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Reduced faecal shedding of *Escherichia coli* O157:H7 in cattle following systemic vaccination with γ-intimin C_{280} and EspB proteins

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ABSTRACT

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is the most prevalent EHEC serotype that has been recovered from patients with haemolytic uremic syndrome (HUS) worldwide. Vaccination of cattle, the main reservoir of EHEC O157:H7, could be a logical strategy to fight infection in humans. This study evaluated a vaccine based on the carboxyl-terminal fragment of 280 amino acids of γ-intimin (γ-intimin C_{280}) and EspB, two key colonization factors of *E. coli* O157:H7. Intramuscular immunization elicited significantly high levels of serum IgG antibodies against both proteins. Antigen-specific IgA and IgG were also induced in saliva, but only the IgA response was significant. Following experimental challenge with *E. coli* O157:H7, a significant reduction in bacterial shedding was observed in vaccinated calves, compared to control group. These promising results suggest that systemic immunization of cattle with intimin and EspB could be a feasible strategy to reduce EHEC O157:H7 faecal shedding in cattle.

1. Introduction

Enterohaemorragic *Escherichia coli* (EHEC) O157:H7 is a major aetiological agent of diseases in humans, whose clinical spectrum includes diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome (HUS), the leading cause of chronic renal failure in children in Argentina and several other countries [1,2]. This bacterium produces Shiga toxins types 1 and/or 2 [3–5], which are responsible for systemic damage, particularly the vascular endothelium of the kidney and brain, with severe renal and neurological sequelae in children and elder people. Current treatment is largely limited to supportive care, as no specific regimen against an *E. coli* O157:H7 infection exists and the use of antibiotics is not recommended because they can cause the release of Shiga toxins from the bacterium, which can worsen the clinical course [6].

The main reservoir for *E. coli* O157:H7 is cattle, which harbour the bacteria in their intestinal tract [7,8], especially in the lymphoid follicle-dense mucosa at the terminal rectum [9]. The bacteria are usually isolated from healthy animals, although episodes of diarrhoea have been observed in young animals [10,11]. Faecal contamination of meat during slaughter, the use of faeces as fertilizers, and the contamination of drinking water are major ways by which this organism can enter the human food chain [7,12].

Besides producing Shiga toxins, *E. coli* O157:H7 is characterized by other virulence-associated traits, which enable it to colonize the intestinal mucosa of humans and animals with a histopathological lesion known as "attaching and effacing" (A/E) [13]. A large chromosomal pathogenicity island called the locus of enterocyte effacement (LEE) is associated with A/E activity [14,15]. The LEE encodes for a type three secretion system (TTSS) that translocates into the host cell effector proteins responsible for the A/E lesion. The A/E lesion is also characteristic of enteropathogenic *E. coli* (EPEC), another category of *E. coli* strains associated with diarrhoea in children [13]. The TTSS forms EspA, a filamentous structure through which effector proteins are translocated into the host cell [16]. Intimin and its bacterially expressed receptor Tir are translocated by the TTSS in the host cell membrane, leading to the formation of the A/E lesion. EspB is translocated into the host cell and contributes, in turn, to the creation of a pore in the eukaryotic cell membrane [17].

Many virulence factors of *E. coli* O157:H7 induce an immune response during the course of natural or experimental infections in animals and patients with HUS. Oral inoculation of calves and steers with *E. coli* O157:H7 promotes an increase in serum antibody titres against the O157 lipopolysaccharide and neutralizing antibodies

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to Shiga toxins [18]. Recently, Bretschneider et al. [19] demonstrated that cattle respond serologically to intimin and EspB of *E. coli* O157:H7 during the course of experimental infection. Antibodies against these proteins have also been detected in serum during both human EHEC [20] and EPEC infections [21] and in colostrum and milk from healthy women [22–25] and cows [26]. In addition, mice infected with *Citrobacter rodentium*, a bacterium that shows virulence determinants and pathological effects in mice highly similar to those of EPEC in humans, develop an immune response against LEE-encoded proteins, which makes them resistant to bacterial reinfection [27].

Vaccination with bacterial colonization factors has been proposed as a strategy to prevent *E. coli* O157:H7 infection. Various vaccine formulations have been assayed, both in cattle [28–33] and in other animal models [34–40], with variable results. In the present study, we evaluated the efficacy of a systemic vaccine composed of γ-intimin C_{280} and EspB, to reduce *E. coli* O157:H7 colonization in cattle.

