Virulence of strong and weakly haemolytic strains of *Brachyspira hyodysenteriae*

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Introduction and Objectives

Brachyspira infections cause substantial economic losses in swine rearing countries. The haemolytic activity of porcine *Brachyspira* isolates is generally associated with *in vivo* pathogenicity.

In this study, 20 *B. hyodysenteriae* isolates were tested in an *in vitro* assay for their haemolytic capacity. Three isolates displayed a significantly lower haemolytic capacity than the other tested strains. To determine whether the haemolytic capacity correlates with *in vivo* pathogenicity, the pathogenic potential of six isolates with different haemolytic capacity was investigated in a mouse model (1).

Material and Methods

In vitro haemolytic capacity: the supernatant of 20 *B. hyodysenteriae* isolates was incubated with a 10% porcine red blood cell suspension for 2 hours. Then absorption was measured to quantify haemolysis (2). In addition, all isolates were screened for the presence of several haemolysis associated genes: the *tlyA* gene, the *hlyA* gene and its flanking *fabG* and *fabF* genes (3).

In vivo pathogenic potential: eighty female C3H/HeN mice were randomly divided into eight groups. Following acclimatisation and spectinomycin treatment in the water for 48-h, all mice were intragastrically inoculated twice with one of three weakly haemolytic or one of three strong haemolytic *B. hyodysenteriae* strains. The highly virulent, strong haemolytic strain B204 was included as reference strain. Control group mice were sham-inoculated. Seven days post-inoculation all mice were humanely euthanized and their ceca were macroscopically and histologically inspected for lesions. Cecal contents were collected and the amount of *B. hyodysenteriae* DNA was determined with qPCR.

Histology: ceca were split longitudinally and sections were stained with hematoxylin and eosin or with Periodic Acid-Schiff reagent (PAS). Sections were evaluated for the following criteria: the presence of widened crypts with flattening of the epithelial cells, the presence of submucosal edema and crypth depth. Using image analysis software (Leica Application Suite 3.8.0), the area occupied by PAS-positive cells relative to the total area of one crypt and villus was determined at eight random sites per cecum for each mouse.

Results

Although all isolates were positive for all the haemolysis associated genes, three isolates displayed a significantly lower haemolytic capacity to near absence of haemolysis in the *in vitro* assay.

Lesions caused by the weakly haemolytic strains were comparable to those caused by strong haemolytic strains and the reference strain of *B. hyodysenteriae.* The percentage of widened crypts, the crypt depth and the percentage of PAS-positive area were significantly greater in all infected groups compared to the control group. However, no statistical difference was observed between infected groups.

Discussion

We describe for the first time weakly hemolytic *B. hyodysenteriae* isolates. Although recent literature proposes a clear association between the degree of haemolysis of *Brachyspira spp.* and its pathogenic potential, this could not be confirmed with the currently used mouse model. The similar pathogenic potential of strong and weakly haemolytic isolates of *B. hyodysenteriae* may suggest that other virulence traits also play an important role in the pathogenesis of *B. hyodysenteriae* infections (4).

References

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