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Short communication

Efficacy of vaccination in preventing giardiasis in calves

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Abstract

The objective of this study was to evaluate the efficacy of a vaccine in the prevention of Giardia duodenalis infection in calves. Six 2-week old calves were vaccinated subcutaneously with a sonicated G. duodenalis trophozoite vaccine. Six 2-week old control calves received a subcutaneous injection of sterile phosphate-buffered-saline mixed with adjuvant. Injections were repeated after 28 days. Eleven days after the second injection, calves were challenged orally with 1 × 10⁵ purified G. duodenalis cysts from a naturally infected calf. Throughout the study, fecal samples were collected at regular intervals and examined for the presence of G. duodenalis. Blood samples were collected weekly until G. duodenalis challenge and bi-weekly following challenge. Calves were euthanized 14 days after challenge and G. duodenalis trophozoites within the small intestines were enumerated. Serum antibody titers were significantly higher in vaccinated compared to non-vaccinated calves. Vaccinated calves tended to excrete more G. duodenalis cysts in their feces than non-vaccinated calves. The number of trophozoites in the small intestine was not different between vaccinated and non-vaccinated calves. Changes consistent of moderate enteritis were found in the intestines of one vaccinated and one non-vaccinated calf. Despite a serological immune response following vaccination, this vaccine was not efficacious in preventing giardiasis or reducing cyst shedding in calves.

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Keywords: Giardia duodenalis; Vaccination; Immune response; Calf

1. Introduction

Giardia duodenalis (syn: G. lamblia) infections are highly prevalent in domestic ruminants throughout the world (reviewed by O’Handley and Olson, 2006). Typically, infections occur in ruminants around one month of age and are chronic or reoccurring, with cyst excretion continuing for months after initial infection.
As with other hosts, the clinical signs associated with *G. duodenalis* infections in ruminants can vary. Subclinical infections are often reported; however, *G. duodenalis* infections are also associated with the occurrence of diarrhea and ill thrift in calves (O’Handley et al., 1999; Geurden et al., 2006). More importantly, previous research has shown that *G. duodenalis* infections can result in significant production losses in lambs, thus raising concern that giardiasis may be a production limiting disease in livestock (Olson et al., 1995; O’Handley et al., 2000a; Aloisio et al., 2006). Additionally, although ruminants are most commonly infected by the livestock specific *G. duodenalis* of genetic assemblage E, they can also be infected with *G. duodenalis* of the zoonotic genetic assemblage A. Thus, domestic livestock have the potential to serve as a reservoir for human giardiasis (O’Handley et al., 2000b; Trout et al., 2004; Uehlinger et al., 2006).

Due to the high prevalence, potential production impact, and zoonotic potential of *G. duodenalis* infections in domestic ruminants, recent research has examined the potential for controlling *G. duodenalis* infections in ruminants through chemotherapy. Paromomycin and the benzimidazole drugs, such as fenbendazole, show efficacy against giardiasis in dairy calves (Xiao et al., 1996; O’Handley et al., 1997; Geurden et al., 2006). However, due to the high level of *G. duodenalis* cysts in their environment, calves are readily reinfected immediately following treatment (O’Handley et al., 2000a). Continuous administration of drugs, such as fenbendazole or paromomycin may allow for long-term control of giardiasis in ruminants. However, this type of treatment regimen is likely not useful or economically feasible for livestock producers. A vaccine to prevent and control *G. duodenalis* in domestic ruminants could be a practical and cost-effective approach. Currently, a sonicated whole *G. duodenalis* vaccine, made with trophozoites isolated from sheep, is available for dogs and cats in North America (Olson et al., 2000). Clinical trials demonstrated that sub-cutaneous administration of this vaccine in young, growing dogs and cats results in the reduction of *G. duodenalis* cyst in the feces, elimination or reduction of *G. duodenalis* trophozoites in the intestines, prevention of clinical disease, and significantly higher weight gains compared to non-vaccinated animals (Olson et al., 1996, 1997). Provided this vaccine had similar efficacy in domestic ruminants, it could reduce or eliminate the role of domestic ruminants as reservoirs for *G. duodenalis* infections in humans and may provide an economic benefit to livestock producers by preventing production losses associated with giardiasis. Therefore, this study evaluates the efficacy of a *G. duodenalis* vaccine in preventing giardiasis in calves.