2. Materials and methods

2.1. Animals

All animal experiments were performed with the ethical approval of the Instituto Nacional de Tecnología Agropecuaria (INTA) Animal Welfare Committee. Eight 6–8-month-old conventionally reared male Holstein-Friesian calves were obtained from a farm in Buenos Aires province, Argentina, and housed at the INTA Experimental Station. Prior to the first immunization and challenge, calves were confirmed twice to be negative for *E. coli* O157:H7 by enrichment of faecal samples followed by immunomagnetic separation as described below. Three days before oral challenge, the calves were allocated in biosafety level 2 rooms and housed in separated pens according to the immunization group. Calves were fed alfalfa pellets, with free access to hay and water.

2.2. Production of recombinant E. coli O157:H7 proteins

Preparation of His-tagged γ-intimin C_{280} and EspB was performed as described previously [26]. Briefly, constructs for the expression of the gene fragment (843 bp) encoding the 280 carboxyl-terminal amino acids of γ-intimin (γ-intimin C_{280}) and the EspB gene were obtained by PCR amplification from the *E. coli* strain 146N (O157:H7). The amplified DNA fragments were cloned into the His-tag expression vector pRSET-A (Invitrogen Corporation, Carlsbad, USA). The resulting constructs were transformed into chemically competent *E. coli* BL21 (D3)/pLysS as described by the manufacturer. Protein expression was then induced by the addition of 1 mM IPTG. The amino-terminal His-tagged proteins were purified from the lysates by affinity chromatography on nickel-agarose columns (ProBond Nickel-Chelating Resin, Invitrogen Corporation), eluted under denaturing conditions and dialyzed in PBS pH 7.4.

2.3. Immunization protocol and oral bacterial challenge

Four calves received two doses of a vaccine composed of the recombinant proteins EspB (100 μg) and γ-intimin C_{280} (100 μg) diluted in 1 ml of PBS and mixed with 1 ml of mineral oil-based adjuvant (Montanide ISA206; Seppic, France) and calcitriol [1α,25(OH)2D3] (2 μg) by the intramuscular route with an interval of 21 days. A control group of four calves that were vaccinated with PBS plus the adjuvant and calcitriol were included. Fourteen days after the second immunization, calves were orally challenged with ca. 1×10^9 colony forming units (CFU) of *E. coli* O157:H7 strain 438/99 (*stx*$_2$, *eae*-γ) in 15 ml of PBS. The challenge strain was isolated from a healthy cow and selected for spontaneous resistance to nalidixic acid.

The magnitude and duration of faecal excretion of viable *E. coli* O157:H7 were followed every other day until 19 days post-challenge. Two methods were used to increase the probability to detect *E. coli* O157:H7 faecal shedding: (1) bacterial counts were performed by plating serial dilutions of faeces in duplicate onto cefixime-tellurite Sorbitol MacConkey agar (Oxoid, Basingstoke, UK) plates containing 20 μg/ml nalidixic acid (Sigma, St. Louis, USA) (CT-SMAC); (2) faecal shedding of the microorganism was also monitored by enrichment at 37 °C for 18 h of rectoanal junction mucosal swabs in Trypticase soy broth (Oxoid) containing 20 μg/ml nalidixic acid. About 1 ml of this culture was subjected to *E. coli* O157 immunomagnetic separation (IMS) with O157 Dynabeads according to the manufacturer's instructions (Invitrogen Dynal AS, Oslo, Norway) and the bead-bacteria mixture was spread onto CT-SMAC. Non-sorbitol-fermenting colonies were tested for *E. coli* O157 LPS by latex agglutination (Oxoid). The selected latex-positive colonies were confirmed by a multiplex PCR for the *stx*$_1$, *stx*$_2$, *eae* and *rfb*$_{O157}$ genes using the primers described elsewhere [41–43]. Briefly, PCR assays were carried out in a 25 μl volume containing 2.5 μl of nucleic acid template, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$; 0.6 μM concentrations of each primer, 0.2 mM concentrations of each deoxynucleoside triphosphate, and 2 U of Taq DNA polymerase (Invitrogen Corp.). Temperature conditions consisted of an initial 94 °C denaturation step for 2 min followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min. Amplified DNA fragments were resolved by gel electrophoresis using 1% (w/v) agarose. Gels were stained with ethidium bromide and visualized with UV illumination.

