Investigation of Adhesion and Invasion Capacity of *Streptococcus agalactiae* in Bovine Mammary Epithelial Cells – BMEC.

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Streptococcus agalactiae is one of the most common pathogen causing mastitis among dairy cattle, reducing milk quality and compromising the udder health. The aim of this study was to analyze the adhesion and invasion capacity in bovine mammary epithelial cells (BMEC) of *S. agalactiae* strains isolated from milk of cows with subclinical mastitis. Thus, we performed the Polymerase Chain Reaction (PCR) for genes that are involved in adhesion and invasion (*hlyb, fbsA, fbsB, pI1, pI2a, pI2b*) and capsular type (Ia, Ib, II-IX) in 145 *S. agalactiae* strains. After genotyping, the strains were grouped in 11 different profiles and 1 strain belonged an each profile were used for adhesion and invasion test. Thus, cells were cultured in 24-well microplates to form confluent layer. The *S. agalactiae* cultures were diluted in Dubelcco Modified Eagle's medium (DMEM) (1.5 x 10^6 CFU/mL) and 1 mL of these suspensions was inoculated into each well, in duplicate, 37°C/5%CO_2. After 3 hours, the wells were washed with PBS buffer and the adhered cells were detached from the plate using trypsin-EDTA for 15 minutes. The trypsin action was stopped adding DMEM and 10 µL of each well and serial dilutions were plated onto TSA plates. For the invasion test, the bacterial suspensions were inoculated in duplicate, and the plates were incubated for 3h/37°C/5%CO_2. After, the wells were washed with PBS buffer, and incubated again for 2 hours with DMEM with 5% FBS and 100µg/ml gentamicin + 5 µg/ml penicilin to remove the extracellular bacteria. Following, the wells were washed with PBS and plated into TSA to check for the absence of bacteria in the extracellular medium. Next, 0.1% Triton X-100 was added to lyse the cells to release the bacteria that invaded them and 10 µL directly from the wells and serial dilutions were plated into TSA. The positive result was determined by the recovering and counting of the colonies, in percentage relation to the values obtained in the adhesion test. Our results showed that all of 11 strains adhered to BMEC but only one of them invaded the cells. The difference between adhesion and invasion rates may be due to the low capacity of *S. agalactiae* to survive into the mammary cells and the unique strain that invaded can be more virulent than others due to its genetic profile. The high adhesion capacity of *S. agalactiae* can contribute the biofilm formation furthering the persistence of bacteria in udder increasing cases of subclinical bovine mastitis.

KEYWORDS: *S. aagalactiae*, mastitis, adhesion, invasion, cell culture.