# Safety and efficacy of a combined paste formulation of a botanical extract of the latex of *Croton lechleri* (SB-300, Neonorm foal™) with probiotics for the treatment of watery diarrhea in nursing foals

## Determinación de la seguridad y eficacia de una formulación en pasta que combina un extracto botánico del látex de *Croton lechleri* (SB-300, Neonorm foal®) con probióticos para el tratamiento de diarrea acuosa en potrillos no destetados

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**Abstract:** Diarrhea is a common disorder in preweaned foals, and there are no antisecretory antidiarrheals currently available. The objective of this study was to evaluate the safety and efficacy of an enteric-coated paste formulation of a standardized botanical extract of the latex of *Croton lechleri* (SB-300) for the treatment of watery diarrhea in foals. This was a randomized blinded multisite, safety and efficacy pilot study of the formulation in client-owned foals with watery diarrhea. Sixty-one foals under 16 weeks of age with watery diarrhea were randomly divided into two treatment groups and one placebo group, approximately 20 foals each. Each foal received either placebo or investigational product/probiotic combination, four times daily. No significant safety issues were noted but the transient production of hard feces that was noticed in foals that continued therapy beyond clinical improvement. A treatment responder was any foal who developed formed stool (fecal score <3), and maintained formed stool for a minimum of 16 consecutive hours within a 24 h period. Statistically significant differences in treatment response were demonstrated at a number of time points between the investigational product treatment and placebo groups. We conclude that a paste formulation of SB-300, a standarized botanical extract of the latex of *Croton lechleri*, when combined with probiotics, is effective in the treatment of watery diarrhea in foals less than 16 weeks of age and warrants further investigation without the addition of probiotics.

**Key words:** diarrhea, foal, *Croton lechleri*, SB-300, chloride channels

**Resumen:** La diarrea es un desorden común en potrillos lactantes, y no se dispone de antidiarreicos antisecretorios específicos. El objetivo del estudio fue evaluar la seguridad y eficacia de una formulación en pasta con cubierta entérica del extracto botánico estandarizado del látex de *Croton lechleri* (SB-300, producto en investigación), para el tratamiento de diarrea acuosa en potrillos. Fue un estudio piloto, ciego, aleatorizado, realizado en distintos sitios, en potrillos de clientes particulares con diarrea acuosa. Sesenta y un potrillos con diarrea acuosa menores a 16 semanas de edad fueron divididos aleatoriamente en dos grupos de tratamiento y uno de placebo, aproximadamente 20 en cada grupo. Cada potrillo recibió placebo o la combinación del producto en investigación y probiótico cuatro veces al día. No se presentaron problemas de seguridad significativos, pero se observó la producción transitoria de heces duras en los que continuaron la terapia luego de la resolución de la diarrea. La respuesta al tratamiento fue considerada en aquellos potrillos que desarrollaron heces formadas (score fecal <3) y mantuvieron las heces formadas durante un mínimo de 16 h consecutivas dentro de un período de 24 h. Se demostraron diferencias estadísticamente significativas en la respuesta al tratamiento en distintos tiempos entre los grupos que recibieron el producto y los que recibieron placebo. La conclusión fue que la formulación en pasta de (SB-300), un extracto botánico estandarizado del látex de *Croton lechleri*, combinado con probióticos, es efectivo para el tratamiento de diarrea acuosa en potrillos menores de 16 semanas de edad y requiere investigaciones complementarias sin la adición del probiótico.