### 2. Materials and methods

#### 2.1. Calves and husbandry

Twelve Holstein–Friesian bull calves, <14 days old, were purchased from eight local dairy farmers. All calves were obtained and transported to the Atlantic Veterinary College (AVC) on day −10 of study, following a satisfactory physical examination. Calves were further evaluated for failure of passive transfer (FPT) of immunity by determining their serum total protein concentrations and were included in the study if their serum protein concentration was ≥5 g/dL (Calloway et al., 2002). Only bovine virus diarrhea virus-negative calves, determined by Buffy coat virus isolation, were included in the study.

Upon arrival at the AVC, calves were housed individually in the isolation unit of the veterinary hospital. The pens consisted of concrete floors and solid walls, which prevented contact between animals. Manure and wet bedding were removed daily and replaced with fresh bedding (wood shavings). Calves were fed a commercial milk replacer twice daily and a feed concentrate according to manufacturers’ instructions. The calves received approximately 0.25 kg loose hay every day and had *ad libitum* access to fresh water (filtered, flocculated, and chlorinated municipal water).

To eliminate any existing *Giardia duodenalis* infections prior to vaccination, and to ensure that calves remained free of *G. duodenalis* until vaccination day, calves were treated with 7.5 mg/kg of fenbendazole per Os (SafeGuard® 10% Suspension, CDMV, St. Hyacinthe, Canada) daily, starting on day −10 until the day of vaccination (day 0).

Medical attention was required during the study for calves with respiratory tract problems and diarrhea. Respiratory problems were treated with florfenicol and, in case of high fever, with ketoprofen, a non-steroidal anti-inflammatory drug. Supportive care was given to diarrheic calves if needed in the form of oral electrolytes. The research project was carried out under the guidelines of the University of Prince Edward Island Animal Care Committee, which bases approval of animal research on the guidelines of the Canadian Council of Animal Care (CCAC). Three days after vaccination, one diarrheic calf died. The results of this calf were excluded from the study, reducing the number of vaccinated calves to five.
2.2. Group allocation, vaccination, and challenge procedure

Calves confirmed negative for *Giardia duodenalis* (absence of *G. duodenalis* cysts in fecal samples taken on 4 consecutive days) (day −7), were randomly allocated into two groups of six, and moved to the AVC Research Barn, where each group was housed in separate but identical rooms. Prior to moving, calves were thoroughly washed with water and a commercially available liquid soap to prevent the mechanical introduction of *G. duodenalis* cysts into the rooms. The two rooms were sanitized with a quaternary ammonium disinfectant (Quatricide® PV-15, Pharmacal Research Laboratories Inc., Naugatuck, USA) and allowed to dry completely prior to the start of the study. Each calf was housed in an individual pen that allowed no contact with the neighboring animal. Each calf was assigned a water and a feed bucket, which were cleaned and disinfected regularly. The feeding and husbandry protocols remained unchanged. Calves were allowed to acclimatize to their new housing for 7 days (days −7 to 0). Strict isolation procedures were followed to make certain *G. duodenalis* cysts were not introduced into the calves’ environment. This included the use of clean coveralls, sanitized footwear, and glove changing between animals.

Following the 7-day acclimation period, one group of calves was injected with a sonicated whole *G. duodenalis* trophozoite vaccine (GiardiaVax™, Wyeth Animal Health, Guelph, Canada) (day 0). One dose (1 mL, according to manufacturer’s instructions for use in dogs) was subcutaneously injected into the neck using a 16-gauge needle. Calves in the other group received a subcutaneous injection (1 mL) of sterile phosphate-buffered-saline (PBS) mixed with adjuvant (Adju-Phos® 80, aluminum-phosphate, Accurate Chemical and Scientific Corp., Westbury, USA). Allocation of vaccination and saline injection to the two groups was made randomly and was blinded to the researchers and caregivers. Twenty-eight days after the initial injection (day 28), the vaccination and saline injections were repeated.

Eleven days after the second injection (day 39), calves in both groups were challenged with an oral inoculation of approximately $1 \times 10^5$ *G. duodenalis* cysts. The day before *G. duodenalis* challenge, feces were collected directly from the rectum of a calf known to be naturally infected with *G. duodenalis*. Cysts from that calf were isolated and enumerated by epifluorescence microscopy using the method of O’Handley et al. (1999). Polymerase chain reaction (PCR) and sequencing of the 16S-rRNA was carried out for the inoculum according to the method of Appelbee et al. (2003), and the cysts corresponded to *G. duodenalis* of assemblage E. Doses comprising $1 \times 10^5$ cysts were mixed in 5 mL of phosphate-buffered-saline. Disposable 5 mL syringes were used to orally inoculate each calf individually.

