Demonstration of Passive Protection in Neonatal Calves against Colibacillosis Following Immunization of Pregnant Heifers at 3 Months of Gestation*

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CLINICAL RELEVANCE

Enterotoxigenic Escherichia coli is one of the primary etiologic agents for diarrhea in neonatal calves. Immunization of dams can provide passive protection in neonatal calves; antibodies transferred through colostrum block colonization of bacteria, thereby preventing disease. In this study, healthy pregnant heifers were vaccinated at approximately 3 months of gestation with either a polyvalent oil-adjuvanted vaccine containing inactivated coronavirus, rotavirus, E. coli K99 sub-unit antigen, and Clostridium perfringens β and ε toxoid or normal saline as a placebo. Calves were allowed to nurse immediately after birth, were orally challenged with virulent heterologous enterotoxigenic E. coli at 1 day of age, and were observed for clinical signs of scours for 10 days. Signs of severe scours were noted in 75% of control calves and 28.6% of vaccinates, and the severity of scours was significantly higher (P = .0382) in the control group. The mortality rate was significantly higher (P = .0007) in the control group (80%) than in the vaccinate group (14%). These findings indicate that the vaccination of pregnant heifers at as early as 3 months of gestation (6 months before calving) provides passive protection in neonatal calves against colibacillosis.

INTRODUCTION

Several etiologic agents have been associated with diarrhea in young calves; the most common include rotavirus, cryptosporidia (particularly Cryptosporidium parvum), coronavirus, Escherichia coli, and Salmonella spp. Enterotoxi-

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Enterotoxigenic *E. coli* can cause acute diarrhea in calves during the first few days of life, and the disease can be fatal if untreated. Enterotoxigenic *E. coli* organisms possess special virulence factors called *pili* (fimbriae), which facilitate the colonization of bacteria to intestinal epithelial cells. The role of K99 pili (F5 fimbriae) in the colonization of bacteria to enterocytes of the small intestine has been firmly established, and colonization is considered an essential step in the pathogenesis of enteric colibacillosis. After colonization, bacteria proliferate and release heat-stable and/or heat-labile enterotoxins, both of which induce fluid loss and electrolyte imbalance. Vaccines containing inactivated whole-cell bacteria expressing K99 pilus antigen, partially purified K99 pilus antigen, or purified K99 pilus antigen have been successfully used to immunize dams, thereby providing passive protection to neonatal calves. Antibodies against K99 pilus antigen block the colonization of *E. coli* to gut epithelial cells, thereby preventing the disease process. Feeding monoclonal antibodies to K99 pilus antigen to calves provides protection against neonatal colibacillosis, confirming the importance of K99 pili in the pathogenesis of this disease.

Duration of immunity is an important factor in selecting a vaccine for immunization. The ability to vaccinate heifers during early pregnancy would give producers more flexibility. Vaccines formulated with aluminum hydroxide adjuvant show shorter duration of antibody titer in the milk compared with vaccines formulated with oil adjuvant. The objective of this study was to confirm the hypothesis that the passively transferred colostral antibodies to *E. coli* K99 pilus antigen confer protection in neonatal calves when heifers are vaccinated at 3 months of gestation (6 months before calving).

**MATERIALS AND METHODS**

**Animals and Vaccines**

Thirty-one 1- to 2-year-old pregnant, cross-bred Angus beef heifers at 3 months of gestation were used. Only healthy heifers with approximately the same calving dates and having low antibody titers to *E. coli* K99 pilus antigen were enrolled in the study. The heifers were blocked by prevaccination antibody titer to *E. coli* K99 pilus antigen and anticipated calving dates and were randomly assigned to treatment groups using random numbers generated by an Excel spreadsheet (Microsoft). One group of heifers (n = 21) was vaccinated with a polyvalent vaccine (Guardian, Schering-Plough Animal Health); a second group of heifers (n = 10) was vaccinated with normal saline as a placebo.

The polyvalent vaccine contained inactivated antigens from two strains of rotavirus (G6 and G10) and two strains of coronavirus (types I and III), *E. coli* K99 subunit antigen, and *C. perfringens* (type C) β and (type D) ε toxoids. The antigens were adjuvanted with water-in-oil emulsion using a homogenizer. The heifers in the treated group were vaccinated subcutaneously with 2 ml of polyvalent vaccine (Guardian) at 75 to 126 days of gestation (154 to 205 days before calving), and a similar booster dose was administered 4 weeks after the initial vaccination. The control heifers were given normal saline in a similar manner. All animals
were handled according to animal welfare guidelines. The study protocol was approved by the attending veterinarian and IACUC chairperson. Both groups of heifers were commingled on pasture and had no direct contact with any other animals at the farm. Animals were supplied with water ad libitum throughout the study and were fed diets that met National Research Council nutrient requirements for cattle at their current production phase.

