Metodologia usada para estudo da Tuberculose
Methodology

During the 2011–2014 hunting seasons we collected submandibular, retropharyngeal, tracheobronchial, and mesenteric lymph nodes as well as lung samples from hunter-killed wild boars. Samples were taken in the field after evisceration of the carcasses, 2–10 hr after death. Tissues were stored in sterile 40ml tubes and refrigerated. Gross pathology evaluation was conducted within 48 hr of sampling. Samples were then frozen at -20ºC until used for bacterial culture.
Age and sex of the animals were recorded for most but not all animals because of constraints of field work. Age was determined by tooth eruption and wear patterns, according to Matschke (1967) and Buruaga et al. (2001). Animals were classified as juvenile (less than 1 yr old), subadult (1–2 yr old) or adult (.2 yr old).

All protocols were performed in a biosafety level 3 laboratory at Life and Health Sciences Research Institute, Braga, Portugal. Tissue samples were thawed and about 3 g of tissue was homogenized in 4 ml sterile water and then decontaminated with 0.75% hexa-decylpyridinium chloride, in a final volume of 35 ml. After a 2 hr-period for decontamination, 2 tubes with Coletsos medium (BioMerieux, Marcy l'E´ toile, France) were inoculated with the sediment/supernatant interface. Culture media were incubated at 37ºC for 15 wk; they were checked after the first week to discard fast-growing bacteria, and then rechecked weekly from week 4 to week 10 to detect bacterial growth. A sample of each positive isolate was preserved at -80ºC in sterile water. An aliquot of this suspension was heat-inactivated at 95ºC for 45 min and DNA was extracted y standard phenol-chloroform method after 2x30 sec agitation with 0.1-mm zirconium beads in a Mini Bead-Beater(Biospec, Bartlesville, Oklahoma, USA). The DNA was stored at -20ºC after quantification with an ultraviolet (UV) spectrophotometer (Beckman DU 650, Beckman Coulter, Fullerton, California, USA).

Bacterial isolates were identified by polymerase chain reaction (PCR) for a panel of selected genes: 16S RNA, IS1081, Rv3120, Rv1510. The PCR protocol described by Huard et al. (2003) was used. Five microliters of Taq buffer 103 (Fermentas, Burlington, Canada), 1.25 U Taq polymerase, 1 ml of each primer at 20 mM, and 1.25 mg of DNA were added to a solution containing 200 mM of each dNTP, 1.5 mM MgCl2, and 5% dimethyl sulfoxide (DMSO), in a final volume of 50 ml. This mix was submitted to the following PCR cycles: initial denaturation at 94 C for 5 min followed by 35 cycles of 94ºC for 1 min, annealing at 60ºC for 1 min, and extension at 72ºC for 1 min with a final extension step of 72ºC for 10 min. Polymerase chain reaction products were visualized by electrophoresis in 1% agarose gel with ethidium bromide and photographed under UV light (Alpha Imager, Alpha Innotech Corporation, San Leandro, California, USA). According to Huard et al. (2003), this set of genes allows for the identification of *M. bovis, Mycobacterium caprae,* other members of the Mycobacterium tuberculosis complex and other mycobacteria not belonging to this complex.

References:

- Buruaga, M.; Calero, A. & Iraizoz, F. (2001). Reconocimiento de sexo y edad en espécies cinegéticas. Edilesa, León, Spain, p.127
- Huard, R.; Lazzarini, L. & Butler, W.; Van Soolingen, D. & Ho, J.(2003). PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. *Journal of Clinical Microbiology,* 41, 1637–1650.
- Matschke, G. (1967). Aging European wild hogs by dentition. *Journal of Wildlife Management,* 31, 109–113.