2.3. Fecal sample collection and procession

To monitor that calves were free of *Giardia duodenalis* prior to vaccination, fecal samples were collected from the rectum of each calf daily and examined for *G. duodenalis* cysts until the day of vaccination (day 0), starting on day −10 (start of study). After vaccination, fecal samples were collected every 3 days to screen for potential *G. duodenalis* infections. To compare *G. duodenalis* cyst excretion levels more accurately between groups following *G. duodenalis* cyst challenge, fecal samples were collected daily from each calf, starting on the day of challenge (day 39) until the study was terminated (day 53). Fecal consistency was monitored for every sample and was scored as either normal (feces holds its form) or abnormal (feces takes shape of the collection container) (O’Handley et al., 1999). *Giardia duodenalis* cysts were isolated from the feces and enumerated by epifluorescence microscopy according to the method of O’Handley et al. (1999).

2.4. Intestinal trophozoite count

Calves were euthanized on day 53 with an intravenous injection of pentobarbital. The method of O’Handley et al. (2001) was used to enumerate *G. duodenalis* trophozoites from the intestines of each calf. In addition, samples of duodenum, proximal jejunum, distal jejunum, and ileum (each 5 cm in length) were immediately placed in 10% buffered formalin. Following 48 h of fixation, tissues were trimmed and processed for histological examination. All tissues were stained with haematoxylin and eosin stain for initial evaluation. Additional slides were examined using a Giemsa preparation. All sections of intestines were examined under a light microscope. Sections were evaluated subjectively for specific increases in lymphocyte, plasma cell and eosinophil populations. All sections were judged either normal or abnormal based on cellular infiltrates; degrees of enteritis were graded as minimal, mild, moderate or severe. Sections which contained protozoal structures were noted.

2.5. Humoral immune response

Blood samples were collected from each calf by venipuncture once a week, beginning on the day of arrival at
the AVC (day −10) until the day of challenge with *G. duodenalis* cysts (day 39). After challenge, venous blood samples were collected twice-weekly until the day of euthanasia (day 53). *G. duodenalis*-specific antibodies in the serum were determined for each calf using a *G. duodenalis*–ELISA, designed previously for use with bovine serum diluted at 1:100 (O’Handley et al., 2003).

### 2.6. Data analyses

Differences in antibody titers (OD values) between vaccinated and non-vaccinated calves were assessed using repeated measure ANOVA. Only titers measured after vaccination or PBS-injection, respectively, were included in that analysis. To approximate the normal distribution, trophozoite and cyst counts were naturally log-transformed. The geometric mean was calculated for the trophozoite counts in each intestinal segment. To analyze geometric means and to perform natural log-transformation, the value 1 was added to the intestinal trophozoite and the fecal cyst counts. Mean numbers of cysts shed in vaccinated and non-vaccinated calves were compared using the non-parametric Wilcoxon test. For this test, only the fecal cyst counts from days 7 to 14 after challenge were included. The same test was also applied to compare trophozoite counts between the vaccinated and non-vaccinated calves in each intestinal segment. Analyses were performed using STATA™ 9.2 (StataCorp, College Station, Texas, USA). Values of *P* < 0.05 were considered statistically significant.

### 3. Results

#### 3.1. Humoral immune response

At the start of the study, mean serum titer OD values were 1.24 and 1.37 for the vaccinated and non-vaccinated group, respectively (Fig. 1). Serum antibody titers decreased during the first 2 weeks of the study and were lowest at 0.83 and 0.85 in the vaccinated and non-vaccinated group, respectively, 4 days after vaccination or PBS-injection. After vaccination, antibody titers increased in the vaccinated calves and were significantly higher than in the non-vaccinated calves (Fig. 1; *P* = 0.006). Antibody titer in the vaccinated group increased over time, while they remained relatively stable in the non-vaccinated group (*P* < 0.001).

#### 3.2. *Giardia* cyst excretion after challenge

No *Giardia duodenalis* cysts were observed in any of the calves before oral challenge. Cyst excretion increased 7 days after challenge, and 10 days after challenge all calves from both groups were shedding *G. duodenalis* cysts. Mean numbers of cysts shed in the feces tended to be higher in the vaccinated calves compared to the non-vaccinated calves (Fig. 2; *P* = 0.07). Mean numbers of cysts shed in vaccinated and non-vaccinated calves between days seven to fourteen after challenge were 7.8 cysts/g of feces (95% confidence interval: 6.1–9.6) and 5.6 cysts/g of feces (95% confidence interval: 3.4–7.8), respectively.