Serum and Colostrum Antibody Measurement

Blood samples were collected from each heifer before vaccination, on day 101 after vaccination, and at calving. Blood samples were collected from all calves 1 day after ingestion of colostrum. Colostrum was collected from each heifer immediately after calving; samples were obtained by milking an approximately equal volume of secretions from each quarter into one tube. Colostrum samples were centrifuged using an ultracentrifuge (5,000 \( \times \) g for 1 hour), and whey was collected for antibody determination. The sera and colostral whey were evaluated for antibody titer to \( E. coli \) K99 pilus antigen using an agglutination assay.\(^\text{17}\) Twofold serial dilutions of positive control, negative control, and test samples were made in U-bottom microtiter plates, and appropriately diluted \( E. coli \) K99 culture grown in pilated phase was added. The plates were mixed and incubated at 36°C ± 2°C for 1 to 3 hours and then at 2°C to 7°C for 18 to 36 hours. After incubation, the plates were read for bacterial agglutination. The titer is expressed as the reciprocal of the highest dilution showing complete agglutination.

Challenge

Each calf was allowed to nurse for approximately 4 to 8 hours after calving. The calves were then separated from the cow for 1 to 3 hours before being challenged with \( E. coli \). A heterologous \( E. coli \) (\( E. coli \) B44 [O9:K30:K99]) challenge culture was used to challenge all calves. Each calf was challenged with 20 ml of challenge culture containing a total of \( 4 \times 10^{12} \) CFU. The challenge culture was administered by depositing the culture in the back of the mouth. The calf was returned to the cow immediately after challenge.

Postchallenge Observation

Calves were observed for mortality and scours for 10 days after challenge. The study was conducted in a blinded manner, and the individual who performed the clinical observations had no knowledge of treatment group assignment. Each calf was assigned a scours score of 0 to 4, depending on the severity of scours:

- 0 = Normal
- 1 = Soft
- 2 = Mild diarrhea
- 3 = Moderate diarrhea
- 4 = Severe diarrhea

Necropsy and Sample Collection for Bacterial Isolation

Necropsies were performed on dead calves to determine the cause of death. An intestinal scraping from each dead calf was collected for isolation of \( E. coli \) organisms. The intestinal scrapings were streaked onto MacConkey agar plates containing nystatin. Colonies resembling \( E. coli \) were picked and restreaked on minimal glucose agar plates, which support the expression of K99 pili. These colonies were tested for the presence of K99 pili by a slide agglutination test using specific K99 antiserum.

Statistical Analysis

Calf mortality and severity of scours were analyzed by the Fisher exact test. Antibody titers to K99 pilus antigen were transformed to number of twofold dilutions by \( \log_2 \) transformation.
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**RESULTS**

K99 antibody titers in the heifers’ serum and colostrum are shown in Figure 1. Control heifers had a prevaccination serum geometric mean titer (GMT) of 4, and the titer did not change at the time of calving. These results indicate that there was no environmental exposure to *E. coli* infection. Vaccinated heifers had a prevaccination serum GMT of 4; at 3 months after vaccination, serum antibody titers in vaccinated heifers ranged from 8 to 256 (GMT, 51) and remained elevated at calving (range, 4 to 256; GMT, 27). The colostrum titer in the vaccinated heifers ranged from 16 to 2,048 (GMT, 92), while colostrum titers in the control heifers ranged from <2 to 64 (GMT, 9). Postvaccination antibody titers in colostrum and serum were significantly higher (*P* < .0001) in vaccinated heifers than in the controls at all time points. All heifers calved 154 to 205 days after the first vaccination, confirming that the antibody responses remain elevated for nearly 7 months.

K99 antibody titers were evaluated in 1-day-old calves after ingestion of colostrum. Sera collected from calves born to the control heifers had a GMT of 4 (range, 2 to 8), whereas sera from calves born to the vaccinated heifers had a GMT of 16 (range, 1 to 256). The serum antibody titer of the calves in the vaccinated group was significantly higher (*P* = .0079) than in the controls.

The *E. coli* challenge administered to the 1-day-old calves was highly virulent, resulting in death in 80% of the calves from control heifers by day 2 (Table 1). In contrast, only 14% (3 of 21) of calves born to vaccinated heifers died. Calf mortality was significantly higher (*P* = .0007) in the control group than in the vaccinated group. All calves with a serum antibody titer >16 survived (Table 2). A total of 83% (5 of 6) of the calves in the control group, which had an antibody titer of ≤4, died after challenge.

The proportion of calves showing varying degrees of scours is shown in Table 3. Two of 10 control calves were not scored for scours because they died before fecal observations could

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**Figure 1.** Geometric mean antibody titer to K99 antigen in sera and colostrum of vaccinated and control heifers. *Significantly higher than controls (P < .0001).*
be made. Seventy-five percent (6 of 8) of the remaining control calves showed signs of severe scours compared with 28.6% (6 of 21) of calves in the vaccinated group. The severity of scours in the control group was significantly higher ($P = .0007$) than in the vaccinated group.

