Efficacy of *Saccharomyces boulardii* for treatment of horses with acute enterocolitis

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**Objective**—To evaluate the viability of *Saccharomyces boulardii* after PO administration in clinically normal horses and its efficacy as a treatment for horses with acute enterocolitis.

**Design**—Prospective study.

**Animals**—5 clinically normal horses and 14 horses with acute enterocolitis.

**Procedure**—Feces were collected from 5 clinically normal horses and submitted for microbial culture for 2 days prior to administration of a lyophilized form of *S boulardii* (25 or 50 g, PO, q 12 h) for 10 days. Feces were collected for microbial culture 5 and 10 days after treatment initiation and 10 days after treatment was discontinued. Fourteen horses with acute enterocolitis were randomly allocated to receive a placebo or *S boulardii* (25 g), PO, every 12 hours for 14 days.

**Results**—*S boulardii* was not detected in feces of clinically normal horses. After administration, yeast survived within the gastrointestinal tract but did not permanently colonize it. In horses with acute enterocolitis, the severity and duration of gastrointestinal tract disease during hospitalization were significantly decreased in horses receiving *S boulardii*, compared with horses receiving the placebo.

**Conclusions and Clinical Relevance**—Administration of *S boulardii* may help decrease the severity and duration of clinical signs in horses with acute enterocolitis. (J Am Vet Med Assoc 2005;227:954–959)

Despite recent advances in intensive care of horses, those with severe enterocolitis may have a guarded prognosis and life-threatening complications may develop even if they survive the primary disease. The reported survival rate for horses with diarrhea is approximately 70%; however, the prognosis is much worse for horses with certain types of colitis, including necrotizing enterocolitis and antimicrobial-associated diarrhea. The cause of colitis is determined in only approximately 35% of horses, and the diagnosis is usually not made in a timely manner. Consequently, treatment is often supportive, consisting mainly of fluids and electrolyte replacement, colloids, anti diarrheal agents, and nonsteroidal anti-inflammatory medications. Specific treatment consists of administration of antimicrobials in selected cases, such as oxytetracycline for equine monocytic ehrlichiosis or metronidazole for enteric clostridiosis. Other treatments administered to help reestablish normal gastrointestinal flora include administration of commercial probiotic paste, live-culture yogurt, or fecal suspensions from a healthy donor. Little has been written concerning the use of biotherapeutic agents in horses, and their efficacy has not been determined for the treatment or prevention of diarrhea in horses.

In human medicine, several biotherapeutic agents have been evaluated for treatment or prevention of diarrhea. Among the various agents being investigated, *Saccharomyces boulardii*, a subtype of *Saccharomyces cerevisiae*, is one of the most promising. *Saccharomyces boulardii* is a nonpathogenic yeast that has been used as an anti diarrheal agent in Europe since 1962 and in the United States. In humans, the yeast can reach high concentrations in the gastrointestinal tract 48 hours after treatment is initiated.

In human medicine, results of several randomized, double-blind, placebo-controlled studies indicate the efficacy of *S boulardii* for prevention of antimicrobial-associated diarrhea and treatment of recurrent colitis caused by *Clostridium difficile* in conjunction with a standard antimicrobial regimen. In addition, *S boulardii* has been used for the treatment of chronic diarrhea in patients with acquired immunodeficiency syndrome and segmental enteritis (*C rohn’s disease*) and for the prevention of traveler’s diarrhea with promising results. The mechanism of action of *S boulardii* in cases of colitis caused by *C difficile* appears to be associated with the release, by the yeast, of a protease able to digest *C difficile* toxins A and B. Proposed mechanisms that could explain the effect of *S boulardii* in other types of colitis include an immunomodulatory effect attributable to the promotion of the release of secretory immunoglobulins within the intestine or activation of the reticuloendothelial and complement systems. The yeast has also been found to have adhesion sites for some strains of *Salmonella typhimurium* and *Escherichia coli* and may be useful in the treatment of enteric infections caused by those organisms. In veterinary medicine, use of *S boulardii* has been restricted to the poultry industry in which it has been found to decrease the frequency of cecal colonization by *Salmonella* spp in broiler chickens experimentally challenged after transport stress. To our knowledge, there are no scientific reports on use of *S boulardii* in horses. *Saccharomyces*
boulardii could potentially be useful for the prevention and treatment of intestinal disorders in horses and help to reestablish the normal homeostasis of the intestinal ecosystem in horses with diarrhea.

