

TITLE: ASSESSMENT OF BLOOD CHANGES POST-CHALLENGE WITH *CORYNEBACTERIUM ULCERANS* AND INFLUENCE OF PHOSPHOLIPASE D AND DIPHTHERIA EXOTOXINS: A COMPREHENSIVE STUDY IN MICE

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ABSTRACT

Diphtheria of zoonotic nature and extrapharyngeal infections caused by *Corynebacterium ulcerans* have been increasingly in different countries. Investigations of pathogenic potential of *C. ulcerans* revealed the ability of interaction with fibrinogen and host cells; however, mechanisms that favor to cross host barriers and get access to deeper tissues need further investigation. Phospholipase D (PLD) is considered as a prominent virulence factor, in addition to diphtheria toxin (DT). Therefore, effects of PLD on haematological profiles (erythrocytes, leucocytes and platelets) of Swiss Webster were analyzed and *C. ulcerans* (n=04) strains were investigated and compared to previously described reference values for mice: DT-negative PLD-positive/wild-type BRAD22 dog isolate and respective PLD-negative mutant due to *pld* disrupted gene ELHA1 strains in addition to the DT-negative and PLD-negative carrying *pld* gene 2590 and DT-positive and PLD-positive 210932 human isolates. Animals were infected through the caudal vein with 0.1 ml of *C. ulcerans* suspension in sterile saline containing 1×10^9 colony-forming units. At the end of one-week period, blood samples were collected by cardiac puncture and mice from each group, including negative controls inoculated only with sterile saline, were euthanized, in accordance with Brazilian ethical policies. Examination of the total number and relative proportions of red blood cells and the percentage of hemoglobin indicated a significant change only for DT-positive 210932 strain as well as formation of neutrophil toxic granulation (that indicate sepsis). Anisocytosis with normocytosis and red blood cell rouleaux formation were observed for all *C. ulcerans* tested, including ELHA1 strain. Leukocytosis, frequently a sign of an inflammatory response, was also observed during *C. ulcerans* infection, independent of DT and PLD production. We report *C. ulcerans*-platelet interaction and aggregation induced at different levels by all strains tested in mice, related to PLD and DT-production. Therefore, in cases of *C. ulcerans* infections platelets retain their cohesiveness but, because of their high numbers, tend to bind to each other, forming clots that can block a blood vessel and cause tissue damage. In conclusion, these changes recorded in the hemogram, leukogram and platelets parameters of mice emphasize the multifactorial nature of *C. ulcerans* pathogenicity.

Keywords: *Corynebacterium ulcerans*, phospholipase D, blood cells, mice.

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