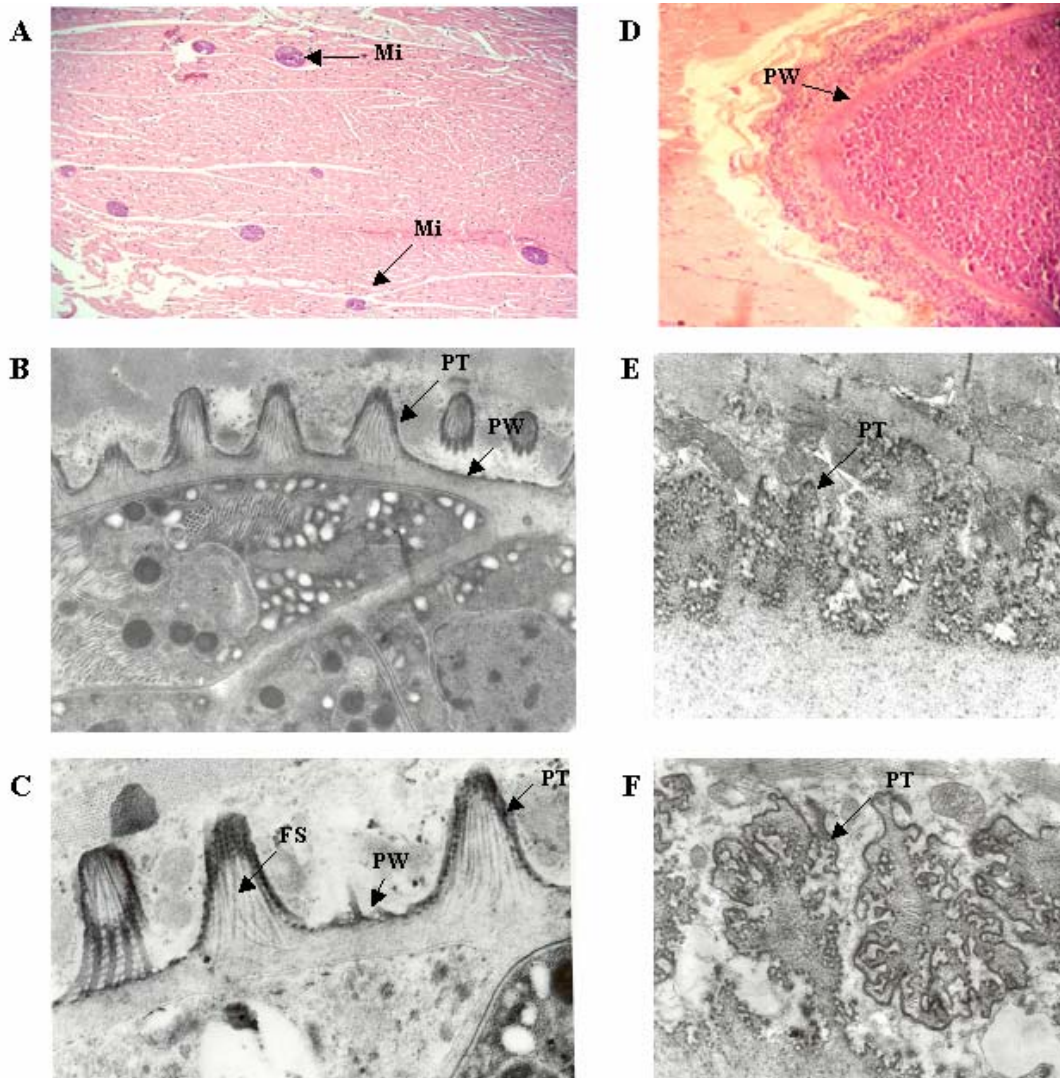


Figure 1 Ultrastructural identification of microscopic cyst belong to *S. lamacanis* (A-C) and macroscopic cyst *S. aucheniae* (D-F). **A** Presence of eight microcysts (*S. lamacanis*) in a transversal section of cardiac muscle of an alpaca at x1,000. **B** Cyst wall of *S. lamacanis*, showing the conical protrusions. x7,500. **C** Magnified part of the primary cyst wall of microscopic cyst, showing protrusions with internal fiber-like structures x8,000. **D** Macroscopic cyst in squeletical muscle observed at x400. **E** Macroscopic cyst showing the primary cyst wall with finger-like protrusions x2,200. **F** Magnified part of the primary cyst wall of macroscopic cyst, showing protrusions x3,500. **Mi**, microcyst; **PT**, protrusion; **PW**, primary cyst wall; **FS**, fiber-like structures.