Short Communication

Influence of Exogenous Melatonin on Horizontal Transfer of Escherichia coli O157:H7 in Experimentally Infected Sheep

Tom S. Edrington, Russell L. Farrow, Ken J. Genovese, Todd R. Callaway, Robin C. Anderson, and David J. Nisbet

Abstract

The objective of the current research was to determine if exogenous melatonin would exert a "protective" effect on the gastrointestinal tract of sheep and prevent or reduce the horizontal transfer of Escherichia coli O157:H7 from experimentally infected to noninfected or "naïve" sheep. Sixteen cross-bred ewes were housed indoors and adapted to a high concentrate ration. Ewes were randomly assigned to one of four rooms and treatment (three ewes/room, six ewes/treatment) and received either control (gelatin capsule only) or melatonin (5.0 mg/kg body weight [BW]/d). Four additional ewes served as "carrier" sheep (one/room) and were experimentally infected via oral gavage with E. coli O157:H7. Three days post-challenge, carrier ewes were housed with naïve sheep and remained with them for the remainder of the experimental period. Treatments were administered to the naïve sheep 1 day prior to introduction of the carrier sheep and on each of the remaining 7 days of the experimental period. Fecal samples were collected via rectal palpation from the carrier sheep daily throughout experiment and from the naïve sheep daily for 5 days, starting 2 days following introduction of the carriers. On day 8 of the experiment, all ewes were euthanized and tissues from the rumen, ileum, cecum, colon, and rectum as well as their respective lumen contents collected. The carrier sheep quickly infected the naïve ewes, which had similar fecal concentrations as the carrier animals throughout the 5-day sampling period. Melatonin treatment had no effect (p >0.10) on daily fecal shedding, luminal content concentrations, or in the percentage of gastrointestinal tract tissue positive for the inoculated strain of E. coli O157:H7.

Introduction

Escherichia coli O157:H7 is found primarily in ruminants (Capriola et al., 2005) with fecal shedding typically more prevalent during the summer months (Chapman et al., 1997; VanDonkergoed et al., 1999). We hypothesize that this seasonal variation is due to physiological responses within the animal in response to changing day-length (Edrington et al., 2006). Previous research conducted in our laboratory with both naturally and experimentally infected cattle and sheep supports our hypothesis and indicated that the hormones melatonin, triiodothyronine, and thyroxin are likely involved (Edrington et al., 2006, 2007, 2008; Schultz et al., 2006).

Melatonin is especially intriguing given that secretion by the pineal gland is seasonal with serum concentrations highest when E. coli O157:H7 prevalence is typically lowest (winter) and vice versa. The gastrointestinal tract (GIT) also produces melatonin, far exceeding pineal production (Bubenik, 2002). Given that certain segments of the GIT are preferred locations for E. coli O157:H7 in the ruminant, current research in our laboratory is investigating if GIT melatonin is secreted seasonally and if tissue concentrations can be correlated to fecal prevalence of E. coli O157:H7. Administration of exogenous melatonin to naturally colonized cattle (Edrington et al., 2008) resulted in modest decreases in fecal shedding of E. coli O157:H7. Melatonin is reported to have a synergistic relationship with the immune system (Lissoni et al., 1997; Drazen et al., 2001) and GIT melatonin has been reported to play a protective role in the GIT (Bubenik 2002). Taken together with results of previous research, this suggests the
The protective role of melatonin in the GIT may reduce the GIT colonization of E. coli O157:H7 and thereby influence fecal prevalence. The objective of the current research was to determine if exogenous melatonin would prevent the horizontal transfer of E. coli O157:H7 from experimentally infected to noninfected or "naive" sheep.

Materials and Methods

Sixteen cross-bred (Suffolk × Rambouillet) ewes (avg. BW = 74 kg) were housed indoors in environmentally controlled facilities and randomly assigned to one of five isolation rooms. Over a 2-week period, ewes were adapted to an 80:20 concentrate to forage ration and adjusted to a 16-hour light and 8-hour dark photoperiod. Following the adaptation period, ewes from four of the five rooms (n = 12, 6 per treatment) were randomly assigned to treatment control (gelatin capsule only) or melatonin (5.0 mg/kg BW/d). The ewes in the fifth room served as the "carrier" sheep and were experimentally infected via oral gavage with 10 mL of tryptic soy broth containing 3.5 × 10^7 colony-forming units (CFU)/mL E. coli O157:H7. Fecal samples were collected from the carrier sheep for two consecutive days to confirm shedding of the challenge strain as well as from the naive sheep to culture for wild-type E. coli capable of growth on rifampicin-supplemented agar. On the third day post-challenge, one carrier ewe was introduced into each room of naive sheep and remained in that room for the remainder of the experimental period. Treatments were administered to the naive sheep 1 day prior to introduction of the carrier sheep and on each of the remaining 7 days of the experimental period. Thus each room contained three E. coli O157:H7 naive and one carrier sheep throughout the course of the experimental period. Fecal samples were collected via rectal palpation from the carrier sheep daily throughout the experiment and from the naive sheep daily for 5 days, starting 2 days following introduction of the carriers. On day 8 of the experiment, all ewes were humanely euthanized (Euthasol®, euthanasia solution; Delmarva Laboratories, Inc., Midlothian, VA) and tissues from the rumen, ileum, cecum, colon, and rectum as well as their respective lumen contents were aseptically collected for bacterial enumeration described below. Care, use, and handling of experimental animals were approved by the Animal Care and Use Committee of the Food and Feed Safety Research Laboratory, U.S. Department of Agriculture.

