# 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 **E**nterocyte 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

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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*[▽]

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# Antigenicity of Intimin and Esp proteins in mice intranasally immunized

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## Efficient immune responses against Intimin and EspB of enterohaemorragic *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

# 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 ($P \leq 0.001$, *t* -test).

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

- An IgG specific response against both proteins was also observed in saliva 21 days after the first immunization. ($P \leq 0.05$, *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.

**Animals**

Holstein pregnant cows confirmed to be negative for EHEC O157:H7 infection
Groups: Vaccinated and Control

**Vaccine**

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 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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Thank you very much for your attention!