TITLE: INVESTIGATION OF DIARRIOGENIC VIRULENCE GENES IN *Escherichia coli* ISOLATED FROM MILK OF COWS WITH CLINICAL MASTITIS

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ABSTRACT:
Bovine mastitis is one of the most important diseases in dairy farms due to the discard of contaminated milk, reduction of production, veterinary assistance, use of antibiotics and animal replacement, accounting for 70% of the producer's economic losses. Besides, there is a potential risk to public health due to the spread of toxins and pathogens, mainly in unpasteurized milk and milk products. In cases of environmental mastitis, *Escherichia coli* is the most prevalent microorganism in clinical mastitis, causing severe symptoms and death. Little is known about the genetic characteristics of these strains, and it can´t be inferred if the isolates responsible for mastitis could also be diarrheagenic, being a zoonotic bacterium and causing diseases to the man, by the ingestion of contaminated milk. Therefore, the aim of this work was to investigate virulence genes associated with intestinal diseases in humans. A hundred strains of *E. coli* previously isolated from milk from cows with clinical mastitis from three farms in São Paulo state were analyzed by the PCR technique for the presence of *eae*, *bfpA*, *stx1*, *stx2*, el*t*, *est*, *ipa*H, *aggR* and *aatA* genes. Each strain was plated onto MacConkey Agar plates, and incubated at 24h/35°C. Subsequently, a single colony was diluted in 200µl sterilized water and boiled for 10 minutes, followed by centrifugation, to collect the supernatant. The samples of *E. coli* ETEC-H10407 (elt^+/est^+), EPEC-E2348/69 ($bfpA^+/eae^+$), EAEC-042 ($aggR^+/aatA^+$), STEC/EHEC-EDL933 ($stx1^+/stx2^+$) and EIEC-EDL1284 ($ipaH^+$) were used as positive controls and laboratory *E. coli* C600 was used as a negative one in all reactions. The genes studied were not found in any of the strains analyzed. So, we conclude that these strains do not present a diarrheagenic zoonotic potential for the humans.

Keywords: *Escherichia coli*, clinical mastitis, virulence genes, diarrheagenic

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