Necropsy findings revealed that death was due to severe dehydration as a result of scours. *E. coli* organisms positive for K99 were isolated from intestinal scrapings of 80% of dead calves, thereby confirming scours induced by *E. coli* as the cause of death.

### DISCUSSION

Duration of immunity is an important criterion to consider when selecting vaccines. Vaccines that provide long-lasting immunity can reduce the cost of labor associated with repeated handling of the animals and also reduce animal stress. The option to vaccinate pregnant cows at the time of pregnancy check (about 6 months before calving) would be an advantage, especially in beef herds. Vaccines blended with aluminum hydroxide adjuvant tend to provide a lower antibody response compared with vaccines made with oil emulsions. Snodgrass and associates showed that an oil-adjuvanted vaccine induced a high level of antibodies to K99 pili in colostrum and milk compared with a similar vaccine formulated with aluminum hydroxide. Multiple other investigators have shown that *E. coli* vaccines (bacterins or partially purified pilus preparations) formulated with oil emulsions induce antibodies in pregnant cows that protect newborn calves against fatal colibacillosis. In our study, we extended this work by using an oil-adjuvanted vaccine containing a more complex mixture of antigens and evaluating the level of protection at about 6 months after vaccination.

### TABLE 1. Percent Mortality in Control and Vaccinate Groups Following Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Calves That Died</th>
<th>% Mortality by Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($n = 10$)</td>
<td>8</td>
<td>80 *</td>
</tr>
<tr>
<td>Vaccinate ($n = 21$)</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

*Mortality was significantly higher in control calves than in vaccines ($P = .0007$).

### TABLE 2. Calf Mortality in Relation to Serum Antibody Titer*

<table>
<thead>
<tr>
<th>E. coli K99 Antibody Titer</th>
<th>No. (% of Calves*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>9 (56)</td>
</tr>
<tr>
<td>8</td>
<td>5 (20)</td>
</tr>
<tr>
<td>16</td>
<td>9 (33)</td>
</tr>
<tr>
<td>≥32</td>
<td>6 (0)</td>
</tr>
</tbody>
</table>

*Antibody titer was not determined for two calves that died before blood could be collected.

### TABLE 3. Severity of Scours Score Following Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Score (No. [%] of Calves)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vaccinates ($n = 21$)</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>Controls ($n = 8^*$)</td>
<td>0 (0)$^*$</td>
</tr>
</tbody>
</table>

*Two control calves died before any fecal observations could be made.

$^*$The severity of scours was significantly higher in control calves than in vaccines ($P = .0382$).
Immunization of pregnant cows is a very effective way to stimulate lactogenic immunity and provide passive protection in neonatal calves through ingestion of colostrum. Lactogenic immunity is a preferred method to provide passive protection against all enteric pathogens affecting neonatal calves. It would be convenient to immunize pregnant cows with a polyvalent vaccine containing all antigens so that neonatal calves are protected when they are most vulnerable to these pathogens.

The data presented in this study indicate that a polyvalent vaccine containing two strains of rotavirus (G6 and G10), two strains of corrnavirus, E. coli K99 subunit antigen, and C. perfringens β and ε toxoids provides lactogenic immunity against a fatal E. coli K99 challenge. Passive transfer for lactogenic immunity is confirmed in this study by demonstrating the specific antibodies to K99 pilus antigen in serum of calves after suckling colostrum from vaccinated heifers and showing the protection after challenge with virulent enterotoxigenic E. coli. The calves born to vaccinated heifers showed significantly less mortality and less severe scourds compared with calves born to placebo controls after challenge.

The findings reported in this study are consistent with the hypothesis that lactogenic immunity against K99 pilus antigen prevents bacteria from colonizing epithelial cells of the small intestine, thereby preventing progression of the disease.1–3 These results are consistent with earlier findings that vaccination of pregnant cattle with whole-cell bacteria expressing K99 pilus antigen or partially purified or purified K99 antigen provides passive protection in neonatal calves.10–15 The data from this study suggest that a correlation exists between the antibody level in calf serum and protection. Calves that had a high antibody titer to E. coli after suckling were better protected against mortality after challenge compared with the calves that had a low antibody titer. The passive transfer of antibodies to neonatal calves is directly related to the colostrum antibody level and the amount of colostrum ingested in the first 12 to 24 hours after birth. This study demonstrated that vaccination with a complex vaccine at 3 months of gestation can induce long-lasting immunity in cows that results in colostral antibodies at levels that will protect calves from a fatal E. coli challenge.1,8

**Conclusion**

This study provides clear evidence that the vaccination of pregnant heifers at 3 months of gestation with a complex oil-emulsion vaccine provides significant passive protection against colibacillosis in neonatal calves.
REFERENCES