Rectal administration of lactoferrin powder but not lactoferrin gel clears *E. coli* O157:H7 in calves.

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INTRODUCTION

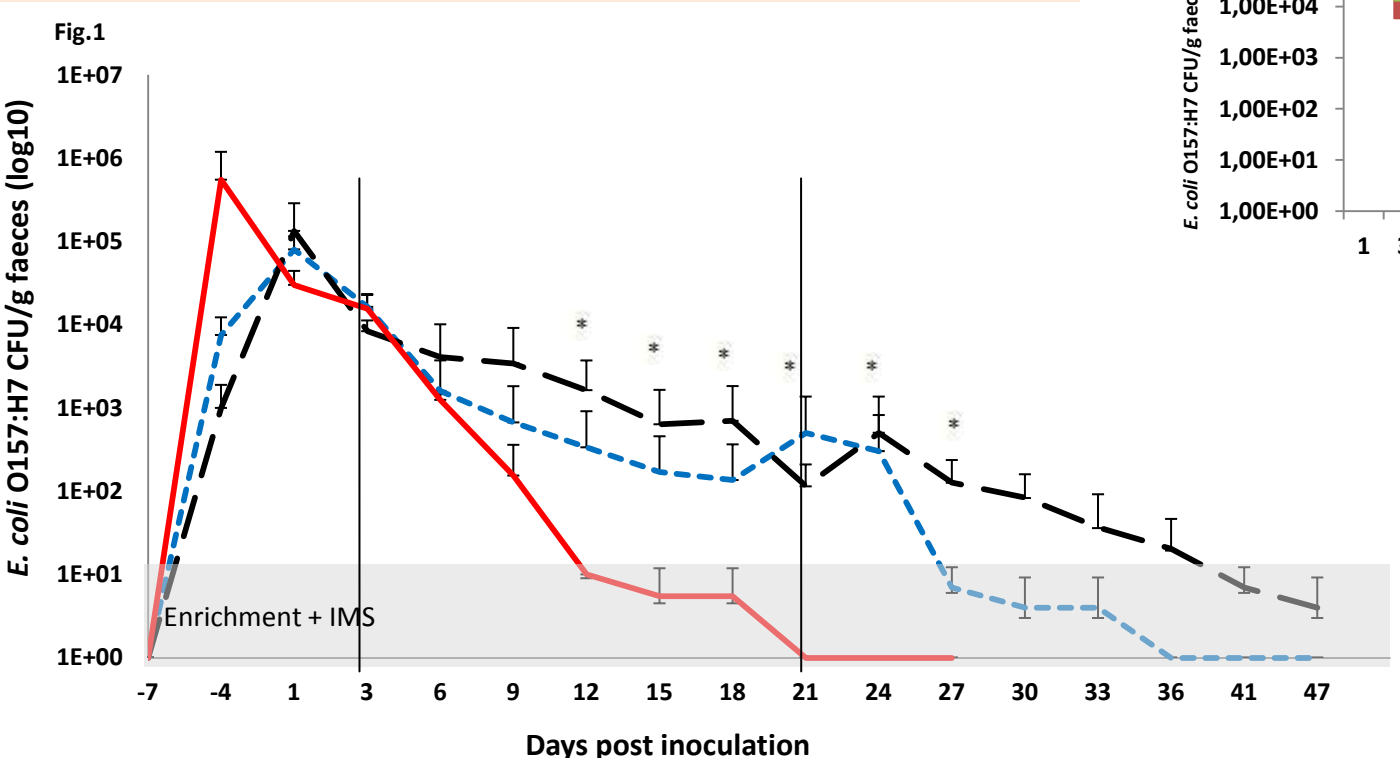
In cattle, the highest numbers of *E. coli* O157:H7 are found in the recto-anal junction, with no bacteria or significantly lower levels detected in luminal contents taken from the gastrointestinal tract. Our previous studies have shown that lactoferrin powder, a natural antimicrobial protein of milk, administered locally in the rectum reduced *E. coli* O157:H7 shedding and cleared the rectal colonization in calves. However, a new formulation form for lactoferrin has to be developed what could simplify the rectal administration of this protein. In this study, the treatment of *E. coli* O157:H7 infected calves using lactoferrin powder was compared with rectal gel, containing 10% of lactoferrin, to evaluate if this new administration form can be as effective in clearance of *E. coli* O157:H7 colonization as it was for the lactoferrin powder.

