INFLAMMATORY CELL PHENOTYPING AND EPITHELIAL PROLIFERATION IN PORCINE *LAWSONIA INTRACELLULARIS* INFECTION

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Introduction

An infection with *Lawsonia intracellularis* (*L. intracellularis*) causes the so-called disease porcine proliferative enteropathy (PPE) leading to major economic losses worldwide. The main objectives of this retrospective study were to compare the current gold standard detection method of *L. intracellularis* (Warthin Starry stain) to *L. intracellularis* immunohistochemistry (IHC) and to immunophenotype the intestinal inflammatory infiltrate.

Material and Methods

- Fourty-two cases of PPE were selected from the archive. HE-stained intestinal samples were evaluated for presence and distribution of several histological parameters.
- Immunohistochemically, *L. intracellularis* antigen expression allowed a categorisation of animals with small (group 1), moderate (group 2), or large amount of *L. intracellularis* antigen (group 3).
- Immunophenotyping of inflammatory cells including regulatory T cells (Tregs) and evaluation of proliferation (Ki67 expression) was performed in selected cases (Table 1).

Marker	Cell population	*L. intracellularis* cases
Ki67	Proliferating cells	Group 3 > groups 1 and 2
CD3	T lymphocytes	Major inflammatory cell population (all groups)
CD20	B lymphocytes	Few cells in mucosa (all groups)
Iba 1	Macrophages	Major inflammatory cell population (all groups)
FOXP3	Regulatory T cells	In mucosa (group 1) and GALT (groups 2 and 3)

Table 1: Cell markers used in immunohistology and their relevance in *L. intracellularis* cases. Group 1 = small amount of bacterial antigen; group 2 = moderate amount; group 3 = large amount.

Results

- Histologically, the hallmark lesion was hyperplasia of crypt enterocytes which was detected in almost every animal.
- Colocalisation of *L. intracellularis* antigen and the macrophage marker Iba1 was detected (Figure 1).
- The major inflammatory cell populations consisted of macrophages and T lymphocytes (Figure 2).
- Tregs were detected in every group in the mucosa (mainly group 1) and in ileal lymphoid tissue (mainly group 2 and 3; Figure 2).
- The number of Ki67-positive cells was higher in group 3 compared to group 1 and 2 (Figure 2).

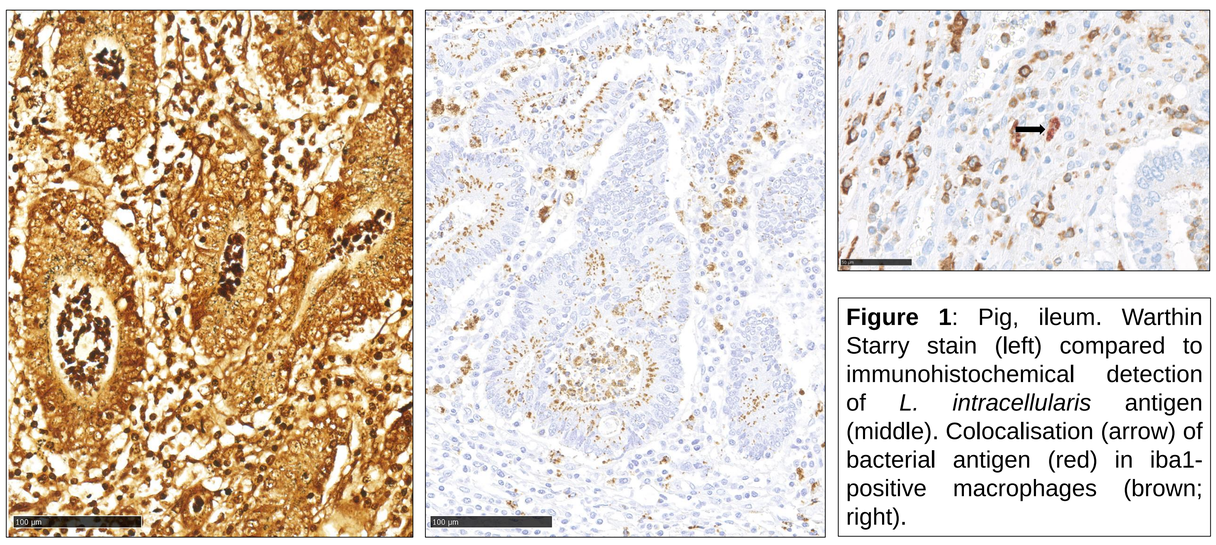

Figure 1: Pig, ileum. Warthin Starry stain (left) compared to immunohistochemical detection of *L. intracellularis* antigen (middle). Colocalisation (arrow) of bacterial antigen (red) in iba1-positive macrophages (brown; right).