In order to monitor antibody responses, serum, saliva and faecal samples were collected before each vaccination, before the oral bacterial challenge, and 19 days post-challenge, when the calves were euthanized. Samples of the ileum, cecum, colon, and rectoanal junction were examined for *E. coli* O157:H7 by direct plating and IMS as described above. Similar intestinal segments were collected for histopathological examination.

2.4. Antibody response

Immediately after their collection, serum samples were stored at −20 °C. Faecal samples were suspended 1:2 (w/v) in sodium acetate buffer (pH 4.5, 10 mM) containing 0.1% (w/v) Protease Inhibitor Cocktail (Sigma–Aldrich Co., Saint Louis, USA), the mixture was centrifuged once at 2500 × *g* for 20 min to sediment larger particles and once at 15,000 × *g* for 30 min; the supernatant was collected and stored at −20 °C until its analysis [19]. Saliva samples were stored at −20 °C until their analysis, when they were centrifuged at 1000 × *g* for 10 min. Serum samples were analyzed for the presence of IgG and IgA antibody responses against γ-intimin C_{280} and EspB by an enzyme-linked immunosorbent assay (ELISA) described previously [40]. Briefly, 96-well Nunc-Immuno MaxiSorp assay plates (Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 100 μl of either γ-Intimin C_{280} or EspB at 10 μg/ml dissolved in PBS pH 7.4. After washing with PBS pH 7.4 containing 0.05% Tween 20 (PBS-T), non-specific binding sites were blocked with PBS-T containing 3% skim milk for 1 h. Serial two-fold dilutions of sera in PBS-T were added (100 μl/well), and plates were incubated for 2 h. For each plate, two wells were incubated with PBS-T alone (negative control), and a known positive sample was included. Each sample was analyzed in duplicate. After washing in PBS-T, wells were incubated for another hour with 100 μl of sheep anti-bovine IgG or IgA conjugated with horseradish peroxidase (Bethyl Laboratories, Montgomery, USA), at dilutions of 1:8000 for IgG and 1:3000 for IgA, in PBS-T. Plates were washed four times with PBS-T. Then, ABTS

[2,2′-azino-di (3-ethyl-benzthiazoline sulphonic acid)] (Amresco, Solon, USA) substrate in citrate-phosphate buffer pH 4.2 plus 0.01% H_2O_2 (100 μl/well) was added. Reactions were stopped after 10 min with 100 μl/well of 5% SDS and read at 405 nm (OD_{405}) in a BioTek ELx808 microplate reader (BioTek Instruments, Winooski, USA). The antibody titre was expressed as the reciprocal of the end-point dilution resulting in an OD_{405} above the cut-off value. The cut-off value was calculated as the average plus two times the standard deviation of the optical densities of the samples measured on day 0.

The amount of EspB- or γ-intimin C_{280}-specific IgA or IgG present in saliva and faeces was determined by a capture ELISA as previously described [40]. To compensate for variations in the efficiency of recovery of secretory antibodies between animals, the results were expressed as mean OD_{405} of undiluted samples normalized to 1 μg of total IgA or IgG present in the sample, respectively. For this, plates coated with sheep anti-bovine IgA or IgG (Bethyl Laboratories) as capture antibody were further incubated with serial dilutions of the samples or a bovine reference serum (Bethyl Laboratories). As secondary antibody, HRP-conjugated sheep anti-bovine IgA or IgG was used, respectively. Plates were developed as described above.

In addition, pools of serum and saliva samples of both vaccinated and control groups were examined by Western blot to confirm the specificity of the antibody response measured by ELISA. One dimension SDS-PAGE was carried out in a 12.0% polyacrylamide gel under reducing conditions [44], loading 2.5 μg γ-intimin C_{280} or EspB proteins per lane, respectively. Proteins were electrophoretically transferred from the gel onto 0.45 μm nitrocellulose sheets (Amersham-Pharmacia, Germany) for immunoblotting as described by Towbin et al. [45]. Nitrocellulose strips were blocked with 5% nonfat dry milk in PBS pH 7.4, for 2 h under agitation, washed three times with PBS-T, and incubated for 2 h with dilutions of serum (1:4000) or saliva (1:4) from immunized and control groups, respectively. After three washes, the membranes were incubated for 2 h with HRP-conjugated rabbit anti-bovine IgG or IgA (Bio-yeda, Rehovot, Israel) diluted 1:1000 in PBS-T. The blots were revealed with 4-Cl-1-naphthol (Pierce, Rockford, USA). Western blotting was performed on pre-immunization and post-immunization samples, from each immunization group to confirm the specificity of the antibody response.