MATERIALS AND METHODS

Eight calves were orally inoculated and re-inoculated 7 days later with 10^{10} CFU *E. coli* O157:H7 for 2 consecutive days. From day 3 after re-infection, 2 calves were rectally treated with 1,5g lactoferrin powder per day for 21 days (powder treatment group) and 3 calves were rectally treated with 3g of lactoferrin gel per day for 18 days (gel treatment group). Thereafter the treatment was changed to 1,5 g of lactoferrin powder per day for 20 days. Three calves served as colonization controls. Fecal excretion was monitored 2 times a week by direct plating and using enrichment.

RESULTS

The fecal *E. coli* O157:H7 shedding (Fig.1) slightly declined from day 6 till day 18 in the gel treatment and colonization control groups. However, in the powder treatment group a fast and significant decline could be seen. On day 21, all animals in the powder treatment group ceased excreting bacteria. The shedding pattern of the powder treatment group and the colonization control group was significantly different from day 12 until day 27. In the gel treatment group, after 18 days of treatment with the rectal lactoferrin gel, we changed the gel for 1,5g lactoferrin powder per day. Subsequently, fecal shedding decreased in this group also, although there was no significant difference in bacterial excretion between the gel treatment group and the colonization control group. On day 36 (16 days after starting the powder treatment), all animals in the gel treatment group ceased excreting the bacteria and stayed negative till the end of experiment (day 47). Calves in the colonization control group were excreting bacteria in the feces at least 47 days. The statistical analysis to examine the bacterial excretion was performed using a repeated measures 2-way ANOVA with Tukey's multiple comparisons test performed with GraphPad Prism software.



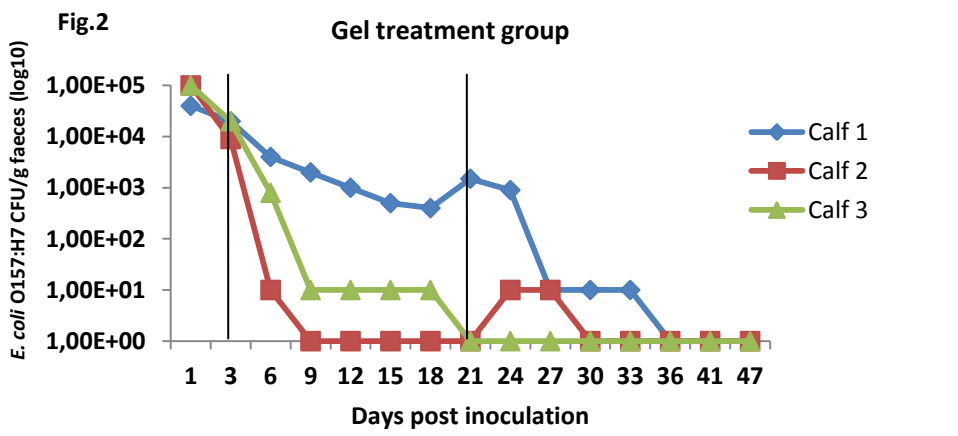


Fig.1 Average of *E. coli* O157:H7 excretion following infection and rectal treatment of calves with lactoferrin powder and lactoferrin gel. The vertical line on day 3 is the start of lactoferrin powder and gel treatment. On the vertical line on day 21 the lactoferrin gel was replaced by lactoferrin powder in the gel treatment group. * Significant differences in bacterial excretion between control and powder treatment groups. **Fig.2** *E. coli* O157:H7 excretion following infection and lactoferrin treatments of individual calves in the gel treatment group.

CONCLUSIONS

This and previous studies performed by our group, demonstrate the reduction of *E. coli* O157:H7 shedding in calves due to rectal administration of lactoferrin powder. For the lactoferrin gel, a new formulation has to be developed that can be more effective in clearance of *E. coli* O157:H7 colonization. That is why further validation of this new strategy has to be performed.