The purposes of the study reported here were to evaluate the viability of S boulardii after PO administration in clinically normal horses and its efficacy as a treatment for acute enterocolitis. The study was performed in 2 parts. A preliminary experiment was performed to evaluate the viability of S boulardii when administered to healthy adult horses. This experiment was designed with the following hypotheses: S boulardii is not a normal inhabitant of the gastrointestinal tract in horses, S boulardii can survive in the gastrointestinal tract of horses after PO administration of a lyophilized form, and S boulardii does not permanently colonize the gastrointestinal tract in horses and is rapidly eliminated after PO administration is discontinued. A prospective, single-blind, randomized, placebo-controlled, parallel-group clinical trial was used to evaluate the efficacy of S boulardii alone and in combination with standard antidiarrheal treatment in horses with acute enterocolitis. The hypothesis was that the addition of a viable, dried preparation of this biotherapeutic agent to the standard treatment would decrease the duration and severity of clinical signs in horses with acute enterocolitis.

Materials and Methods

Horses—Five clinically normal horses from 10 to 14 years old and weighing 300 to 530 kg (660 to 1,166 lb) were used to evaluate the viability of S boulardii administration. Horses were owned by the University of Pennsylvania. Horses were housed in individual indoor stalls, were fed mixed hay ad libitum, and received 0.5 kg (1.1 lb) of concentrate feed twice a day. Water was available ad libitum. None of the horses had received any medication or nutritional supplementation for at least 14 days prior to the beginning of the experiment. Horses were determined to be clinically normal on the basis of physical examination findings, PCV, and total solids concentration performed prior to the beginning of the experiment.

The clinical trial was performed at the George D. Widener Hospital for Large Animals, with horses recruited among cases referred for evaluation of acute onset of diarrhea. Each owner gave written informed consent. Horses were > 4 months old and weaned. No breed or sex restrictions were applied. Horses were eligible for enrollment in the study if they had an acute onset of diarrhea, which was defined as at least 3 consecutive episodes of loose or watery feces prior to enrollment. Horses were excluded from the study if they had gastrointestinal illness for > 48 hours, were receiving PO antifungal treatment, or were in gestation. The protocol and procedures used for this study were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Viability of S boulardii administration—The yeast was provided as a live lyophilized powder prepared by the company and obtained from actively growing broth cultures of S boulardii. Evaluation of the contents of the lyophilized powder was performed by an independent laboratory by use of polymerase chain reaction assay and microbiologic culture. The yeast was kept in a lightproof container and stored at 4°C.

Horses were randomly allocated to 2 groups and received their respective dosages as a top dressing on their concentrate feed for 10 days. Three horses were given 10 X 10^6 yeast cells (25 g), PO, every 12 hours (group 1). Two horses received 20 X 10^9 yeast cells (50 g), PO, every 12 hours (group 2). The 2 doses of the lyophilized form of S boulardii were extrapolated by weight from the recommended doses used in humans. The high dose was administered to further evaluate the possible development of adverse reactions associated with PO administration of this microorganism. Heart and respiratory rates, rectal temperature, general attitude, appetite, and fecal production and consistency were monitored during the treatment period.

Fecal samples were collected daily for 2 consecutive days prior to treatment. Subsequent collections were performed 5 and 10 days after administration of the initial treatment. One final sample was collected 10 days after the last treatment. The specimens were fresh feces collected from the stall floor, stored at 4°C, and submitted to the clinical microbiology laboratory within 24 hours for isolation of S boulardii. Microbiological assessment was performed on fecal samples from horses and a control sample of dehydrated S var boulardii reconstituted with sterile water. All submissions, including the control, were directly plated to safron dextrose agar and inhibitory mold agar with gentamicin and incubated at 30°C for 48 to 72 hours. All colonies were screened by Gram stain for yeast cells. Colonies with positive results for yeast cells were subcultured to safron dextrose agar and incubated at 30°C for 48 hours. Yeast was identified by use of a yeast assimilation system in accordance with the manufacturer’s instructions by use of inocula derived from a 48-hour safron dextrose agar culture. Yeast inocula were subcultured to cornmeal agar with polysorbate 80 to establish purity and morphology controls.

