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Isolation of immunogenic glycopeptidolipids of *Mycobacterium avium* subsp. *hominissuis*

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Introduction

Mycobacterium avium (MA) is an important pathogen in both animals and humans which belongs to the *M. avium* complex (MAC). Diagnosis of MA-infections in pigs is traditionally based on the detection of granulomatous lesions in lymph nodes seen by eye at slaughter. Since sensitivity and specificity of this approach is questioned (Komijn et al., 2007) we developed as an alternative a serodiagnostic ELISA assay on the basis of a polar lipid fraction from MA (Wisselink et al., 2009). We isolated glycopeptidolipids (GPLs) from *M. avium* subsp. *hominissuis* (MAH) and analyzed them for their suitability for serodiagnosis of MA infections in pigs.

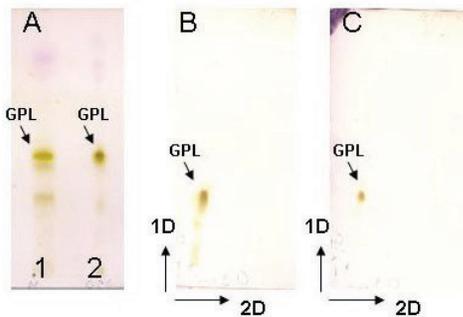
Materials and Methods

MAH serotype 4 strain 17404 was grown at 37°C in Dorset Henry medium. Glycolipid antigens were extracted, analyzed by one and two dimensional thin layer chromatography (TLC) and further purified (Nishiuchi et al., 2004; Papa et al., 1993; Kitada et al., 2002). As reference, GPLs from a MAH serotype 4 strain were used (Kindly supplied by Y. Nishiuchi, Osaka, Japan). The immunogenicity of the GPLs was evaluated in ELISA tests using reference serum samples obtained from pigs held under experimental and field conditions.

Results

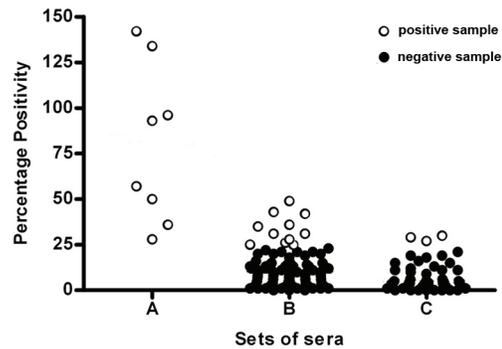
The TLC-pattern of GPLs from MAH strain 17404 and the reference GPL (Nishiuchi et al., 2004) showed both a Retention Factor (Rf) value of 0.42 (Fig. 1)

Fig.1. One – dimensional (A) and two-dimensional TLC analysis (B and C). Reference GPLs from MAH serotype 4 (lane 1) and MAH serotype 4 strain 17404 (lane 2) had both a Rf value of 0,42. The purity of GPLs was examined by two-dimensional TLC (B: MAH strain 17404; C: reference GPL).



To evaluate the GPLs for their immunogenicity the cut-off value for negative and positive test results was calculated (Wisselink et al., 2009) which appeared to be 24.5 percentage positivity (PP). In all eight sera, experimentally infected with MA, high anti-GPL antibody titers were found. On the two infected farms 10.6% (11/104) of the pigs tested serologically positive and on the five farms, free for an MA infection 3.3% (3/92) pigs (Fig. 2).

Fig. 2. Results of ELISA tests with 1 ug GPLs per well from MAH using serum samples from pigs experimentally infected with MA (A; n=8), from two farms with pigs known to be infected with MA (B; n=104) and pigs from five farms free for an MA infection (C; n=92).



Discussion

Earlier we developed an ELISA test on the basis of polar lipids for serodiagnosis of MA infections in pigs. Here we show that purified GPLs can also be used as antigen in a serodiagnostic test because of its clear recognition by serum antibodies in experimentally infected pigs. For use under field conditions, the results of the GPL-ELISA indicate that MA-infected farms can be discriminated from MA-negative farms. Further work is needed to optimize and evaluate the GPL-ELISA for use in the field.

References

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