Homeopathic treatment as an alternative prophylactic to minimize bacterial infection and prevent neonatal diarrhea in calves

Bruno F. Fortuoso\textsuperscript{a}, Andreia Volpato\textsuperscript{b}, Luana Rampazzo\textsuperscript{a}, Patricia Glombowsky\textsuperscript{a}, Luiz Gustavo Griss\textsuperscript{a}, Gabriela M. Galli\textsuperscript{a}, Lenita M. Stefani\textsuperscript{a,b}, Matheus D. Baldissera\textsuperscript{c}, Emanuel B. Ferreira\textsuperscript{d}, Gustavo Machado\textsuperscript{e}, Aleksandro S. da Silva\textsuperscript{a,b,}\textsuperscript{*}

\textsuperscript{a} Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Brazil
\textsuperscript{b} Post-Graduate Program in Animal Science, Universidade do Estado de Santa Catarina (UDESC), Brazil
\textsuperscript{c} Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Brazil
\textsuperscript{d} Medical Veterinarian, Londrina, Brazil
\textsuperscript{e} Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, USA

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\textbf{ABSTRACT}

Bovine neonatal diarrhea is common due low immunity in newborn calves, poor management (or absence) of sanitary barriers, and other factors. Newborn calves with diarrhea in the first days of life suffer failure to thrive and may die if left untreated. The aim of this study was to evaluate whether prophylactic administration of a homeopathic product (Dia 100\textsuperscript{®}) can control bovine neonatal diarrhea in calves born on a farm with substantial sanitary challenges. We counted total bacteria and protozoan parasites in fecal samples. We measured serum glucose, total protein, globulin, albumin, cholesterol and triglycerides on days 1, 7 and 14 of life. Twenty newborn calves were maintained in individual stalls, and were divided in two groups: ten untreated animals (control) and ten animals treated with Dia 100\textsuperscript{®}. Fecal consistency was evaluated daily. We diagnosed diarrhea in five animals in the treated group, and in all animals from the control group. Infections with \textit{Escherichia coli} and \textit{Giardia duodenalis} were identified as the responsible organisms. The \textit{E. coli} count was low in the treatment group on day 7 of life compared with the control group. Antibiotics were given to eight animals in the control group, and to two animals in the treatment group. On day of life 7, serum levels of total protein and globulins were higher in the control group, but were lower on day 14. Serum levels of glucose and triglycerides were greater in treated animals on days 7 and 14, suggesting that the homeopathic product contributes to improvement of intestinal health and absorption and nutrients. We conclude that Dia 100\textsuperscript{®} controls diarrhea with 50\% of efficacy, and reduces antibiotic utilization.

\textbf{1. Introduction}

The production of calves is a fundamental part of the life cycle of dairy cattle. The suckling period is particularly critical. Newborn calves commonly suffer from neonatal diarrhea due to low immunity, handling problems, and absence of sanitary barriers [1]. Bovine neonatal diarrhea is associated with economic losses due high mortality, therapeutic failures, weight loss, and impaired development. Several factors are associated with neonatal diarrhea, including viral, bacterial and protozoan infections [2]. The common pathology is intestinal hypersecretion, which interferes with digestion and absorption [3].

Antibiotics are commonly used to treat bovine neonatal diarrhea, but excessive use has been linked to adverse effects on human and animal health, as well as adverse effects on the environment [4].

Alternative treatments have emerged as new approaches for treatment of bovine neonatal diarrhea. Our hypothesis is that the use of homeopathic agents decreases the use of chemical drugs, and reduces the occurrence of diarrhea. Homeopathic treatments are permitted in cattle raised in an organic production system [5]. According to [6], homeopathic agents stimulate animal immune systems and protects them from infectious diseases.

However, the effects of homeopathic products during the newborn calf suckling period remain unknown. Thus, the aim of this study was to evaluate the effects of prophylactic administration of a homeopathic product on bovine neonatal diarrhea on a farm with substantial sanitary challenges.

\textsuperscript{*} Corresponding author. Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Brazil.
\textit{E-mail address:} aleksandro.ss@yahoo.com.br (A.S. da Silva).

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2. Materials and methods

2.1. Product

The homeopathic product (Dia 100®) was purchased from the REAL H. company. According to the manufacturer, the product is indicated for prevention and/or cure of diarrhea, without side effects.

2.2. Animals and experimental design

The study was conducted on a commercial farm located in Xanxerê, Santa Catarina, Brazil. The farm was selected because it has adequate facilities for our experiments, and because the farm is known to deal with considerable sanitary challenges that have led to a substantial incidence of diarrhea in their suckling animals.

Twenty newborn calves (day 1 of life; births at intervals of 5 days) were divided in two groups with ten animals each: an untreated group (control group), and a treatment group. The treatment group was fed orally with Dia 100® in two divided doses (18 g) on days 1 and 7 of life. The first dose was administered twenty minutes after ingestion of colostrum. All newborns ingested high qualitycolostrum (21%-31% fat), as measured by a refractometer (Brix®).