Determination of efficacy of S boulardii—The treatments, consisting of S boulardii mixed with molasses or a plain molasses placebo, were administered PO in combination with standard antidiarrheal treatment. Saccharomyces boulardii was given at a dosage of 25 g (10 X 10^6 yeast cells) every 12 hours for 14 days even if resolution of clinical signs of diarrhea was detected. Nurses administered the yeast or placebo. Clinicians and veterinary students were unaware of treatment group allocation. If needed, the yeast was provided after discharge to complete the treatment. Data required to complete the study were obtained via personal communication with the owner or agent of the horse.

At the time of enrollment in the study, a medical history was obtained and a physical examination was performed on each horse. Fecal samples were collected and tested for Salmonella spp and C difficile and Clostridium perfringens via standard microbial cultures and toxin assays. Serodiagnostics was performed for Neorickettsia risticii on suspected cases on the basis of time of the year, clinical signs (eg, fever and laminitis), and history of vaccination. The specific antidiarrheal treatment administered was at the discretion of the clinician and was given in combination with S boulardii or placebo.

Prior to enrollment in the study, acute diarrhea was defined as at least 3 consecutive episodes of watery or loose feces. During the initial evaluation and the hospitalization, fecal production and consistency were classified as follows: watery feces, loose feces, no feces, and formed feces. The end of diarrhea was defined as a return to normal fecal production and consistency. Study treatment failure was defined as diarrhea that did not resolve or reoccurred before the end of the 14 days of study treatment with or without other antidiarrheic treatments. The study treatment was discontinued if the horse had clinical signs of adverse reactions.

The specimens were fresh feces collected from the stall floor, stored at 4°C, and submitted for microbial culture within 24 hours of collection. Specimens collected at enrollment were inoculated onto trypticase soy agar with 5% sheep blood, Columbia agar with colistin and naladixic acid, and
thioglycollate broth for isolation of \textit{C. perfringens}. Specimens submitted for isolation of \textit{C. difficile} were inoculated onto trypticase soy agar with 5% sheep blood, Columbia agar with colistin and naladixic acid, and \textit{C. difficile} agar. The plates were incubated in an anaerobic jar system for 48 hours at 35 to 37°C. Organisms that had typical colonial morphology and Gram stain results for \textit{C. difficile} and \textit{C. perfringens} were sub-cultured, and aerotolerance testing was performed. A rapid identification system was used for final identification.

An ELISA developed for detection of \textit{C. perfringens} enterotoxin and \textit{C. difficile} toxins A and B in feces was used. Tests were performed according to manufacturer’s instructions.

Fecal samples were collected for 3 consecutive days after enrollment for microbial culture for detection of \textit{Salmonella} spp. Specimens were placed in 100 mL of selenite F and incubated for 18 to 24 hours at 35°C. Selenite was sub-cultured and streaked for isolation onto xylose lysine desoxycholate and MacConkey agar plates. Plates were incubated for 18 to 24 hours at 35°C. Plates were checked at 24 and 48 hours for morphology typical of \textit{Salmonella} spp. Plates from which no growth was detected after 24 hours were reincubated from reincubated selenite. Colonies believed to be \textit{Salmonella} spp were inoculated into a tube of sterile demineralized water. The suspension was standardized to a 0.5 McFarland standard, and 0.05 mL of the suspension was inoculated onto an identification plate. A purity check was performed by use of a xylose lysine desoxycholate plate. Plates were incubated at 35°C for 18 to 24 hours and then read via an automated system. \textit{Salmonella} species were grouped by use of \textit{Salmonella} antisera groups B, C1, C2, D, and E. Serum was submitted for indirect fluorescent antibody testing for detection of \textit{N. risticii} infection at enrollment in the study.

