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Effect of hemolytic and non-hemolytic E. coli on canine endometrial epithelial and stromal cells
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Canine pyometra E. coli isolates are mainly assigned to phylogenetic group B2 and are characterized by a high number of uropathogenic Escherichia coli virulence factor genes and pathogenicity-associated islands (PAIs) markers (1). One of these genes is α-hemolysin (hlyA) which was detected in 48% of pyometra E. coli strains compared to 30% and 19% of E. coli from urinary tract infection and fecal origin, respectively (1). The objective of this study was to evaluate the effect of α-hemolysin in canine epithelial and stromal cells. Endometrial epithelial and stromal cell populations were isolated as previously described (2) with modifications and in vitro stimulated for 1 and 4 hours with Pyo14 (non-hemolytic E. coli), Pyo 18 (hemolytic E. coli) and Pyo18ΔhlyA (isogenic mutant of Pyo18, constructed as previously described (3)). At the end of each time of incubation the following parameters were evaluated: (1) adhesion and internalization of E. coli; (2) cells morphology and number. Results were analyzed using the Mann–Whitney U test. In epithelial cells, the percentage of adhesion of the 3 strains was similar at 1 and 4h (3-5%). This was also the case for stromal cells incubated with Pyo14 and Pyo18ΔhlyA. Incubation of stromal cells with Pyo18 for 4h resulted in dead of the majority of cells, so no adhesion, internalization and cell number quantifications were obtained. A very low percentage of bacterial internalization was observed in epithelial and stromal cells for Pyo14 and Pyo18ΔhlyA and only at 4h of incubation (0.1% – 0.2%). Incubation with Pyo14 and Pyo18ΔhlyA did not induce alterations in epithelial (Pyo 14 1h: 107.1±2.5%; 4h: 96.3±6.3%; Pyo18ΔhlyA 1h: 109.6±2.7; 4h: 97.9±4.4) or stromal (Pyo 14 1h: 102.7±5.9%; 4h: 98.9±2.5%; Pyo18ΔhlyA 1h: 104.5±3.0%; 4h: 95.4±3.5%) cells number and morphological aspect, when compared to control cells. Incubation with Pyo 18 induced a 51.1% reduction in the number of epithelial cells after 4h of incubation, a result not observed after 1h of incubation (105.9±3.3%) (p< 0.0001). Morphological aspect of epithelial cells after incubation with Pyo18 was not altered after 1 or 4 hours. Although the number of stromal cells (102.9±4.8%) was not affected after 1h incubation with Pyo18, cells started to lose the elongated fibroblast shape, becoming spherical. After 4h of incubation with Pyo18, all stromal cells were detached, not allowing the quantification of the number of cells. Our results suggest that α-hemolysin might contribute to E. coli virulence in the uterus. Its cytotoxic activity especially towards to stromal cells may contribute to the damage of endometrium, facilitating bacteria progression in the uterine tissue. 


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