#### 3.3. Trophozoite count

Geometric mean *G. duodenalis* trophozoite count per centimeter was higher in all sections of the small intestines in vaccinated compared to non-vaccinated calves (Table 1). The Wilcoxon test was, however, not

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**Fig. 1.** Mean (±S.E.M.) *G. duodenalis*-specific serum antibody titers (optical density) in vaccinated and non-vaccinated calves. Days 0, 28 and 39 (bold print) are the days of vaccination, booster injection and challenge, respectively.

**Fig. 2.** Mean count of *G. duodenalis* cysts (natural log) per gram of feces in vaccinated and non-vaccinated calves.
neonatal diarrhea, dehydration, and cardiovascular
findings observed were consistent with an acute
vaccination. The clinical signs, gross and microscopic
mortem examination of the calf that died after
diagnosis. Further diagnostic tests to pursue other causes of
parvum oocysts were detected in the feces of this calf.

Before challenge had a short bout of diarrhea on the
calf that experienced diarrhea before challenge with
G. duodenalis cysts before challenge and vaccinated calves had a tendency to shed
more cysts than non-vaccinated calves. There were no
severe clinical signs observed due to giardiasis.

In dogs and cats, subcutaneous administration of a G. duodenalis vaccine resulted in a reduced number of
cysts shed in the feces, a lower number of trophozoites in the intestines, an increased feed intake as well as
weight gain and a shorter time during which abnormal
feces were passed (Olson et al., 1996, 1997). A study
has also been conducted on the use of a G. duodenalis vaccine as a therapeutic agent in dogs and cats. Olson
et al. (2001) found that dogs which suffered from
chronic giardiasis and which had received treatments
with a G. duodenalis vaccine ceased to shed fecal cysts, and clinical signs disappeared. However, numerous
other studies have failed to demonstrate a significant
effect (Payne et al., 2002; Stein et al., 2003; Anderson
et al., 2004). Reminiscent of the results presented in this
paper, Anderson et al. (2004) reported that more dogs in
the vaccinated group shed G. duodenalis compared to
the non-vaccinated group, although fewer vaccinated
dogs were positive for G. duodenalis at the end of that
study. To our knowledge, use of a vaccine against
giardiasis in calves has not been reported previously.

We can only speculate as to why this vaccine failed to
prevent G. duodenalis infection in calves. The vaccine
failure in the calves may have been due to the large
inoculation dose used for challenge or due to continuous
exposure to cysts within the environment following
challenge, despite regular cleaning and disinfection.
However, higher challenge doses (10^6 G. duodenalis
cysts) were used in other vaccination studies where the
vaccine was efficacious (Olson et al., 1996, 1997).
Antibody titers in dogs and cats increased rapidly within
3 weeks of vaccination (Olson et al., 1996, 1997). Titers

| Small intestinal section | Vaccinated | Non-vaccinated |
|-------------------------|------------|---------------|
| Duodenum                | 54.93 (4.01, 2.45) | 4.97 (1.60, 1.60) |
| Proximal jejunum        | 120.43 (4.79, 2.95) | 21.55 (3.07, 1.94) |
| Distal jejunum          | 2,479.83 (7.82, 1.98) | 429.23 (6.06, 1.93) |
| Ileum                   | 206.47 (5.33, 2.19) | 89.19 (4.49, 2.01) |

Data are presented as geometric mean and (mean natural log ± S.E.M.) trophozoites per centimeter of intestinal segment.

Significant for the differences between the counts of the vaccinated and non-vaccinated groups in any intestinal
segment (smallest P = 0.26 in distal jejunum).

3.4. Histology of intestinal sections

Two calves demonstrated changes in the duodenum consistent with moderate enteritis; one calf was in the
vaccinated group and one calf was not vaccinated. No abnormalities were found upon examination of other
histological preparations from any of the other calves and no trophozoites were found in any of the slides.

3.5. Clinical signs and fecal score

In the vaccinated group, two calves experienced
diarrhea and respiratory tract problems before G. duodenalis challenge, while two other calves in this
group had only respiratory tract problems or diarrhea
before G. duodenalis challenge. One calf in the non-
vaccinated group had diarrhea and respiratory tract
problems before challenge, while three calves in this
group had only respiratory tract problems or diarrhea
before G. duodenalis challenge. No G. duodenalis cysts were detected in the feces of the five calves that
experienced diarrhea before challenge. No G. duodenalis cysts or G. duodenalis oocysts were detected in the feces of any of the other calves.