**Statistical analyses**—For the clinical trial, baseline characteristics (age, breed, and sex), elements of the clinical history before enrollment (prior surgery, prior treatments, and other medical conditions), results of diagnostic tests (serologic testing for \textit{N. risticii}, microbial cultures of feces, and toxin assays), treatments used and duration of treatment during hospitalization, recurrence of diarrhea, and outcome were compared between treatment groups by use of the Fisher exact test. Continuous outcome tests were performed by use of the Kruskal-Wallis test. These comparisons included duration of diarrhea prior to enrollment, classification of fecal production during the study, and duration of hospitalization by study group. Survival analyses (\(\chi^2\) tests) were used to determine differences in disease persistence by treatment. All statistical analyses were performed with computer software. Values of \(P < 0.05\) were considered significant.

**Results**

All horses used to evaluate the viability of \textit{S. boulardii} administration remained healthy, and there was no evidence of adverse effects during or after the treatment period. The yeast top dressing did not affect appetite, and horses did not have clinical signs of gastrointestinal illness during this portion of the study.

\textit{Saccharomyces boulardii} was not identified on microbial culture of feces from any horse prior to initiation of treatment. \textit{Cryptococcus laurentii} was identified on microbial culture from 1 horse prior to the study. The clinical importance and pathogenicity of this yeast are not known; however, this microorganism did not interfere with the subsequent growth of \textit{S. boulardii}. \textit{Saccharomyces boulardii} was identified on microbial cultures of feces from all 5 horses on the 5th and 10th days of treatment. No other yeasts were identified from any fecal samples during the treatment period. All of the yeast isolates, including the control sample, had an excellent identification profile for \textit{S. cerevisiae}. All yeast isolates did not assimilate galactose, which identified the yeast as \textit{S. cerevisiae}. In the 4 horses from which fecal samples were obtained for culture 10 days after the last treatment, \textit{S. boulardii} or other yeasts were not isolated. One horse was not available for the last fecal collection.

Fourteen horses were enrolled in the clinical trial to evaluate the efficacy of \textit{S. boulardii} for treatment of horses with acute enterocolitis from July 2001 through August 2002. Seven horses were allocated to receive \textit{S. boulardii}, and 7 were allocated to receive the placebo. Of the 14 horses studied, 13 completed the trial and were discharged. One horse receiving \textit{S. boulardii} was euthanized after development of severe laminitis on the third day of hospitalization. Clinical data from that horse were included in the analyses.

The mean age of horses treated with \textit{S. boulardii} was not significantly different from the mean age of horses receiving the placebo. Sex and breed distribution were also not significantly different in horses receiving \textit{S. boulardii}, compared with those receiving the placebo.

The 2 groups were statistically similar with regard to the clinical history, including history of recent surgery, presence of medical conditions other than gastrointestinal disease, and use of antimicrobials and other treatments prior to enrollment. Five horses included in the \textit{S. boulardii} group received antimicrobial treatment prior to hospital admission. One horse developed clinical signs of enterocolitis while receiving antimicrobial treatment after surgery for distal limb deformity. Two horses were receiving antimicrobials for treatment of a documented pulmonary disease, and 2 horses were treated with antimicrobials for fever of unknown origin and diarrhea. Four horses included in the placebo group were treated with antimicrobials prior to hospital admission. One horse received antimicrobial treatment after surgery for umbilical hernia repair. Three horses received antimicrobial treatment for pneumonia (\(n = 1\)), grain overload (\(1\)), and fever of unknown origin (\(1\)). All horses from both groups received other treatments prior to hospital admission, including nonsteroidal anti-inflammatory medications, fluids administered IV, and gastrointestinal protectants.

At the beginning of the study, the severity of diarrhea was not significantly different between the 2 groups; all horses had watery feces. The duration of diarrhea prior to enrollment in the study was significantly longer in horses allocated to receive \textit{S. boulardii}, compared with horses allocated to receive the placebo (Table 1).

