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Characterization of Polyclonal and Monoclonal Antibodies Against *Lawsonia intracellularis*

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Introduction & Objectives

Proliferative enteropathy is an intestinal infectious disease caused by the obligate intracellular bacterium *Lawsonia intracellularis* (Lawson & Gebhart, 2000). PE is routinely diagnosed in veterinary diagnostic laboratories based on histologic lesions detected by hematoxylin and eosin stain and Warthin Starry silver stain, and by polymerase chain reaction (PCR) in fecal and intestinal samples. An immunohistochemical (IHC) stain using a monoclonal antibody against *L. intracellularis* (IG4) (McOrist et al, 1987) detected positive antigen label among several animal species (Cooper & Gebhart, 1998). The IHC stain has been shown to have superior sensitivity than hematoxylin and eosin and Silver staining for detecting *L. intracellularis* in histological sections (Jensen et al, 1997; Guedes et al, 2002). However, the monoclonal antibody used in those studies is not commercially available. Therefore, the objective of this study was to produce and characterize new polyclonal and monoclonal antibodies against *L. intracellularis*.

Materials & Methods

The new monoclonal (2001 MAb) and polyclonal antibodies (1999 PAb) were compared to the IG4 antibody using Western blot analysis of outer membrane proteins (OMPs) of 6 *L. intracellularis* isolates (Table 1), *Bilophila wadsworthia* and *Brachyspira hyodysenteriae*, IHC of known positive and negative histologic samples and pure cultures of *L. intracellularis*, *B. wadsworthia*, *B. hyodysenteriae*, *Salmonella choleraesuis*, *S. typhimurium* and *Escherichia coli* K88. An immunogold stain using the 2001 MAb was performed to show the specificity of the antibody against a *L. intracellularis* surface protein.

Results & Discussion

Western blot analysis showed that the new monoclonal antibody (2001 MAb) targeted an OMP of 77 kDa, which was different from the 1987 MAb that targeted a 18 kDa OMP, in all *L. intracellularis* isolates except a hamster isolate (Table 1). The immunogold stain demonstrated the specificity of the 2001 MAb to a surface protein of *L. intracellularis*. The polyclonal antibody (1999 PAb) targeted five OMPs (77, 69, 54, 42 and 36 kDa). Both the 2001 MAb and the 1999 PAb stained the known positive but not the known negative histologic samples. Both the 2001 MAb and the 1999 PAb stained a pure culture of *L. intracellularis* and did

not stain any other common enteric pathogens. These two new antibodies will be useful in immunodiagnosis of *L. intracellularis*.

Table 1: Isolate information and Western blot results

| Isolate tested | Origin | Western blotting | | |
|---------------------------|--------|------------------|--------|---------|
| | | IG4 | 2001MA | 1999PAb |
| <i>L. intracellularis</i> | | | | |
| Pigs isolate (PHE) | | | | |
| VPB4 | USA | + | + | + |
| PHE/MN1-00 | USA | + | + | + |
| 15540D | Den* | + | + | + |
| Pig isolate (PIA) | | | | |
| 963/93 | UK | + | + | + |
| Hamster isolate | | | | |
| HAM-1 | USA | - | - | + |
| Foal isolate | | | | |
| Foal/96 | USA | + | + | + |
| <i>B. wadsworthia</i> | USA | - | - | - |
| <i>B. hyodysenteriae</i> | USA | - | - | - |

* Denmark

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