Title: AN EXPERIMENTAL VACCINE AGAINST *E. COLI* O157:H7 FOR CATTLE COMPOSED BY INTIMIN, ESPB AND STX2B.

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ABSTRACT

Escherichia coli O157:H7 is a zoonotic pathogen of global importance and the serotype most frequently associated with cases of Hemolytic Uremic Syndrome. The main reservoir of this pathogen is bovine cattle.

Our group has demonstrated that vaccination with the carboxy-terminal fraction of Intimin γ (IntC280) and EspB can reduce fecal shedding of *E. coli* O157:H7. Furthermore, Stx2 has been shown to exert a local immunosuppressive effect on the bovine intestine, so an immune response against the toxin could improve the protective ability of the vaccine. In a previous work, we have demonstrated that vaccination of cattle with Stx2B fused to Brucella Lumazine Synthase (BLS-Stx2B) induces humoral immune response able to neutralize Stx2 cytotoxicity in Vero cells *in vitro*.

The objective of this work is to determine the protective efficacy in terms of fecal shedding after experimental challenge, in calves vaccinated with IntC280, EspB and BLS-Stx2B. To this end, 15 3-month-old male Holando Argentino calves were immunized as follows: EspB + IntC280 (n = 5); EspB + IntC280 + BLS-Stx2B (n = 5); Control (n = 5). Animals received two doses with 100 µg of IntC280 and EspB and 300 µg of BLS-Stx2B, separated by 15 days. Blood samples were taken periodically to evaluate the humoral immune response. At 24 days post immunization (dpi), calves were intragastrically challenged with 10^9 CFU of *E. coli* O157:H7. Shedding of *E. coli* O157:H7 was followed in recto-anal mucosal swabs by direct plating and enrichment followed by immunomagnetic separation. Subsequent multiplex PCR was performed to confirm the presence of EHEC O157:H7.

Vaccination generated a significant increase in the level of specific antibodies against IntC280 and EspB in both groups, and against Stx2B in the group that received the 3 antigens. Furthermore, the antibodies against Stx2B showed neutralization of Stx2 cytotoxicity in Vero cells after 15 dpi, significantly different from the control group and from animals vaccinated with 2 antigens. Both vaccine formulations induced a protective response reducing the shedding of *E. coli* O157:H7 compared to the control group. However, the addition of Stx2B to the vaccine formulation did not induce greater protection than the protection conferred by IntC280 and EspB alone.

In conclusion, despite a significant humoral response against Stx2B with neutralizing ability of Stx2 *in vitro* was elicited in calves vaccinated with this antigen, no further increase in protection was observed when compared with the group that received only 2 antigens. Based on this evidence it is necessary to pursue the search for alternatives that improve the elicitation of immune

responses against Stx2 in order to reduce the colonization of the bovine gastrointestinal tract by these zoonotic bacteria.

Keywords: EHEC, vaccine, antigens, protection

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