STRAIN DIVERSITY IN CAPRINE PARATUBERCULOSIS – CORRELATION OF *MYCOBACTERIUM AVIUM* SSP. *PARATUBERCULOSIS* GENOTYPES WITH MORPHOLOGICAL LESIONS

M. Rasper-Hössinger*, S. Scherrer†, R. Stephan‡ and F. Seehusen*

*Institute of Veterinary Pathology, †Institute for Food Safety and Hygiene, Section of Veterinary Bacteriology and ‡Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, CH
frauke.seehusen@uzh.ch

Introduction

Paratuberculosis is an infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) causing chronic enteritis in domestic ruminants worldwide. In goats, paratuberculosis is mainly caused by the C (cattle)-type and to a lesser extent by the S (sheep)-type. Ten Swiss caprine MAP isolates were characterised by mycobacterial interspersed repetitive units and variable number tandem repeats (MIRU-VNTR) profiling as well as single nucleotide polymorphism assay (SNP) and correlated with histologic lesions.

Material and Methods

Testing for MAP was performed by qPCR targeting IS*900* and F57. Strain diversity of MAP was determined by applying the MIRU-VNTR typing method to classify different INRA Nouzilly MIRU-VNTR (INMV) profiles. C- and S-type strains were differentiated by SNPs based on PCR and enzymatic restriction. Histological scoring of intestinal lesions was performed as described previously. Additionally, phenotyping of the inflammatory infiltrate was applied (CD3 for T lymphocytes, CD79a for B lymphocytes and Iba1 for macrophages).

Results

- All goats showed moderate to severe lymphohistiocytic and plasmacytic mucosal infiltration.
- Ziehl-Neelsen stain revealed no to moderate numbers of acid-fast bacilli (Figure 1).
- MIRU-VNTR profiling displayed presence of “new” strains (INMV218/220) and “classic” strains (INMV1), belonging to S- (INMV218/220) and C-types (INMV1), accordingly. Both types showed diffuse multibacillary/3b or diffuse lymphocytic paucibacillary/3c histological lesions (Figure 2).
- The mucosal and submucosal inflammatory infiltrate was mainly composed by macrophages and T lymphocytes (Figure 3).

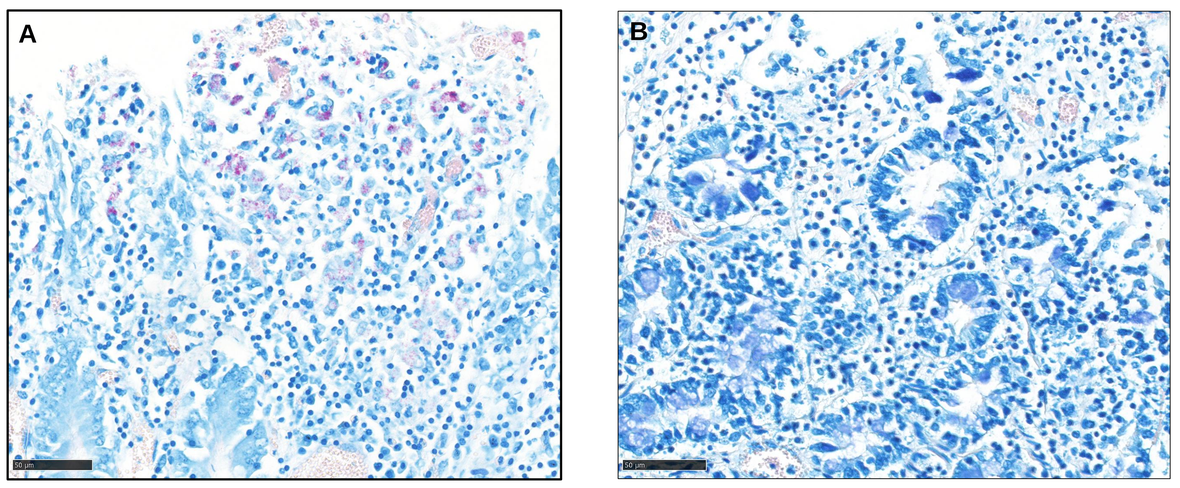

Figure 1: Ziehl Neelsen stain of histological scores diffuse mulitbacillary/3b (A) and diffuse lymphocytic/paucibacillary/3c (B) .

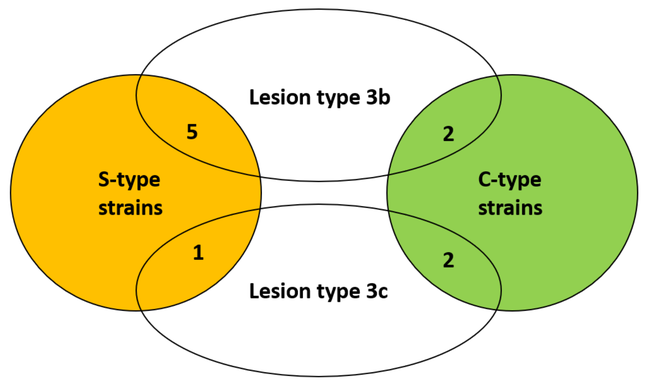

Figure 2: Distribution and number of histological lesions type 3b and 3c associated with S-type (yellow) or C-type strains (green).

