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CAMPYLOBACTER INFECTION IN INTESTINAL ORGAN CULTURES

A thesis presented in partial fulfilment (50%) of the requirements for the degree of Master of Philosophy in Veterinary Pathology at Massey University Palmerston North New Zealand

Jabbar Gh. Kuhait
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ABSTRACT

Six different media (T199 medium, T199 + 10% foetal calf serum, T8 medium, T8 + 10% foetal calf serum, RPMI medium and RPMI + 10% foetal calf serum) were tested for their ability to maintain foetal lamb intestine in organ culture. T199 medium + 10% foetal calf serum was chosen because it gave more consistent results in maintaining the foetal intestine for a period of six days in culture.

Two groups of foetal lamb intestine were cultured, a control group and a group infected with Campylobacter jejuni. The effects of the microorganisms on the intestinal culture were assessed at 6 hours, 13 hours and 15 hours post-culture.

Light, Transmission and Scanning Electron Microscopy were used to study the pathogenicity of C. jejuni at the cellular level. Light microscopic studies showed that C. jejuni were attached and colonised the tips of the villi and the crypt epithelium of the intestinal cultures at 6 hours, 13 hours and 15 hours. The epithelial cells showed marked necrosis at the tips of the villi. The microorganisms also invaded the cytoplasm of epithelial cells of the villi and the intestinal crypt.

Transmission Electron Microscopy revealed degeneration of the microvilli in the infected cultures. The microorganisms were found attached to the tips of the microvilli of the villous epithelial cells by pilus-like structure. Microorganisms were present within phagolysosomes of macrophages in the lamina propria. Various cytoplasmic changes were observed at 6, 13 and 15 hours post-infection.

Scanning Electron Microscopy confirmed the different changes in the morphology of the infected epithelial cells. The microorganisms were observed adhering to the surface of the epithelial cells at 6, 13 and 15 hours post-culture.
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epithelial cells show a moderate loss of microvilli (arrow). (SEM X4840).

3.54. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of C. jejuni, showing changes that have occurred 6 hours post infection. A filament-shaped microorganism can be observed attached to the tips of the epithelial cells. The infected epithelial cells (C) show a severe loss of microvilli. (SEM X8,250).

3.55. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of C. jejuni, showing changes that have occurred 13 hours postinfection. The infected epithelial cells (C) are disorganised and have lost their normal polygonal pattern and their microvilli when compared with the 13 hour control culture (Figure 3.49). One normal epithelial cell (arrow) remains covered with densely packed rod-shaped microvilli (MV). (SEM X7,700).

3.56. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of C. jejuni, showing changes that have occurred 13 hours postinfection. The infected epithelial cells (C) are swollen and protrude towards the luminal surface. A layer of thick white mucus (mu) can be observed covering the surface of the infected cells. Pits and holes are present (arrow) and represent goblet cells (G). Occasional spiral-shaped (s) to rod-shaped (r)
microorganisms can be seen within the mucus. (SEM X12,100).

3.57. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of C.jejunii, showing changes that have occurred 13 hours postinfection. The surface of the villous epithelium is roughened and irregular and covered with thick mucus (arrow). The epithelial cells (C) show a severe loss of microvilli. Spiral (s), filamentous (f) and ring-shaped (r) microorganisms can be observed attached to the surface of the infected epithelial cells. (SEM X6600)

3.58. Higher magnification of Figure 3.57. The infected epithelial cells (C) are swollen and protrude into the luminal surface. They show a severe loss of microvilli and are covered with thick, white strands of mucus. (SEM X12,100).

3.59. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of C.jejunii, showing changes that have occurred 15 hours postinfection. The epithelial cells (C) are severely disorganised, round to elongate in shape (arrow) and some show a severe loss of microvilli (MV). A few are exfoliated (Ex). The external surface of the epithelial cells is roughened and covered with thick strands and plugs of white mucus (M). Occasional spiral-shaped microorganisms (MO) are attached to the surface of the epithelial cells. (SEM X2640).
3.60. Higher magnification of Figure 3.59 showing the irregular, ridged surface of the absorptive epithelial cells (arrow). A severe loss of microvilli can also be observed. (SEM X9,900).

3.61. Organ culture of foetal lamb intestine in T199 medium + 10% foetal calf serum, infected with approximately $10^6$ CFU/ml of $C.\text{jejuni}$, showing changes that have occurred 15 hours post-infection. Necrosis and exfoliation of some of the epithelial cells can be observed at the tips of the villi (arrow). The lamina propria (L) is exposed and protrudes above the remaining epithelial cells (C). Some of the intact epithelial cells show a disorganised pattern (long arrow) when compared to the 15 hour control culture (Figure 3.50). Occasional shreds and plugs of mucus (MU) cover the desquamated cells (small arrow). (SEM X6050).

3.62. Histogram comparing the means of epithelial villus heights in foetal lamb intestinal organ cultures infected with $C.\text{jejuni}$, and controls, at 6, 13 and 15 hours post-infection.

3.63. Histogram comparing the means of epithelial cell widths in foetal lamb intestinal organ cultures infected with $C.\text{jejuni}$, and controls, at 6, 13 and 15 hours post-infection.

3.64. Histogram comparing the means of epithelial cell heights in foetal lamb intestinal organ cultures infected with $C.\text{jejuni}$, and controls, at 6, 13 and 15 hours post-infection.