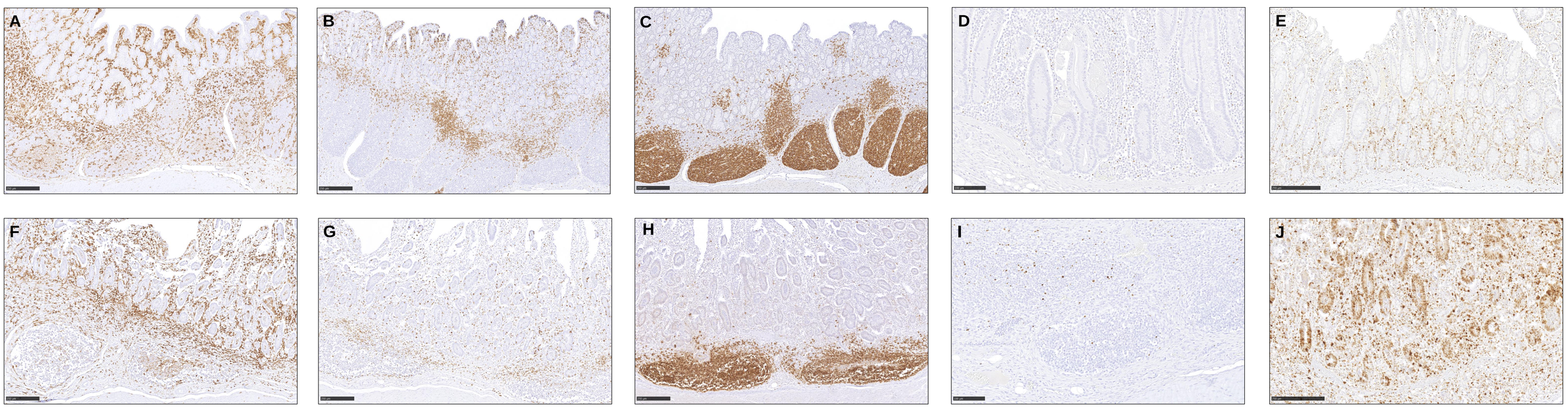

Figure 2: Pig, ileum. Inflammatory cell phenotyping and expression of proliferation marker Ki67 in *Lawsonia intracellularis* infected animals with low (group 1, A-E) and high (group 3, F-J) bacterial load. High number of Iba1-positive macrophages in mucosal and submucosal tissue of both groups (A, F). Moderate number of CD3-positive T lymphocytes in mucosal and submucosal tissue in both groups (B, G). CD20-positive B lymphocytes in Peyer`s patches, only few mucosal lymphocytes in both groups (C, H). Single FOXP3-positive regulatory T cells in lamina propria (D) and submucosa (I). Low number of Ki67 expressing crypt epithelial cells and inflammatory cells in group 1 (E). High number of Ki67-positive cells (crypt epithelial and mucosal inflammatory cells in an animal of group 3 (J). ABC method, chromogen = DAB, counterstained with hematoxylin.

Conclusions

The immunohistochemical method to detect *L. intracellularis* is equivalent to the Warthin Starry stain which serves as a gold standard. The bacterial load correlated with the severity of histological lesions and the proliferation of crypt epithelial cells. The detection of *L. intracellularis* in macrophages may contribute to a bacterial persistence in chronic infections. The immunomodulatory effects of Tregs and Treg dysregulation in early and late phases of the infection may affect the proliferation and spread of bacteria.

References

1. Brandt et al. Evaluation of *Lawsonia intracellularis* infection in a group of pigs in a subclinically affected herd from weaning to slaughter. Veterinary Microbiology (2010) 146, 361-365.
2. Vannucci and Gebhart. Recent advances in understanding the pathogenesis of *Lawsonia intracellularis* Infections. Veterinary Pathology (2014) 51, 465-477.
3. Guedes et al. *Lawsonia intracellularis* in pigs: Progression of lesions and involvement of apoptosis. Veterinary Pathology (2017) 54, 620-628.
4. Pedersen et al. Association between faecal load of *Lawsonia intracellularis* and pathological findings of proliferative enteropathy in pigs with diarrhoea. BMC Veterinary Research (2012) 8, 198.
5. Pereira et al. Survival of *Lawsonia intracellularis* in porcine peripheral blood monocyte derived macrophages. PLOS ONE (2020) 15, e:0236887.