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Correction page 505

5 % pentobarbital should read 0.5 % pentobarbital

line 2 in Abstract
line 10 in Materials and methods
line 1 in Table 1
line 2 in Table 2
line 7 in Discussion

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A monoclonal antibody reacting with the cell envelope of spirochaetes from intestinal spirochaetosis

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Introduction

Intestinal spirochaetosis results from infection with weakly haemolytic spirochaetes that are distinct from both *Serpulina hyodysenteriae* and *Serpulina innocens* (1, 2, 3). These bacteria only have 4-6 axial flagellae, and belong to a newly-recognised genetic group with the proposed name *"Anguillina coli"* (3). Disease is mainly seen in weaner and grower pigs, which develop a sloppy mucoid diarrhoea and suffer a reduction in weight gain (1). Similar bacteria are associated with intestinal spirochaetosis in humans (4).

The purpose of the present work was to prepare and characterise a specific monoclonal antibody that could be used to identify isolates of *"A. coli"* in the faeces of affected individuals.

Materials and methods

"A. coli" strain 3295, isolated from an Australian pig with intestinal spirochaetosis, was used in the preparation of monoclonal antibodies (Mabs). The cell envelope was extracted with Triton X-114, as described for *Serpulina hyodysenteriae* (5), and was used to immunise Balb C mice. Hybridomas were prepared using standard techniques, and their products were screened with an ELISA, using a cell envelope extract from strain 3295 as the plate-coating antigen. Positive hybridomas were selected, cloned out, and cultured *in vitro*. The class of one Mab (BJL/AC1) was determined using a commercial mouse monoclonal sub-isotyping kit (Bio-Rad Laboratories). This Mab was tested for specificity in Western blots with cell envelope preparations from ten strains of *"A. coli"* from pigs and humans, and with ten strains of *Serpulina* spp. (including the type strains of *S. hyodysenteriae* and *S. innocens*). The Mab then was used in immunofluorescence and immunogold labelling studies directly on cells of these isolates, as described for a 16kDa antigen of *S. hyodysenteriae* (6). Pig faeces from a healthy animal were also seeded either with cells of *"A. coli"* strain 3295, *S. hyodysenteriae* strain B78, or *S. innocens* strain B256. After blending the samples, cotton-tipped swabs were placed into each, and thin smears made on glass microscope slides. These were air-dried, fixed in acetone, and then examined using the same indirect fluorescent antibody technique as applied to cells.

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Results

The Mab BJL/AC1 reacted in Western blot analysis with a molecule of approximately 25 kDa in cell envelope preparations from all porcine and human isolates of *"A. coli"*, but did not react with any component of the envelope of isolates of the *Serpulina* spp.

In immunogold electron microscopic analysis, Mab BJL/AC1 attached infrequently and irregularly to the outer surface of isolates of *"A. coli"*. Using immunofluorescence, the monoclone was also shown to bind to the surface of *"A. coli"* cells. No specific fluorescence occurred on the cells of the *Serpulina* spp. isolates. *"A. coli"* strain 3295 was detected in faeces seeded with this strain. The faecal samples seeded with *S. hyodysenteriae* and *S. innocens* were recorded as negative.

Discussion

The Mab BJL/AC1 specifically attached to a component of the cell envelope of isolates of *"A. coli"*, which were recovered from pigs and humans suffering from intestinal spirochaetosis. Using immunogold and immunofluorescence labelling this component was shown to be exposed on the surface of the *"A. coli"* isolates, but was sparce, and irregularly distributed along the length of the cell. The nature of this material was unclear. Nevertheless, the Mab to the material could be used in indirect fluorescent antibody tests to specifically identify isolates of *"A. coli"*. Furthermore this procedure was shown to give positive results only in faeces seeded with an isolate of *"A. coli"*. As such, this technique may prove useful as a simple and rapid diagnostic test for the detection and identification of spirochaetes associated with cases of intestinal spirochaetosis in pigs and humans.

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