

Abstract P-04.34

VIRULENCE ATTENUATION OF AN INOCULUM PREPARED FROM GUT MUCOSA AFTER BACTERIAL CULTURE IN EXPERIMENTAL OVINE PARATUBERCULOSIS

Fernandez M.^[1], Delgado L.^[1], Fuertes M.^[1], Castaño P.^[1], Royo M.^[1], Garcia Marin J.F.^[1], Benavides J.^[1], Ferreras M.C.^[1], A. Sevilla I.^[2], **Perez V.*^[1]**

^[1]Universidad de León ~ León ~ Spain, ^[2]Neiker-Tecnalia ~ Derio ~ Spain

Abstract text:

The inoculum used in experimental infections with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is one of the factors that can influence notably the outcome of the challenge. The aim of this study is to compare the results of an experimental challenge in lambs using either an inoculum prepared from gut mucosal tissue or cultured bacteria obtained from the same intestinal homogenate.

A total of 12 one-month-old lambs were divided into two groups and infected with a total amount of 1×10^8 mycobacteria per lamb of a suspension obtained from the ileal mucosa of a diseased sheep (group M) or the low passage isolate (group C) obtained after the culture of the mucosal homogenate employed in group M. A third group of 6 animals was kept as uninfected control. The peripheral immune response was evaluated and pathological and nested PCR analysis were carried out in three lambs from each group culled at 120 days post infection (dpi) and in the remaining animals euthanized at 220 dpi.

All lambs from group M showed specific peripheral cellular (evaluated by IFN- γ production) and humoral (indirect ELISA) immune responses from day 90 and 120 respectively, whereas only some lambs from group C showed positive IFN- γ response at 90 dpi and no antibody response was detected in this group. Lesions were observed in all lambs from group M: at 120 dpi animals have multifocal lesions, with granulomas widespread to several areas of the intestinal mucosa and at 220 dpi, one lamb had a focal form and the remaining multifocal lesions. However, only focal lesions, with no acid fast bacteria, were seen in the Peyer's patches of three lambs from group C, two killed at 120 and one at 220 dpi. MAP DNA was detected by nested PCR in tissues from all lambs from both groups.

The MAP isolate cultured from an intestinal homogenate clearly had significantly lower virulence than the inoculum directly prepared from the intestinal homogenate.

Keywords:

Virulence, Culture, Homogenate