2.5. Histological studies

Tissues were fixed in neutral buffered 10% formalin for 24–48 h, embedded in paraffin, sectioned, and stained with haematoxylin and eosin for routine histology.

2.6. Statistical analyses

Faecal shedding counting data were $\log_{10}$ transformed and analyzed as a repeated measures design [46] using Proc Mixed of SAS (SAS v.9.1; SAS Institute Inc., Cary NC, USA). Fixed effects were treatment, and linear, quadratic, and cubic terms for time of measure interacting with treatment. Animals were considered independent random variables. The covariance structure for the repeated measures within animals that gave the best fit displayed heterogeneity of variance across times. The values of each treatment and the difference between treatments at any given day were calculated as estimable linear contrasts [47]. A similar procedure was used to test the difference between vaccinated and control animals for the logarithm of shedding during the complete experiment, as originally proposed by Bono et al. [48]. The procedure of Kenward and Roger [49] was used to correct the degrees of freedom of all linear hypotheses. ELISA data within each immunization group and between groups were performed with the Student's *t*-test. In all cases *P* values of <0.05% were considered significant.

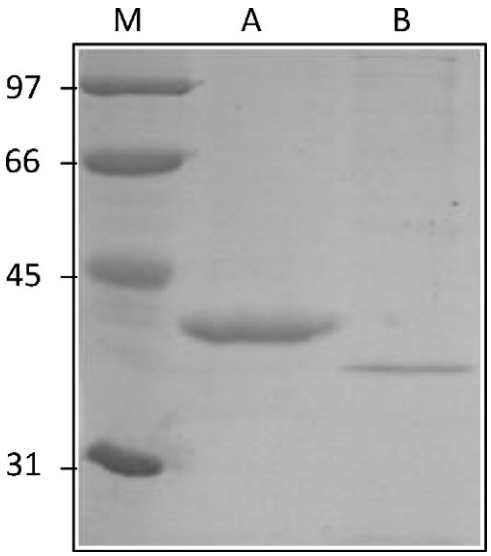

Fig. 1. Coomassie blue-stained polyacrylamide gel of purified γ-intimin C_{280} (A) and EspB (B) antigens used in immunizations.

3. Results

3.1. Purification of recombinant proteins

EspB and γ-intimin C_{280} proteins were analyzed by SDS-PAGE and Coomassie blue staining, and single bands of the predicted molecular weight were observed (Fig. 1).

3.2. Immune response elicited after intramuscular immunization with EspB and γ-intimin C_{280}

The systemic immunization of 6–8-month-old calves with two doses of EspB and γ-intimin C_{280} antigens formulated with ISA206-D3 induced a strong and significant increase in serum IgG titres against both proteins after the first immunization ($P < 0.05$) but not IgA antibodies (data not shown). IgG-antibody titres showed a 13-fold increase on day 21 and remained at the same level throughout the experiment (Fig. 2). A small but not significant increase in EspB and γ-intimin C_{280}-specific IgG titres was observed in the control group after *E. coli* O157:H7 oral administration.

Mucosal anti-EspB and anti-γ-intimin C_{280} IgA and IgG antibodies were quantified in saliva and faecal samples. Specific salivary IgA antibodies against both proteins showed a significant 4–7-fold increase after the first immunization but were undetectable on day 19 post-challenge ($P < 0.05$) (Fig. 3). Salivary anti-EspB and anti -γ-intimin C_{280} IgG antibodies were also detected but the increase after vaccination was not significant (Fig. 4). The bacterial challenge did not result in any detectable increase in salivary antigen-specific IgG or IgA antibody responses. The specificity of the immune response was confirmed by western blot analysis of pools of antisera and saliva from vaccinated and control groups (Fig. 5). Faecal IgG or IgA response to both antigens was not detected (data not shown).