**Palabras clave:** diarrea, potrillo, *Croton lechleri*, SB 300, canales de cloro

## Introduction

Diarrhea in foals is very common and there are many causative agents (viral, bacterial, protozoa, parasites, drug or dietary associated, toxins) that manifest with clinical signs of watery diarrhea (Atherton *et al*., 2009a, 2009b; Lester, 2001; Wohlfender *et al*., 2009). An intestinal insult (virus, bacteria, toxin) results in stimulation of intracellular cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and cytosolic calcium and/or protein kinases, which in turn cause an increase in chloride secretion through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel located on the apical membrane of enterocytes (Barrett & Keely, 2000) (Figure 1). Other apical channels such as calcium-activated chloride channels (CaCCs) and type-2 chloride channels also produce intestinal chloride secretion. CaCCs appear to play a role in the complex etiology of secretory diarrhea caused by rotavirus (Lorrot & Vasseur, 2007). Activation of the chloride channels and resultant increased secretion of chloride into the intestinal lumen results in osmotic movement of water into the lumen. This increase in intestinal water overwhelms the absorptive capacity of the small and large intestine with resultant diarrhea (Cottreau *et al*., 2012).

Latex of the *Croton lechleri* tree, (Dragon's blood), which is found in the western Amazonian region of South America (Jones, 2003), has been used by indigenous tribes as a remedy for diarrhea for many years (Mark *et al*., 2000). Botanical extracts of this latex have been shown *in vitro* to be effective inhibitors of both CFTR and CaCCs channels (Fisher *et al*., 2004). This mode of action suggests that it may be an aid in reducing the water content of feces during episodes of secretory diarrhea.

Specific treatments for diarrhea in foals are aimed at causative agents and include the administration of antimicrobials; while supportive care may include fluid therapy and anti-inflammatories combined with antidiarrheal products. Current antidiarrheal treatments have a variety of actions including adsorbents, probiotics and toxin binders.

Bismuth subsalicylate has been shown to have some antisecretory effects but the exact mode of action is unknown (DuPont, 1987; Ericsson *et al*., 1990). Currently there are no products available for foals with specific antisecretory properties to normalize the intestinal secretion of chloride.

The rationale for this study was that if intestinal secretion of chloride in response to an insult can be controlled or normalized, then the feces produced would have a lower water content which would in turn aid in avoiding diarrhea associated dehydration and secondary complications associated with it.

## Materials and methods

This was a randomized, blinded, multisite, safety and efficacy pilot study of enteric-coated SB-300 paste in client-owned foals with active watery diarrhea. This study was performed in the province of Buenos Aires, Argentina, during the 2015 breeding season. Five sites were initiated. Site and treatment by site interaction terms were included in the models.

### *Foals*

Following identification of potential participants and the signing of an informed consent form, screening assessments were performed to determine eligibility for enrollment into the study. Inclusion criteria were: male or female foals of any breed between birth and 16 weeks of age with a baseline fecal score of 3 or 4 (Table 1). Foals of foal heat diarrhea age (6-15 days) were only considered for enrollment if they showed signs of complication of the foal heat diarrhea (fever, absence of nursing or other clinical signs such as dehydration). Foals were excluded if they were suffering from nongastrointestinal disease or injury, fractious, moribund, >16 weeks of age, weaned, orphaned, treated with oral antidiarrheals within 7 days of the first dose administration or had a fecal score of <3 or >4.
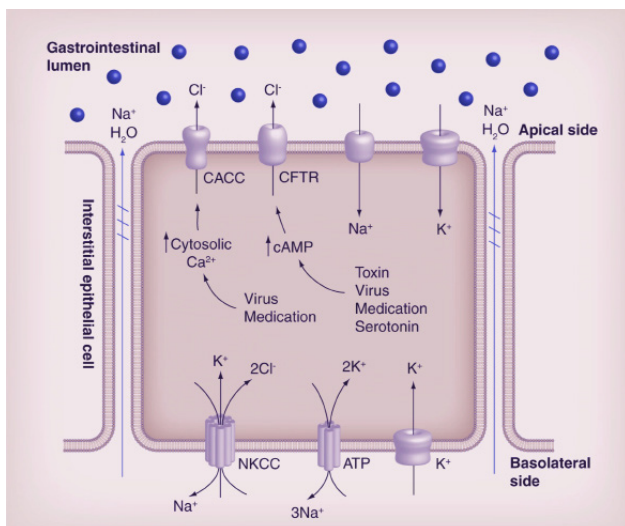
Following enrollment foals were randomly



Figure 1. Mechanisms involved in intestinal epithelial cell chloride secretion.SB-300, a botanical extract of *Croton lechleri* (blue circles) binds to the apical surface of the cystic fibrosis transmembrane conductance regulator and the calcium-activated chloride channels, blocking secretion of chloride into the intestinal lumen.