Cryptosporidium parvum oocysts were observed in the feces of four of these calves. One calf in the non-vaccinated group that experienced diarrhea before challenge had a short bout of diarrhea on the
fourth day after challenge. No G. duodenalis cysts or C. parvum oocysts were detected in the feces of this calf.

Further diagnostic tests to pursue other causes of
diarrhea were not performed in any of the calves.
Diagnostic Services at the AVC performed a post-
mortem examination of the calf that died after
vaccination. The clinical signs, gross and microscopic
findings observed were consistent with an acute neonatal diarrhea, dehydration, and cardiovascular
collapse. The etiologic agents detected from submitted
specimens were C. parvum and coronavirus.

There was no difference in fecal scores between the
two groups. All calves continued to eat after the
challenge and severe clinical signs were not observed
after infection with G. duodenalis.

4. Discussion

Despite a significantly higher humoral immune response in vaccinated compared to non-vaccinated
calves, subcutaneous vaccination against giardiasis in calves was not efficacious in reducing cyst shedding.

There were no significant differences between vacci-
nated and non-vaccinated calves with respect to the
number of trophozoites present per cm of small intestine. All calves shed G. duodenalis cysts after
challenge and vaccinated calves had a tendency to shed
more cysts than non-vaccinated calves. There were no
severe clinical signs observed due to giardiasis.

In dogs and cats, subcutaneous administration of a G. duodenalis vaccine resulted in a reduced number of
cysts shed in the feces, a lower number of trophozoites in the intestines, an increased feed intake as well as
weight gain and a shorter time during which abnormal
feces were passed (Olson et al., 1996, 1997). A study
has also been conducted on the use of a G. duodenalis vaccine as a therapeutic agent in dogs and cats. Olson
et al. (2001) found that dogs which suffered from
chronic giardiasis and which had received treatments
with a G. duodenalis vaccine ceased to shed fecal cysts, and clinical signs disappeared. However, numerous
other studies have failed to demonstrate a significant
effect (Payne et al., 2002; Stein et al., 2003; Anderson
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paper, Anderson et al. (2004) reported that more dogs in
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inoculation dose used for challenge or due to continuous
exposure to cysts within the environment following
challenge, despite regular cleaning and disinfection.
However, higher challenge doses (10^6 G. duodenalis
cysts) were used in other vaccination studies where the
vaccine was efficacious (Olson et al., 1996, 1997).
Antibody titers in dogs and cats increased rapidly within
3 weeks of vaccination (Olson et al., 1996, 1997). Titers
increased more rapidly after a booster injection on day 21. In our study, calves were given a booster injection on day 28, 4 weeks after initial vaccination, and 1 week later than in the cats and dogs. Nevertheless, the serological response in the calves followed a similar trend as in the dogs and cats: the titers increased within a week after vaccination and increased more rapidly after a booster injection. It is possible that the presence of maternal antibodies in the calves could have interfered with vaccination in this study. Both groups had a similar mean antibody titer, presumably maternal antibodies, at the beginning of the study, which declined prior to vaccination. Although a previous study has demonstrated that maternal antibodies to \textit{G. duodenalis} decline within 30 days in dairy calves, sufficient maternal antibodies may have been present when vaccinations were carried out in this group of calves. However, the impact of maternal antibodies on vaccine efficacy is controversial, and recent studies have indicated good vaccine efficacy despite administration in the presence of maternal antibodies (Martelli et al., 2006; Zimmerman et al., 2006), while other studies reported a negative impact of maternal antibodies on vaccine efficacy. Few studies have examined the immune response of ruminants following \textit{G. duodenalis} infection, but a weak serum immune response was observed following natural infection in both lambs and calves and this is thought to account, in part, for the chronicity of infection in ruminants (Yanke et al., 1998; O’Handley et al., 2003). Although calves mounted a serological immune response following vaccination, it is possible that vaccination did not stimulate immunity locally within the gut, and a localized immune response is important in the elimination of infection (Gottstein et al., 1990). In addition, recent research has shown that ruminants are most commonly infected with \textit{G. duodenalis} corresponding to the genetic assemblage E (O’Handley et al., 2000b; Appelbee et al., 2003; Trout et al., 2004, 2005, 2006), which was also the genotype used to challenge the calves in this study. Although the genotype of \textit{G. duodenalis} comprising the vaccine is not known, it would not be assemblage E as this assemblage has yet to be cultured \textit{in vitro}. Thus, antigenic differences between the genetic assemblages could account for the failure of this vaccine to prevent \textit{G. duodenalis} infection in calves.

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