3.3. Effect of systemic immunization on E. coli O157:H7 shedding

Two weeks after the second immunization, animals were orally challenged with 10^9 CFU of *E. coli* O157:H7. Bacterial shedding post-challenge was calculated quantitatively by direct plating of serial

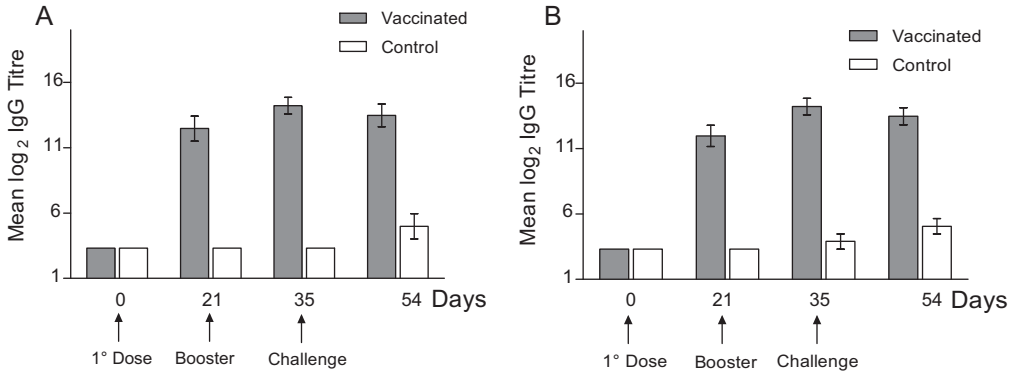

Fig. 2. Serum IgG responses in calves vaccinated with γ-intimin C_{280} and EspB measured by ELISA. (A) Antibody titres against γ-intimin C_{280}. (B) Antibody titres against EspB. Results are presented as mean $\log_2$ of IgG titres and SEM is indicated by vertical lines. A significant increase in serum IgG against the two antigens was observed at the moment of the second immunization ($P < 0.05$).

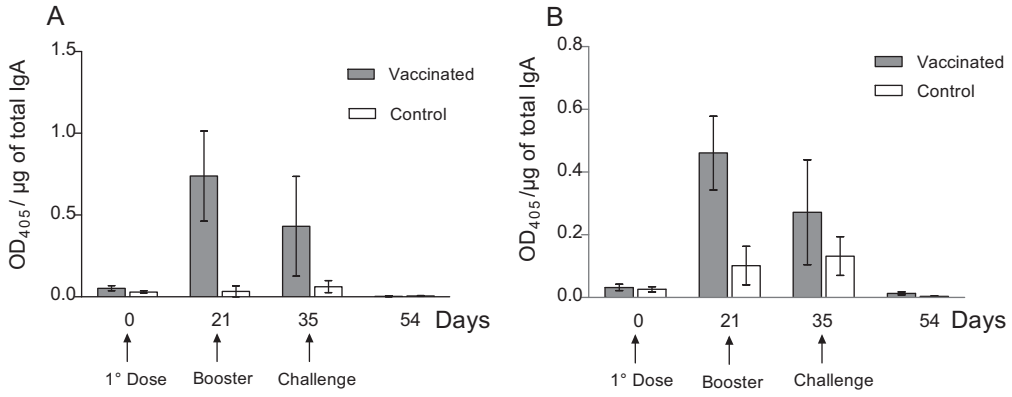

Fig. 3. Saliva IgA response in calves vaccinated with γ-intimin C_{280} and EspB measured by ELISA. (A) Antibody titres against γ-intimin C_{280}. (B) Antibody titres against EspB. Results are presented as mean OD_{405} of undiluted samples normalized to 1 µg of total IgA. SEM is indicated by vertical lines. A significant increase in IgA antibodies against the two antigens was observed at the moment of the second immunization ($P < 0.05$).