$Ca^{2+}$: calcium; CACC: calcium-activated chloride channels; cAMP: cyclic adenosine monophosphate; CFTR: cystic fibrosis transmembrane conductance regulator; $Cl^-$: chloride; $H_2O$: water; $K^+$: potassium; $Na^+$: sodium; NKCC: sodium-potassium-chloride-cotransporter.

Table 1. Fecal score. Foals with a fecal score of 3 or 4 were eligible for enrollment. Foals with a fecal score of 1 or 2 were considered to have insufficient diarrhea for enrollment and those with a grade 5 were excluded due to the possibility of hemorrhagic enterocolitis.

| Fecal description | Score |
|---|---|
| Well-formed feces | 1 |
| Soft or very soft, moist (cow patty consistency) | 2 |
| Watery, liquid feces with some particulate matter either evident adhered to the tail or perineum or upon the surface of the bedding | 3 |
| Severe watery diarrhea with no particulate matter visible or no diarrhea seen but watery staining of the tail, perineum or walls evident. | 4 |
| Hemorrhagic diarrea | 5 |

divided into one of three groups. A randomization schedule was generated using SAS proc plan (SAS for Windows, version 9.3 or higher, Cary, NC). Medical history was obtained and a complete physical examination was performed on all eligible foals. Baseline blood work and fecal analyses were performed. Blood work consisted of white blood cell differential percentages and absolute counts, hematocrit and a serum biochemistry profile (albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin/globulin ratio, calcium, chloride, creatinine, direct bilirubin, gamma glutamyltransferase (GGT), globulin, glucose, iron, potassium, sodium, total bilirubin, total protein, triglycerides, urea nitrogen, serum amyloid A and plasma fibrinogen.

Fecal collection for analyses was conducted at baseline only, except for Salmonella spp. cultures which were conducted at baseline, 24 and 48 hours (+/- 6) hours post t=0 (time of first treatment administration). Rotavirus antigen test and clostridial antigen/toxin ELISA tests were also conducted. Fecal aerobic cultures for detection of other pathogens were also performed. Additional blood samples were collected and analyzed at the end of the treatment period (T72 h) and at the end of the observation period (T144 h) and at post-treatment observation period (72-144 h). Following enrollment, the foal entered the treatment period (0-72 h), which was followed by a post-treatment observation period (72-144 h). From the onset of the treatment period to the end of the observation period, ongoing fecal scoring (four times daily), clinical assessments including adverse events (four times daily), and concomitant medications were recorded. Clinical assessments were made based on a standardized form with heart rate, respiratory rate, mucous membrane color, capillary in refill time, rectal temperature and body condition score being recorded. Total time on

study for each foal was 6 days. Fecal scoring was conducted jointly with the dosing times (four times a day with a minimum of 4 hours in between assessments) during the treatment period, followed by twice a day thereafter until the end of the observation period.

### Treatments

The study design included 3 treatment groups. Treatment groups were divided such that approximately 20 foals each would receive: investigational product/probiotic (IVP/P) four times daily (QID) (Group 1), IVP/P two times daily (BID) and placebo BID (Group 2), or placebo QID (Group 3). Group 2 therefore received alternating doses of IVP/P and placebo that represents half the daily dose of IVP/P received by Group 1. Each foal received a 30 ml syringe of either IVP or placebo QID. There were two test materials: one contained the IVP and a probiotic (DigestivTM), the second was a placebo.

Both test materials were formulated as a paste and packaged in identical 30 ml metered syringes. Both test materials contained the same excipients. The IVP (SB-300) is a proprietary standardized botanical extract of sustainably harvested *Croton lechleri*. All formulation components were considered safe for use in animals.