A potential etiologic agent was detected in 4 of the 14 horses evaluated. There was no difference between treatment groups regarding the recovery rate of fecal pathogens or horses with positive results for \textit{N. risticii}. \textit{Salmonella} spp and \textit{C. difficile} toxin A was detected on analysis of the feces of 1 horse included in the \textit{S. boulardii} group. In the same group, 1 horse had positive results for \textit{C. perfringens} enterotoxin and a horse unvaccinated for Potomac Horse Fever had a positive \textit{N. risticii} titer at enrollment in the study. \textit{Salmonella} spp
was detected on microbial culture of feces obtained during hospital admission from 1 horse in the placebo group.

Although the type, number, and duration of standard antidiarrheic treatments varied, no significant difference was detected among study groups. Horses from both study groups were treated with fluids administered IV, supplemental electrolytes, and nonsteroidal anti-inflammatory drugs. Most horses received hyperimmune plasma and polymyxin B as well as gastrointestinal protectants and antiulcer medications. Other treatments included pentoxifylline, probiotic paste, sodium bicarbonate, and supplemental dextrose. The number of horses treated with antimicrobials during hospitalization was not significantly different between the 2 groups. Of the 7 horses included in the S boulardii group, 6 received antimicrobial treatment. All horses in the placebo group were treated with antimicrobials during hospitalization. Most horses were treated with metronidazole alone or in combination with other classes of antimicrobials, including beta-lactams, aminoglycosides, tetracyclines, and quinolones.

During hospitalization, the severity of the gastrointestinal disease was determined by the appearance of feces and duration of clinical signs. The duration of clinical signs after enrollment in the study was significantly different between the 2 groups; the number of days horses had diarrhea was significantly higher in horses receiving the placebo, compared with horses receiving S boulardii (Table 1). The duration of watery diarrhea was less in horses receiving S boulardii, compared with horses receiving the placebo. No significant difference in the duration of production of nonformed feces was detected between the 2 groups. The median number of days of production of formed feces during hospitalization was significantly higher in horses receiving S boulardii, compared with horses receiving the placebo. Of the 7 horses receiving S boulardii, no significant adverse reaction was reported. In 2 horses treated with S boulardii, no fecal production was noticed for at least 24 hours.

No significant differences in the duration of hospitalization, recurrence of diarrhea during hospitalization, and outcome were detected between the 2 groups. The median number of days of hospitalization was 10 for the patients included in the S boulardii group and 9 for the group receiving placebo. Of the 14 horses enrolled in the study, 3 of the 7 horses receiving placebo had recurrence of diarrhea, whereas none of the horses receiving S boulardii had recurrence of diarrhea during hospitalization. Six of the 7 horses receiving S boulardii were discharged from the hospital, and 1 was euthanatized because of development of severe laminitis. All horses in the placebo group were discharged from the hospital.

### Discussion

Results of the first experiment indicated that S boulardii is not a normal inhabitant of the gastrointestinal tract in horses, which is consistent with a previous report of normal equine intestinal flora. Results of the study reported here indicated that PO administration of a lyophilized form of S boulardii results in viable yeast within the gastrointestinal tract in horses. Saccharomyces boulardii was detected in feces of each horse within 5 days of the initiation of treatment and in every subsequent fecal sample obtained during the treatment period. These findings are consistent with results of a study in humans indicating that S boulardii reaches maximum steady-state concentration 3 days after administration. Microbial cultures prior to day 5 of treatment were not performed in our study, and results of future studies may indicate that viable S boulardii can be detected earlier in the treatment period. In human patients, S boulardii is no longer detectable in the stool 2 to 5 days after treatment has been discontinued. In the study reported here, results of microbial cultures indicated that S boulardii was not detected in the feces 10 days after administration was discontinued. Permanent survival in the intestinal tract did not occur in any of the horses available for sampling. Contamination of the stall may have been a problem in our study and may have confounded our findings. Saccharomyces boulardii was given to horses via top dressing of the feed concentrate, which may have resulted in contamination of the stall floor, and hence feces, with S boulardii; therefore, collection of feces from the rectum should have been performed. Adverse reactions attributable to S boulardii administration were not observed in any of the horses included in the first experiment.