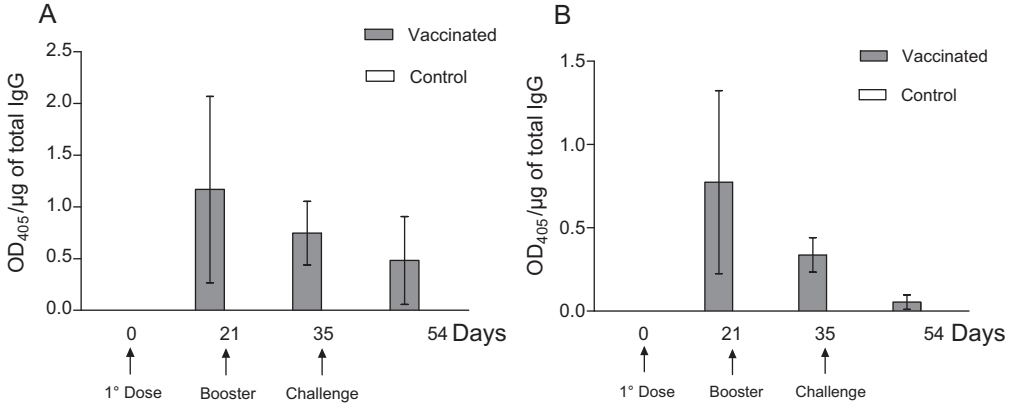

Fig. 4. Saliva IgG response in calves vaccinated with γ-intimin C_{280} and EspB measured by ELISA. (A) Antibody titres against γ-intimin C_{280}. (B) Antibody titres against EspB. Results are presented as mean OD_{405} of undiluted samples normalized to 1 µg of total IgG. SEM is indicated by vertical lines. The response to γ-intimin C_{280} and EspB increased on day 21 in vaccinated animals as compared with control calves.

dilutions of faeces as well as by broth enrichment of rectoanal mucosal swabs followed by IMS during the 19-day post-challenge period. Specimens containing less than the detection limit (*E. coli* O157:H7 found only by enrichment) were assigned a value of 10. Negative specimens by both methods were assigned a value of 1. No calf had diarrhoea after inoculation. Calves of the placebo group were successfully colonized by *E. coli* O157:H7. This is supported by the observation that bacterial shedding was detectable at least until day 11 following oral inoculation in all the animals. Fig. 6B shows the trend in the estimates of the level of $\log_{10}$ (CFU/g) for control and vaccinated animals on any day. The animals of the control group displayed a larger value of bacterial shedding. The differences between the animals in the control and vaccinated groups were significant ($P < 0.05$) up to day 13. Later, the difference became smaller and not significant. Total bacterial shedding, expressed as the area under the curve, was estimated to be 22.2 for the vaccinated animals and 35.7 for the control group, showing a significant difference ($P < 0.05$). At necropsy, *E. coli* O157:H7 was not recovered from any intestinal sample from animals of both groups. The individual values of bacterial excretion can be seen in Fig. 6A. In