The following medications were prohibited beginning at baseline through to the end of the observation period: additional probiotics, oral electrolytes (IV fluids were allowed) and other oral antidiarrheal treatments (within 7 days prior to the first dose being administered and while on study).

The weight range for foals enrolled in the study was 37-140 kg, which resulted in a dosing range of 2-18 mg/kg.

Descriptive statistics (number of subjects, mean, standard deviation, standard error of the mean, median, minimum and maximum values) were presented for age (weeks), baseline body weight (kg) and baseline fecal score, in each treatment group.

Safety was assessed by the following parameters: adverse events, plasma chemistry and hematology. Descriptive statistics were presented by treatment group, for each parameter for the screening visit, end of treatment period, end of observation period and for the changes from screening to end of treatment period and end of observation period. Possible differences between treatment groups in the changes from screening were assessed by ANCOVA modeling. The model contained baseline fecal score as a covariate with treatment, time point and treatment by time point as a fixed effect. Residuals from the model were investigated for normality using the Shapiro-Wilk test. If the normality assumption was rejected at an alpha level of 0.05, the degree of departure from normality was

investigated and a rank transformation was applied. Additionally, clinically significant changes from screening were investigated for white blood cell count (change from screening >12,500 cells/µl), serum amyloid A (change from screening >30 µg/ml) and percentage of banded neutrophils (change from screening >5 %). The number (%) of foals with clinically significant changes was presented by treatment group. Possible differences between treatment groups were assessed with Fisher's Exact (2-tail) test.

To standardize care and where possible any foal that received antibiotics was placed on ceftiofur (5 mg/kg q8h) and amikacin (22 mg/kg q24h). A treatment responder was defined as a foal that developed formed feces or had no feces (fecal score <3), and maintained formed feces or no feces (i.e. no fecal score of 3, 4 or 5) for a minimum of 16 consecutive hours within a 24 hours period during the 72 hours treatment period. However, due to the difficulty in determining if "no observed feces" actually corresponded to "no feces", only those foals with observable feces that could be scored as <3 were considered to be responders.

All tests of significance were assessed at alpha = 0.05, 2-sided. No adjustments were applied for multiple comparisons. All statistical analyses were conducted using SAS for Windows.

For the treatment responder parameters, the number (%) of successes was presented for each treatment group. Possible differences between treatment groups were assessed using a generalized linear mixed model assuming a binomial distribution and a logit link. The model contained baseline fecal score as a covariate with treatment, time and treatment by time interaction as fixed effects.

Subgroup analyses, including only those foals with a baseline fecal score of 4 were also completed.

## Results

At study completion site 1 through 5 had enrolled 22, 6, 21, 6 and 6 foals respectively, resulting in a total of 61 enrolled foals. One foal was the subject of an early withdrawal due to debilitation and worsening clinical condition, with a total of 60 foals completing the study. Data from all foals were used in the analysis of safety. The safety population included all animals that received at least one dose of IVP or placebo. The safety population included 20 animals treated with the IVP QID, 21 animals treated with IVP BID and placebo BID, and 20 animals treated with placebo QID. An efficacy population was defined as a subset of the safety population excluding 6 foals that had ≤ 1 recorded fecal score through the 144 hours of the study. The efficacy population included 19 animals treated with IVP QID,

19 animals treated with IVP BID and placebo BID and 17 animals treated with placebo QID.

An arbitrary scoring system was established with "no observation of feces" being noted as just that. In the 0-72 h time frame, there were a total of 732 possible fecal scores with 496 recorded as "No fecal sample (NFS)". For each treatment group, the following scores were recorded as NFS: IVP/P QID (161 of 240), IVP/P BID/placebo BID (171 of 252), and placebo QID (164 of 240).

Age, body weight and baseline fecal score were similar among treatment groups for both the safety and efficacy populations. The chi-square p-value was not statistically different between the groups for those that received antibiotics, indicating a similar distribution.

