ABSTRACTS

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DETECTION OF EXPRESSION OF P FIMBRIAE IN VITRO USING A HAEMAGGLUTINATION ASSAY

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Introduction - P fimbriae are commonly associated with the attachment of E. coli to urinary tract epithelial cells in humans (5) and dogs (3). The gene encoding P fimbriae (the pap gene (4)) has also been detected in a significantly larger proportion of strains of E. coli isolated from the uteri of bitches with pyometra than from faecal isolates (2). However, any role for P fimbriae in the attachment of E. coli to the uterine epithelial cells has not been demonstrated. The expression of P fimbriae in pap-positive E. coli cultures is phase-regulated, and is affected by environmental conditions such as temperature and the nutrient composition of growth media (1). P fimbriae, when expressed in bacterial cultures, cause mannose-resistant haemagglutination in vitro (6).

Aims - The aims of this study were to a) determine the growth phase for pap-positive E. coli cultures that gave maximal expression of P fimbriae, and b) optimise the haemagglutination assay for detection of expression of P fimbriae in such cultures.

Materials and Methods - Two strains of E. coli - P3 and F8 were used. The P3 strain was isolated from the uterus of a bitch with pyometra and carried five uropathogenic virulence factor genes (pap, sfa, hlyA, cnf1 and fim) as determined by the polymerase chain reaction (2). The F8 strain was isolated from the faeces of a clinically healthy dog and did not carry any known UVF genes. Both strains were incubated on LB broth or LB agar at 37 °C and grown to stationary, log and mixed growth phase cultures. These cultures were then titrated and incubated with sheep and dog erythrocytes at different temperatures (4, 22 and 38.5 °C) for one hour.

Results - The results were interpreted in haemagglutination units (HAU), with one HAU being the lowest concentration capable of mediating haemagglutination. All growth phase cultures of strain P3 agglutinated sheep erythrocytes at 4 °C, however the mixed growth phase culture yielded higher haemagglutination titres (8 HAU) than the stationary (2 HAU) and the log (2 HAU) growth phase cultures. None of the cultures of strain F8 agglutinated sheep erythrocytes. Contrarily, both P3 and F8 strain cultures agglutinated dog erythrocytes at 4 °C indicating that such agglutination was mediated by factors other than P fimbriae. A total of eleven strains of E. coli (four pap-positive and seven pap-negative strains) isolated either from the uteri of bitches with pyometra or from the faeces of clinically healthy dogs were then grown to mixed growth phase cultures. The cultures were incubated with sheep and dog erythrocytes at 4 °C for an hour followed by the addition of 0.5% mannose suspension. All of the four pap-positive strains (including strain P3) and none of the seven pap-negative strains (including strain F8) of E. coli agglutinated sheep erythrocytes at 4 °C in the presence of mannose. The study thus demonstrated that the expression of P fimmbriae could be obtained in pap-positive E. coli cultures when grown on LB broth or agar at 37 °C. This expression could be detected in vitro using the optimised haemagglutination assay, and was maximal in mixed growth cultures.

References