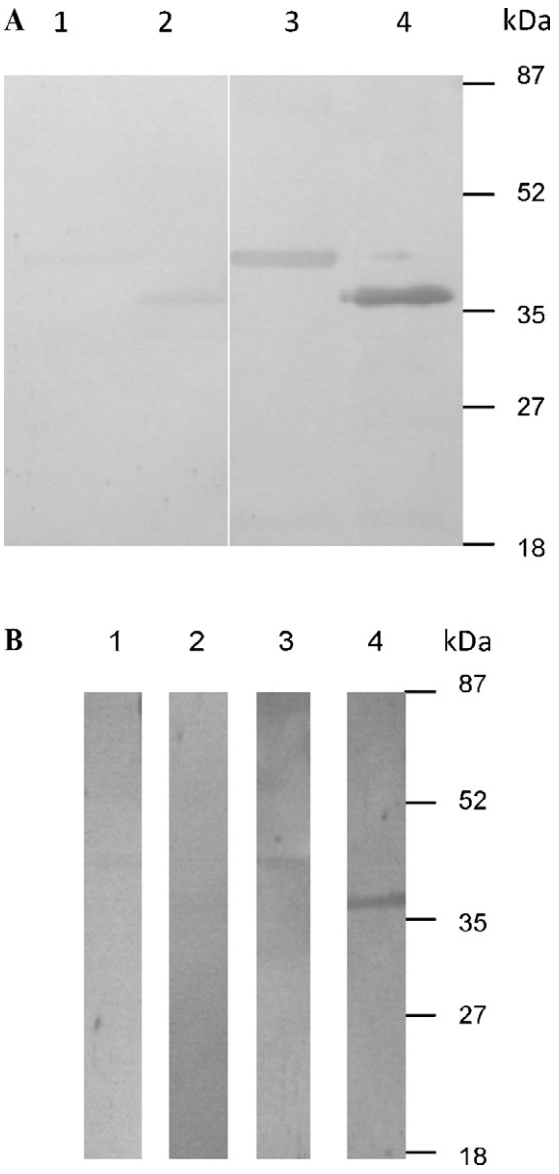

Fig. 5. Western blotting of EspB and γ-intimin C_{280} with pools of serum (A) or saliva (B) samples, demonstrating the specificity of the immune response measured by ELISA. (A) Serum IgG; (B) saliva IgA. Lanes 1 and 2, pre-immunization samples reacting with EspB and γ-intimin C_{280}, respectively; lanes 3 and 4, post-immunization samples reacting with EspB and γ-intimin C_{280}, respectively. Serum and saliva samples were diluted 1:4000 and 1:4, respectively.

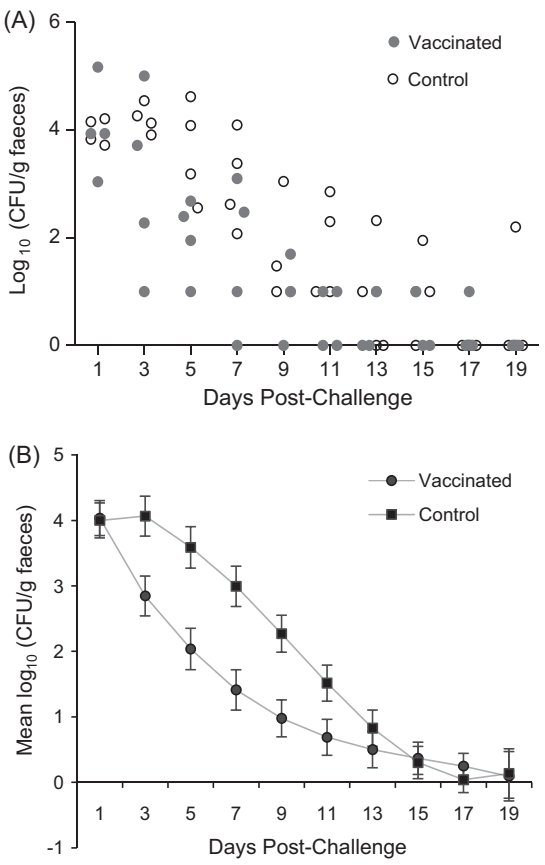

Fig. 6. Faecal shedding of *E. coli* O157:H7 of calves immunized with EspB and γ-intimin C_{280}. (A) Dot plot of $\log_{10}$ CFU/g data for each calf over the entire trial period. In most animals of both groups, no bacteria were detected after day 17 post-challenge. (B) The modeled faecal excretion of *E. coli* O157:H7 shows the trend in the estimates of the level of $\log_{10}$ (CFU/g) ± SEM for control and vaccinated animals at any day of the experiment. The animals of the control group displayed a larger value of bacterial shedding. The differences between the animals in the control and vaccinated groups were significant ($P < 0.05$) up to day 13. Later, the difference became smaller and not significant. One animal in the control group had a random pattern of measures, thus increasing the heterogeneity of the variance. This also explains why curves cross each other on day 15. Total bacterial shedding, expressed as the area under the curve, was estimated to be 22.2 for the vaccinated animals and 35.7 for the control group, showing a significant difference ($P < 0.05$).

most animals of both groups, no bacteria were detected after day 17 post-challenge.

3.4. Histopathology

No histological changes consistent with attaching and effacing lesions or adherent bacterial layers were seen in intestinal sections of either group of animals at necropsy on day 19 post-challenge.

4. Discussion

Many efforts have been directed to the development of vaccines against enteric pathogens that are able to induce strong mucosal immune responses capable of preventing intestinal colonization. In this sense, immunization of cattle to reduce *E. coli* O157:H7 shedding should be aimed to block the adhesion and colonization process, thereby reducing the risk of microbial transmission to other susceptible hosts. As reported, intimin is a major antigen of bacteria producing attaching and effacing lesions and a key virulence factor that allows strong binding of *E. coli* O157:H7 to the rectal mucosa of calves [53–55]. Thus, intimin and other LEE encoded type III secreted proteins, including Tir, have been tested as vaccine antigens in cattle, with variable results [31–33]. With respect to EspB, although its central role in *E. coli* O157:H7 colonization of the intestinal epithelium has been demonstrated [56,57], this protein has not yet been evaluated as antigen in *E. coli* O157:H7 vaccines for cattle.

