Recombinant vaccine reduces the excretion of *Escherichia coli* O157:H7 in cattle and elicits high titers of bacteria-targeted antibodies in colostrum

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- Enterohemorrhagic *Escherichia coli* O157: H7 was the most prevalent EHEC serotype recovered from patients with hemolytic uremic syndrome (HUS) in the world.

- Argentina is a country with a very high incidence of HUS in children, 14 cases per 100 000 children younger than 5 years old (2006).

- Pre-slaughter vaccination of cattle, the main reservoir of EHEC O157: H7, could be a logical strategy to reduce the incidence of infection in humans.

- Vaccination of pregnant cows could be also a tool to obtain antibodies-targeted dairy products.

- This study evaluated a vaccine based in a fragment of Intimin and EspB, two key colonization factors of *E. coli* O157: H7.
Enterohemorrhagic *Escherichia coli* O157:H7

Shiga toxins and Attaching and Effacing lesion

LEE (Locus Enterocyte Effacement)

Type Three
Secretion System
(TTSS)
Previous works

- Immunoglobulin enriched cow colostrum protects mice against *E. coli* O157:H7 infections by prevention of bacterial attachment, colonization and growth in the intestinal tract in mice (Funatogawa *et al.*, Microbial Immunol 2002; 46: 761)

- Neutralizing activity of bovine colostral antibody against verotoxin derived from enterohemorrhagic *E. coli* O157:H7 in mice (Kuribayashi *et al.*, J Infect Chemother 2006; 12:251)

- Bovine colostral antibody against verotoxin 2 derived from *E. coli* O157:H7: resistance to proteases and effects in beagle dogs (Kuribayashi *et al.*, Comp Med 2009; 59:163)
Presence of specific antibodies against Intimin and Esp proteins in colostrum from cows non experimentally immunized

Bovine Colostrum Contains Immunoglobulin G Antibodies against Intimin, EspA, and EspB and Inhibits Hemolytic Activity Mediated by the Type Three Secretion System of Attaching and Effacing Escherichia coli

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Antigenicity of Intimin and Esp proteins in mice intranasally immunized

Efficient immune responses against Intimin and EspB of enterohaemorrhagic Escherichia coli after intranasal vaccination using the TLR2/6 agonist MALP-2 as adjuvant

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1° Vaccine Trial

REduced EXCRETION OF *Escherichia coli* O157: H7 IN CATTLE AFTER VACCINATION WITH INTIMIN AND EspB PROTEINS

**Animals**
- Holstein calves 6–8 months old
- Negatives for *E. coli* O157:H7
- Groups: Vaccinated and Control

**Vaccine**
- Recombinant proteins EspB and Intimin
  + adjuvant
- Control: PBS

**Containment Facilities**
- BS II
Vaccine: two doses

Challenge $10^9$ CFU *E. coli* O157:H7 438/99 Nal$^R$

Samples: serum, saliva, feces, rectoanal mucosal swabs
A significant reduction in total bacterial shedding of *E. coli* O157:H7 excreting animals were observed in the vaccinated group compared to the control group over the sampling period.

High titers of Intimin and EspB –specific IgG antibodies were observed after the first immunization in the vaccinated calves, compared to control animals (*P* ≤ 0.001, *t* -test).

A significant IgA response against both proteins was observed in saliva after the first immunization (*P* ≤ 0.05, *t* -test).

An IgG specific response against both proteins was also observed in saliva 21 days after the first immunization. (*P* ≤ 0.05, *t* -test).
Holstein pregnant cows confirmed to be negative for EHEC O157:H7 infection
Groups: Vaccinated and Control

Recombinant proteins EspA, EspB and Intimin and inactivated Shiga toxin type 2 + adjuvant
Control: PBS + adjuvant

Dairy herd at Rafaela Experimental Station- INTA
Vaccine: three doses

Samples cows: serum, colostrum, milk

Samples calves: serum, feces
RESULTS

- Cows mounted high colostrum IgG titers to Intimin, EspA and EspB
- Cows and colostrum-fed calves also exhibited serum IgG antibodies against EspA, EspB and Intimin
- Western blotting confirmed the specificity of the responses measured by ELISA
- Colostrum from Stx2-vaccinated cows exhibited high Stx2-neutralizing antibodies titers compared with the control group
Immunization with recombinants Intimin and EspB proteins seems to be a feasible strategy to reduce EHEC O157:H7 fecal shedding in cattle.

Hyperimmune colostrum from cows immunized with Intimin and EspB proteins is a source of antibodies against EHEC O157:H7 which could block the colonization and toxic activity of that bacterium in the human intestine.

Dairy products with EHEC O157:H7-targeted antibodies could be a useful tool to decrease the risk of the progression of diarrhea to HUS on children.
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