### Safety parameters

Hematology and serum amyloid A (SAA). The comparison of the number of foals with clinically significant white blood cell count and serum amyloid A values were not found to be statistically different between groups at enrollment or by responder/nonresponder.

Serum biochemistry. No clinically significant differences were found at screening or between treatment/placebo groups at T 72 h or T 144 h.

### Adverse events

An adverse event was defined as any change in physical of clinical pathology evaluations from baseline. These were classified as mild, moderate or severe. Adverse events noted included alopecia of the perineal area, fevers and increases/decreases in white cell counts. Most adverse events noted were deemed to be related to the underlying disease/diarrhea rather than having a direct relationship with the investigational product other than the development of transient hard feces, which was noted in a number of foals. No severe events were reported during either the treatment or observation period.

### Treatment outcome parameters and analyses

The number of responders in each group and statistical comparisons for the evaluated population are presented in Table 2. The percentage of responder from 0-72 h was greater in the IVP BID group (68 %) than in the placebo group (35 %) (p= 0.03). The percentage of responders from 0-96 h was also found to be greater in IVP BID group (79 %) when compared to the placebo group (47 %) (p= 0.03). From 0-120 h, the percentage of responders in the IVP QID group was 84 % versus 53 % in the placebo group, which was found to be significant (p= 0.04). No significant differences were found between groups for the periods 0-24 h and 0-48 h.

Table 2. Number and percentage of responders (a foal that developed formed feces -fecal score < 3-, and maintained formed feces for a minimum of 16 consecutive hours within a 24 hours period during the 72 hours treatment period), during the treatment and observation periods. Also, statistical comparison of each treatment group to the placebo.

| Period | Active QID | Active BID | Placebo QID | Pairwise comparison to placebo Fisher's Exact (2-tail) p-value | |
| --- | --- | --- | --- | --- | --- |
| | | | | Active QID | Active BID |
| | Neo 101 | Neo 101 | Neo 101 | Neo 101 | Neo 101 |
| 0-24 h | 0/19 (0.0 %) | 2/19 (10.5 %) | 3/17 (17.6 %) | 0.96 | 0.46 |
| 0-48 h | 2/19 (10.5 %) | 6/19 (31.6 %) | 3/17 (17.6 %) | 0.55 | 0.21 |
| 0-72 h | 9/19 (47.4 %) | 13/19 (68.4 %) | 6/17 (35.3 %) | 0.40 | 0.03 |
| 0-96 h | 13/19 (68.4 %) | 15/19 (78.9 %) | 8/17 (47.1 %) | 0.20 | 0.03 |
| 0-120 h | 16/19 (84.2 %) | 14/19 (73.7 %) | 9/17 (52.9 %) | 0.04 | 0.10 |
| 0-144 h | 17/19 (89.5 %) | 15/19 (78.9 %) | 10/17 (58.8 %) | 0.04 | 0.17 |

Table 3. Number and percentage of responders of the subset of foals that had a fecal score of 4 upon enrollment, during the treatment and observation periods and shows statistical comparison of each treatment group to the placebo.

| Period | Active QID | Active BID | Placebo QID | Pairwise comparison to placebo Fisher's Exact (2-tail) p-value | |
| --- | --- | --- | --- | --- | --- |
| | | | | Active QID | Active BID |
| 0-24 h | 0/16 (0.0 %) | 1/11 (9.1 %) | 0/13 (0.0 %) | 0.99 | 0.98 |
| 0-48 h | 2/16 (12.5 %) | 3/12 (25.0 %) | 1/13 (7.7 %) | 0.78 | 0.22 |
| 0-72 h | 8/16 (50.0 %) | 9/13 (69.2 %) | 4/13 (30.8 %) | 0.31 | 0.05 |
| 0-96 h | 12/16 (75.0 %) | 11/13 (84.6 %) | 5/13 (38.5 %) | 0.05 | 0.02 |
| 0-120 h | 15/16 (93.8 %) | 10/13 (76.9 %) | 6/13 (46.2 %) | 0.02 | 0.10 |
| 0-144 h | 15/16 (93.8 %) | 11/13 (84.6 %) | 7/13 (53.8 %) | 0.03 | 0.10 |

