



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 0157: 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 0157: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* 0157: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* 0157: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

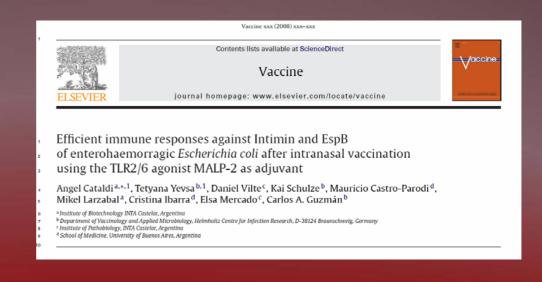
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Bovine Colostrum Contains Immunoglobulin G Antibodies against Intimin, EspA, and EspB and Inhibits Hemolytic Activity Mediated by the Type Three Secretion System of Attaching and Effacing *Escherichia coli* ⁷

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Antigenicity of Intimin and Esp proteins in mice intranasally immunized



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 10⁹ CFU *E. coli* O157:H7 438/99 Nal^R

Samples. serum, saliva, feces, rectoanal mucosal swabs

RESULTS

- 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 ($P \le 0.001$, t-test).
 - ightharpoonup 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. ($P \le 0.05$, t-test).

2°Vaccine Trial

SYSTEMIC IMMUNIZATION OF COWS WITH EspA, EspB, INTIMIN and Stx2 PROTEINS FROM *Escherichia coli* O157:H7 INDUCES SPECIFIC COLOSTRAL ANTIBODIES THAT ARE EFFICIENTLY TRANSFERRED TO NEWBORN CALVES.

Holstein pregnant cows confirmed to be negative for

EHEC 0157:H7 infection

Groups: Vaccinated and Control

Vaccine

Animals

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

Control: PBS + adjuvant

Containment Facilities

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

Final Conclusions

- ➤ Immunization with recombinants Intimin and EspB proteins seems to be a feasible strategy to reduce EHEC 0157:H7 fecal shedding in cattle.
- ➤ Hyperimmune colostrum from cows immunized with Intimin and EspB proteins is a source of antibodies against EHEC 0157: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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Thank you very much for your attention!