Lesion type 3b was found in five S-type strains and two C-type strains. Lesion type 3c was found in one S-type strain and two C-type strains.

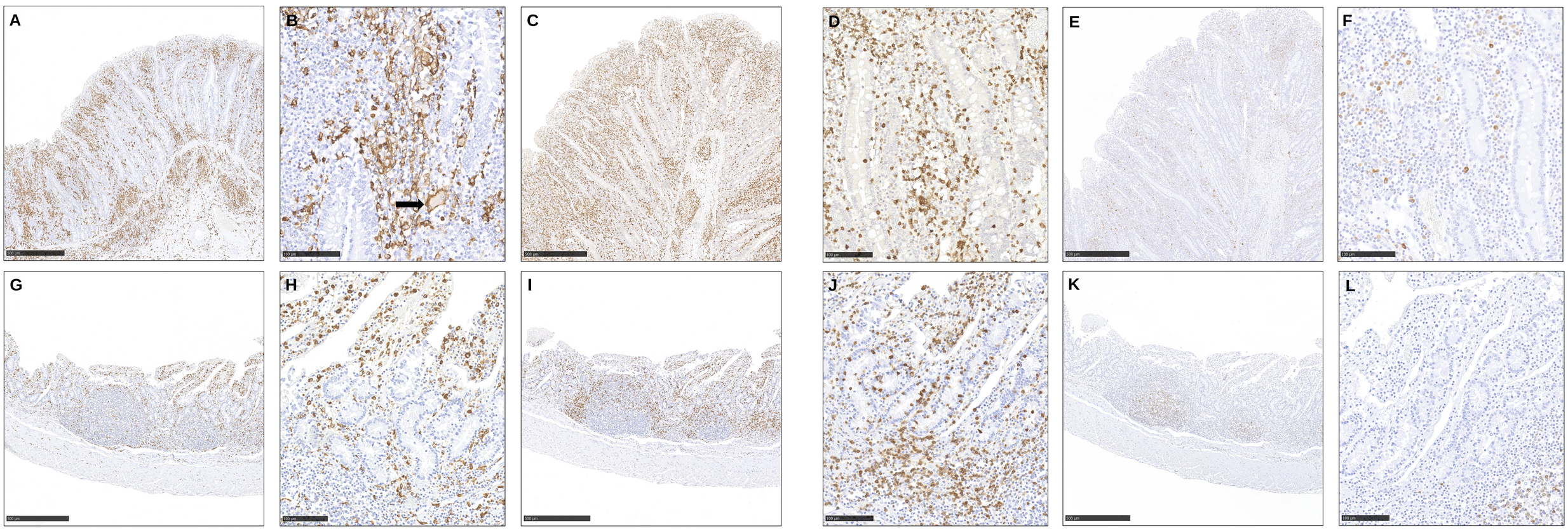

Figure 3: Immunohistology, goat, ileum. Upper row: type 3b lesion. Lower row: type 3c lesion. High number of Iba1-positive macrophages in a type 3b lesion (A overview, B detail, arrow = multinucleate giant cell). Moderate to high number of CD3-positive T lymphocytes in a type 3b lesion (C overview, D detail), Low number of CD79a-positive B lymphocytes in a type 3b lesion (E overview, F detail). Moderate number of Iba1-positive macrophages in a type 3c lesion (G overview, H detail). Moderate to high number of CD3-positive T lymphocytes in a type 3c lesion (I overview, J detail). Low number of CD79a-positive B lymphocytes in a type 3c lesion (K overview, L detail). ABC method, chromogen = DAB, counterstained with hematoxylin.

Conclusions

Histological scoring of lesions as well as immunohistological investigations did not reveal any obvious differences between S-/C-type and INMV strains in Swiss goats so far. However, detection of MAP by PCR proved to be a reliable approach to the identification of infected animals regardless of the histological lesion type. Morphometrical analysis of enteric tissue and investigations in immunomodulatory properties of inflammatory cells will be further performed to characterise the possible strain diversities in more detail.

References

1. Pérez et al. Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. J Comp Pathol. (1996) 114:107–122.
2. González et al. Histopathological classification of lesions associated with natural paratuberculosis infection in cattle. J Comp Pathol. (2005 133:184–196.
3. Thibault et al. New variable-number tandem-repeat markers for typing *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* strains: comparison with IS900 and IS1245 restriction fragment length polymorphism typing. J Clin Microbiol (2007) 45:2404–2410.
4. Leão et al. Novel single nucleotide polymorphism-based assay for genotyping *Mycobacterium avium* subsp. *paratuberculosis*. J Clin Microbiol. (2016) 54:556–564.
5. Scherrer et al. Morphological and molecular characterization of a new *Mycobacterium avium* subsp. *paratuberculosis* S-type strain genotype in goats. Front Vet Sci. (2019) 6:250.
6. Rasper-Hössinger et al. Strain diversity in *Mycobacterium avium* subsp. *paratuberculosis*-positive bovine fecal samples collected in Switzerland. Front Vet Sci. (2023) 10:1154516.