Feeding horses S boulardii can result in transient viability of the yeast in the intestinal tract. However, our results did not permit us to determine whether the doses administered corresponded to the appropriate quantities necessary to reach adequate concentrations of yeast in the intestines for prophylactic or therapeutic purposes. Evaluation of colonic contents and mucosa sampling was not performed, and the quantity of colony-forming units of S boulardii per kilogram of feces was not investigated in our study because of the expense associated with those procedures. Doses of S boulardii used in our study were extrapolated from results of a study in humans suggesting that the minimum therapeutic dose was $1 \times 10^8$ colony-forming units/d to $1 \times 10^9$ colony-forming units/d.

The second experiment was designed to assess the intrinsic efficacy of S boulardii when administered in combination with standard antidiarrheic treatment for acute enterocolitis in horses. Comparison with a placebo, randomized allocation of treatments, and conditions in which those administering treatments were unaware of treatment group allocation permitted adequate assessment of the efficacy of the yeast.

### Table 1

<table>
<thead>
<tr>
<th>Duration</th>
<th>S boulardii</th>
<th>Placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea (median, hours) prior to enrollment</td>
<td>24</td>
<td>12</td>
<td>0.023*</td>
</tr>
<tr>
<td>Diarrhea (median, days)</td>
<td>5</td>
<td>7</td>
<td>0.041*</td>
</tr>
<tr>
<td>Watery diarrhea (median, days)</td>
<td>2</td>
<td>3</td>
<td>0.035*</td>
</tr>
<tr>
<td>Loose feces (median, days)</td>
<td>2</td>
<td>3</td>
<td>0.054</td>
</tr>
<tr>
<td>No feces (median, days)</td>
<td>2</td>
<td>0</td>
<td>0.141</td>
</tr>
<tr>
<td>Formed feces (median, days)</td>
<td>7</td>
<td>4</td>
<td>0.040*</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.05) different between groups.
Treatment with *S. boulardii* significantly decreased the duration of watery diarrhea and the duration of gastrointestinal illness after enrollment in the study. Because of the difficulty of accurately monitoring the frequency and volume of fecal production in our hospital setting and after discharge, these parameters, although important, were not evaluated in our study. The duration of gastrointestinal illness was determined from enrollment of horses in the study. This appeared to the authors to be the best way to avoid potential confounders, such as delay in recognizing the presence and severity of gastrointestinal illness before referral or inadequate medical management of horses prior to hospital admission. Because horses were hospitalized in our facility, they all received optimal standard antidiarrheic treatment and were submitted to the same intensive monitoring. By measuring the duration of clinical signs of enterocolitis after hospital admission, our goal was to assess the progression of gastrointestinal illness while horses were receiving the study treatment. For this reason, data concerning the duration prior to and after enrollment in the study were analyzed separately. Six of the horses that survived their primary disease and were receiving *S. boulardii* made a complete recovery without recurrence of diarrhea, compared with 4 of 7 horses allocated to the placebo group.

Results of other studies performed in animals, human volunteers, and ill patients indicate that *S. boulardii* is safe and effective for the treatment and prevention of diarrhea. In our study, 13 of 14 horses recovered completely from their gastrointestinal illness and were discharged from the hospital. The authors attributed development of severe laminitis in 1 horse to the placebo group. Minor adverse effects reported in humans treated with *S. boulardii* include increased thirst and constipation. Most of the horses receiving *S. boulardii* were not performed. Transient constipation for a short duration occurs frequently after resolution of diarrhea in horses. Therefore, constipation in those 2 horses may not have been associated with administration of *S. boulardii*. Microbial culture of blood for *S. boulardii* was not performed. Transient fungemia could not be ruled out but was unlikely because of the uncomplicated clinical progression of most of the horses receiving *S. boulardii*.

Results of the second experiment suggested that *S. boulardii* may be a useful treatment in the management of acute enterocolitis in adult horses. Successful results of the first experiment attributed to the use of probiotics, *S. boulardii,* for prevention of diarrhea in critically ill tube-fed patients. Additional controlled clinical trials are required to further assess the use of *S. boulardii* as a therapeutic and prophylactic antidiarrheic agent.

### References

11. Buts JP, Cortijus G, Delme M. *Saccharomyces boulardii* for...