For the subset of foals with a baseline fecal score of 4, the percentage of responders from 0-96 hours was found to be significantly different (p= 0.02) between the IVP BID (85 %) and placebo groups (39 %). For the interval from 0-120 hours, the IVP QID group was found to be significantly different (p= 0.02) from the placebo group (94 % versus 46 %, respectively; Table 3). No significant differences were found for the periods 0-24 h, 0-48 h and 0-72 h.

## Discussion

This trial design allowed for 72 hours of treatment and a further 72 hours observation period. This was designed to allow extended assessments of foals post-treatment, as the duration of an acute diarrhea episode in foals is commonly 5-6 days. A key component and often, key difficulty in any diarrhea study, is the ability to evaluate fecal production. The diarrhea scoring system used in this trial was derived from a scoring system that had been used in a previous safety study for the same product carried out in a group of older foals (Divers; personal communication). Extensive literature searches revealed little in terms of scoring fecal production in horses or foals. The most common difficulties encountered and not adequately addressed by this scoring system are the following:

1. No obvious fecal production due to the feces simply not being observed and thereafter mixed with bedding or the fecal production is of such watery consistency that if not directly observed it soaks directly through the bedding,

2. No fecal production associated with ileus in the acute phase of illness, and

3. No fecal production, which frequently occurs in the recovery phase.

While in each of these scenarios we may expect to find some other clinical indicators such as improvement in attitude and appetite that may indicate if the

animal is improving it was felt that many of these indicators are subjective and frequent exceptions were likely.

Given the strict use of the term "NFS" for any period when no fecal production was witnessed, many foals had missing fecal scores, which resulted in 6 foals being excluded from the efficacy analysis due to only having a single fecal score. Therefore, it is possible that these foals responded or reached clinical resolution before such time as was recorded for them. A recommendation for future studies would be the use of cameras with recording systems in the stalls to facilitate detection of defecation.

The product was palatable and easily administered in the paste formulation. There were no significant safety issues related to administration. One foal was withdrawn due to a worsening clinical condition. The foal had marked signs of sepsis and a cardiac arrhythmia was noted at withdrawal that had not been present at enrollment. The foal died 18 hours later and a necropsy indicated septicemia with cultures yielding a *Pseudomonas sp*. Unblinding of the foal's treatment group was requested at withdrawal and it was revealed that the foal had received placebo. It was noted that a number of foals in the study produced hard feces, which in some cases was noted to have a small amount of surface blood, likely related to mucosal bleeding. In total, 5 foals were noted to have this change within the 144 hours of the study. The production of hard feces in all cases was transient and none of the foals experienced any long-term medical problems related to the production of hard feces. In the opinion of the trial veterinarian, the production of hard feces was more likely to occur in cases where the diarrhea had resolved before the end of the treatment period but use of the product was continued past the resolution of the diarrhea.

Analysis of plasma biochemistry and hematology revealed that foals across the three groups had similar laboratory results on admission indicating a similar level of systemic illness across the groups. Through the regulation of chloride channels, SB-300 prevents hyper-stimulation of electrolyte secretion in response to an intestinal insult. This helps to control fluid balance and prevent dehydration but is not a direct treatment for the cause of the diarrhea. Depending on the etiology of diarrhea, antibiotics may be required and as such where deemed necessary based on clinical and laboratory findings foals were placed on antibiotic therapy.