In this study, we demonstrated that two doses of a systemic vaccine containing the carboxyl-terminal domain of γ intimin and EspB reduces bacterial colonization and shedding following challenge with *E. coli* O157:H7 in cattle. The immunization induced high serum IgG antibody titres and a mucosal response consisting of both IgA and IgG antibodies against the two antigens. These results are consistent with previous observations that antibodies against intimin are capable of blocking *E. coli* O157:H7 adherence to cultured cells [58,59]. In other studies, systemic vaccines that elicit seric and mucosal IgG antibodies against TTSS antigens have demonstrated the ability to protect streptomycin-treated mice or cattle against *E. coli* O157:H7 colonization [31,34]. The possible explanation for the reduced bacterial shedding observed in the

current study could then be the presence of IgG and IgA antibodies in the intestinal mucosa. Although copro-antibodies were undetectable despite the appropriate processing of faecal samples, we hypothesize that the detection of anti-EspB and anti-γ-intimin C_{280} IgA and IgG in saliva would be related to the presence of specific antibodies also in intestinal mucosa. Vaccination studies where samples were taken by rectal swabbing for antibody determination have shown that systemic immunization of cattle produced similar antibody responses in different mucosal secretions [39].

The published efficacy of systemic vaccines to elicit a mucosal IgA response is variable [31–34]. Although the IgA response after intramuscular immunization is induced mainly in systemic lymphoid tissues, an increased homing of antigen-specific IgA-secreting cells (ASC) to the gut-associated lymphoid tissue has been observed when the antigen is supplemented with 1α,25(OH)2D3 [60]. It has been shown that this steroid hormone can enhance the antigen-specific IgA response in serum as well as in mucosal secretions after intramuscular immunization. This molecule is the active metabolite of vitamin D, a lipophilic steroid hormone classified as a Th2-immunomodulating adjuvant [61]. In our study, the addition of 1α,25(OH)2D3 to the vaccine formulation could shift the immune response to EspB and γ-intimin C_{280}, favouring an antibody response and stimulating mucosal immunity. We also speculate that calves could be mucosally primed to both proteins. It has been shown that prior to mucosal priming it is required to generate an intestinal antigen-specific IgA response to systemic immunization [62,63]. In a previous study, we found that 77% of colostrum samples from cows from farms in Buenos Aires contained IgG antibodies against EspB and γ-intimin C_{280} [26]. These data indicate a high exposure of cattle to either *E. coli* O157:H7 or other *E. coli* strains encoding highly homologous EspB and γ-intimin proteins.

We observed the highest circulating antibody response at challenge. However, although the seric IgG antibodies remained high through the whole experiment, mucosal antibodies apparently decreased after the second immunization, until it became almost undetectable on day 54. The time between the second immunization and the next sampling may have been too long to detect a possible increase in mucosal antibodies.

The time-course of the excretion showed an initial increase followed by a sharp drop in bacterial shedding. A similar pattern of excretion has been observed in older calves or adult cattle [50–52] but not in very young (1–2 months) calves, which shed *E. coli* O157:H7 for longer periods after experimental inoculation [50]. The absence of histopathological lesions in the intestinal samples was expected if it is considered that the inoculum bacteria were recovered on day 19 post-challenge only from one animal of the control group, indicating low intestinal levels of *E. coli* O157:H7, probably insufficient to observe A/E lesions.

5. Conclusions

Cattle vaccination would be a feasible pre-slaughter intervention key to reduce *E. coli* O157:H7 faecal shedding and a good strategy to lower the risk of contamination for humans. Our results in a cattle infection model suggest that systemic immunization with two doses of a vaccine containing recombinant intimin and EspB could reduce *E. coli* O157:H7 colonization and shedding in cattle. Further field trials are needed to evaluate the efficacy of the vaccine to reduce bovine *E. coli* O157:H7 carriage under natural conditions.

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