Bacterial cultures and enumeration

The challenge strain of E. coli O157:H7 (strain 2336 obtained from the Field Disease Isolation Unit, Pullman, WA) was made resistant to rifampicin via successive cultivation in tryptic soy broth containing 25 mg/mL rifampicin. Fecal and luminal content samples (1 g) were homogenized and serially diluted (10-fold increments) in sterile phosphate-buffered saline, plated on MacConkey agar supplemented with 25 mg/mL rifampicin, and incubated (24 hours, 37°C) to enumerate E. coli O157:H7 concentrations. Tissue samples were incubated (24 hours, 37°C) in 20 mL GN Hajna with rifampicin, prior to plating and incubating as above for qualitative determination of the challenge strain.

Statistical analysis

Data were analyzed using SAS Version 8.02 (SAS Inst. Inc., Cary, NC). Data for quantitative fecal shedding and qualita-

### FIG. 1

Fecal shedding of E. coli O157:H7 (colony-forming units [CFU]/g feces log_{10}) in naive sheep administered either 0 (control) or 5 mg melatonin/kg body weight (melatonin) and exposed to experimentally infected carrier sheep.

Discussion

Results of the current research highlight the ease of horizontal transmission of E. coli O157:H7 from infected to naive animals when animals are housed in relatively confined spaces. The failure of our melatonin treatment to reduce or prevent horizontal transmission of E. coli O157:H7 could be a result of a number of factors. Our hypothesis, based on previous reports of melatonin's protective effects on the GIT (Lissoni et al., 1997; Bubenik 2002), was that we could prevent colonization and subsequent shedding in naive sheep with administration of exogenous melatonin. In hindsight, only 1 day of melatonin treatment prior to exposure to carrier animals was probably not sufficient time for the melatonin to exert a protective effect on the GIT. Considering the relatively
MELATONIN AND E. COLI O157:H7

TABLE 1. Populations of E. coli O157:H7 from the Luminal Contents of the Rumen, Ileum, Cecum, Colon, and Rectum in Naïve Sheep Administered Either 0 (CONTROL) or 5 mg Melatonin/kg BW (MELATONIN) and Exposed to Experimentally Infected Carrier Sheep

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Melatonin</th>
<th>SEM</th>
<th>p &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen</td>
<td>1.1</td>
<td>1.6</td>
<td>0.3</td>
<td>0.26</td>
</tr>
<tr>
<td>Ileum</td>
<td>3.7</td>
<td>3.4</td>
<td>0.39</td>
<td>0.63</td>
</tr>
<tr>
<td>Cecum</td>
<td>3.3</td>
<td>3.5</td>
<td>0.39</td>
<td>0.63</td>
</tr>
<tr>
<td>Colon</td>
<td>3.8</td>
<td>3.7</td>
<td>0.5</td>
<td>0.92</td>
</tr>
<tr>
<td>Rectum</td>
<td>3.1</td>
<td>3.7</td>
<td>0.48</td>
<td>0.4</td>
</tr>
</tbody>
</table>

BW, body weight; SEM, standard error of the mean; CFU, colony-forming units.

slow timeframe in which melatonin naturally responds to decreasing day-length, naïve ewes should have received the melatonin treatments for a longer time frame prior to E. coli O157:H7 exposure. Although, in previous research (Edrington et al., 2008) we demonstrated an effect on fecal shedding in cattle during a 4-day dosing regimen of melatonin. Possibly the lack of effect observed in the current research was a result of melatonin degradation in the rumen with little melatonin reaching the lower GIT. However, based on previous research (Edrington et al., 2008) in which we did observe an effect on fecal shedding of E. coli O157:H7 following dosing with an oral melatonin bolus, we hypothesized that the dose used in this experiment would be satisfactory and elicit similar effects.

The high concentration of E. coli O157:H7 shed by the seeder ewes (which would classify them as “super-shedders”) could have overwhelmed any protective effects the melatonin may have had on the GIT. Exposure to seeder animals shedding lower concentrations might have produced a more “subtle” challenge to the naïve animals. Possibly the melatonin dose administered was not large enough to alter GIT melatonin concentrations. As we did not have a viable GIT melatonin assay at the time of the experiment we can only speculate. However, in previous research (Edrington et al., 2008), melatonin administered to cattle at the same rate (5 mg/kg BW) was sufficient to influence fecal shedding of E. coli O157:H7 and we hypothesized that this dose would likewise be sufficient to influence horizontal transfer of this pathogen.

In evaluating which hormones (known to respond to changing day-length) to examine, melatonin based on the secretion patterns that are inverse to E. coli O157:H7 shedding patterns, stands out as a logical choice. However, a number of hormones offer intriguing possibilities, some of which we have demonstrated can also influence fecal shedding of E. coli O157:H7 (Edrington et al., 2007). The most probable scenario likely involves multiple hormones, or other compounds yet to be discovered, functioning in a cascade of events to influence E. coli O157:H7 populations.

Acknowledgment

Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Disclosure Statement

No competing financial interests exist.

References


Address correspondence to: Tom S. Edrington, B.S., M.S., Ph.D., Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, 2881 FEB Rd., College Station, TX 77845
E-mail: edrington@ffsr5.tamu.edu