Another challenge when performing trials evaluated foals with diarrhea is the range of etiologies that may be involved. Diarrhea may be infectious or noninfectious and the animals may show minimal clinical signs other than the diarrhea or may be severely systemically ill. Standardization of the degree of illness

and etiology is impossible. Animals may appear to only be mildly ill if presented soon after the onset of diarrhea and deteriorate after admission or vice versa. All efforts were made to try to enroll foals with a comparative level of illness. To this effect only foals with diarrhea scores of 3 or 4 were enrolled. Foals with lesser scores were likely to be not as ill and foals with fecal scores of 5 frequently carry a poor prognosis which could result in a negative bias. Every effort was made not to enroll foals with 'foal heat' diarrhea which is regarded as being a normal physiological event. Foals with foal heat diarrhea are normally presented from 6-15 days but are bright, actively nursing with no changes in physical parameters such as heart rate or body temperature. If a foal of that age group was presented with a diarrhea score of 3 or 4, it had to demonstrate some indication of complication of the foal heat diarrhea such as fever to be eligible for enrollment. Comparison of physical and laboratory parameters between the treatment groups indicated that the animals were similarly ill across all groups.

Standardization *per etiology* is also impossible. Definitive determination of the cause of diarrhea is not possible in many cases (Savage, 2009; Sellon, 2014). In this trial an etiology was determined in 28.4 % of cases (Deferrari *et al*., 2016). An additional complication is the likely mixed etiology of many cases of foal diarrhea and new research indicating the presence of previously unrecognized viruses as causes of foal diarrhea (Vega *et al*., 2016).

The argument could be made that administration of antibiotics to some foals and not to others could impact the outcome and while this is indeed true if one treatment group contains a large number of foals that receive antibiotics compared to other groups, the similar distribution between groups in this trial for those that received antibiotics; IVP/P QID 13 of 19, IVP/P BID/placebo BID 13 of 19 and placebo QID 9 of 17, indicates that the likely impact of antibiotics on the overall comparative analysis was minimal. Similarly, the argument could be made that younger foals may have a tendency towards more severe illness than older foals. When the responders were divided by age group (<1 month and >1 month), there was little numerical difference between the responders by age group but there was a numerical difference between treated and placebo for foals <1 month of age. The placebo group of foals >1 month of age only consisted of 2 foals and no inferences could be drawn (Table 4).

Probiotics are live organisms that, when administered at adequate concentrations, provide a beneficial effect beyond that of their nutritional value. They are used widely in the treatment of diarrhea in humans, small animals and equines. To standardize their use across this study, probiotics were included

Table 4. Number and percentage of responders (a foal that developed formed feces -fecal score <3-, and maintained formed feces for a minimum of 16 consecutive hours within a 24 hours period during the 72 hours treatment period), during the treatment and observation periods with foals <1month of age on the left and foals >1month of age on the right.

| Foals less than 1 month (age) | | | |
|---|---|---|---|
| Period | Active QID Neo 101 | Active BID Neo 101 | Placebo Neo 101 |
| 0-24 h | 0/9 (0.0 %) | 0/10 (0.0 %) | 2/15 (13 %) |
| 0-48 h | 0/9 (0.0 %) | 3/10 (30 %) | 3/15 (20 %) |
| 0-72 h | 3/9 (33.3 %) | 7/10 (70 %) | 6/15 (40 %) |
| 0-96 h | 6/9 (66.7 %) | 8/10 (80 %) | 8/15 (53 %) |
| 0-120 h | 7/9 (77.8 %) | 7/10 (70 %) | 9/15 (60 %) |
| 0-144 h | 8/9 (88.9 %) | 7/10 (70 %) | 9/15 (60 %) |

with the investigational product in the dosage syringe. The positive or negative effect of the probiotics on the outcome of this trial is debatable and unknown. At this point in time the only conclusion that can be drawn was that the combination of the investigational product with probiotics appears to be effective in the treatment of foal diarrhea.

In conclusion, the administration of a paste formulation of SB-300 was well tolerated with minimal adverse events and showed numerical and statistically significant improvement in the resolution of diarrhea in nonweaned foals less than 4 months of age. It is recommended that a larger trial be performed to further evaluate the benefits of the product without additional probiotic.

## Acknowledgements

## Conflict of interest

Siobhan McAuliffe provides consultation services to Jaguar Animal Health Inc. None of the other authors have any conflicts of interest to declare.

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