The calves were assigned to individual stalls in a covered shed, and were fed the same milk-based food. From the fifth day of life, the animals received feed (14% of protein) and water ad libitum. All animals were monitored daily to evaluate fecal consistency in order to diagnose diarrhea [7].

2.3. Sample collection

Feces were collected directly from the rectal ampulla on days 7 and 14 of life. Samples were stored in plastic pots and refrigerated until analysis. One portion of feces were stored in sterile plastic pots for total bacterial count.

On days 1, 7 and 14 of life, blood was drawn from the jugular vein, using collection tubes without anticoagulant. Whole blood was centrifuged at 3500 rpm × 10 min. Supernatants (serum samples) were stored at - 20 °C until analysis.

2.4. Serum biochemistry

We measured serum levels of total protein, albumin, urea, cholesterol, glucose and triglycerides using commercial kits in a semi-automated device (BioPlus 2000®). Serum globulin level was calculated as total serum protein minus serum albumin.

2.5. Bacterial count

One gram of feces was used to count bacteria (total fecal coliforms and E. coli). Fecal samples were prepared using 3M Petrifilm™, according to manufacturer’s instructions. Counts are expressed as UFC per gram of feces.

2.6. Parasitological examination

A centrifugal-flotation technique was used evaluate the presence of protozoans in calf feces [8]. A hypersaturated sugar solution was used to dilute 2 g of feces. Results were defined as “positive” or “negative”, depending on the presence or absence of protozoans observed by light microscopy.

2.7. Statistical analyses

The data from two groups of animals, treated and control, were analyzed first using descriptive statistics. Data are expressed as mean and standard deviation for CFU (E. coli and total coliforms), total protein, globulin, glucose, triglycerides, albumin, cholesterol and urea. All variables for each group and day of observation were tested for normality using the Shapiro-Wilk test. We applied the Levene test (log transformation) to evaluate for skewness, kurtosis and homogeneity when needed. A t-test was used to analyze all parameters on days 1, 7, and 14. P < 0.05 was defined as statistically significant. Statistical analysis was performed using R-language, v.3.1 (R Development Core Team 2012).

3. Results

3.1. Clinical signs

We observed diarrhea in five animals in the treatment group and all animals in the control group. Two calves in the treatment group presented with severe diarrhea and pyrexia (> 39.5 °C), and were treated for five days with antibiotic (trimethoprim-sulfamethoxazole, 1.3 mg/kg, IM) and anti-inflammatory (flunixin meglumine: 2.2 mg/kg, IM). In the control group, eight animals suffered severe diarrhea, and were treated with antibiotic.

3.2. Bacterial count and parasitological examination

Bacterial counts are shown in Fig. 1. The number of total fecal coliforms were not significantly different between groups (P > 0.05), though total coliforms numbers were greater in the control group on days 7 and 14. The E. coli count was greater in the control group on day 7 compared to treated group (P < 0.05), different on day 14 (Fig. 1).

The parasitological examination performed on day of life 14 demonstrated roughly equivalent infection with G. duodenalis in both groups (seven animals in the control group and five animals in the treatment group).

3.3. Serum biochemistry

The control group had higher serum total protein and globulin levels (P < 0.001).
levels on day of life 7, compared with the treatment group (P < 0.001). By day 14, both levels were significantly lower in the control group (P < 0.001, Fig. 2). Serum glucose and triglycerides were greater in the treatment group compared with the control group at all time points (Fig. 3). Serum albumin, cholesterol, and urea did not differ between groups (P > 0.05, Table 1).

4. Discussion

Bovine neonatal diarrhea is associated with infections caused principally by bacteria and protozoa belonging to the genera *Escherichia* and *Giardia*, respectively [9–11]. Newborn calves are particularly susceptible to enterotoxigenic *E. coli* (ETEC) during the first days of life, manifesting as watery diarrhea [12]. The toxin produced by ETEC after colonization of intestinal epithelium increases the secretion of chloride, and osmotic pressure draws water into intestinal lumen [12]. In our study, the homeopathic product Dia 100® helped avoid or minimize bovine neonatal diarrhea. The homeopathic agent we used is formulated with *Colibacillinum, Mercurius dulcis, Chinchona officinalis, Enterococcinum, Podophyllum peltatum* and *Colocynthis*, diluted on the centesimal scale. Dilution to this scale makes it difficult explain how the agent prevents or cures diarrhea. Homeopathy attempts to explain these effects by reference to the electromagnetic energy of these substances, in which the nervous system captures the energy [13]. Another mechanism is described by the Law of Similars (*Simillimum*), which states that substances causing similar symptoms of disease when provided to healthy animal can heal and maintain normal function in sick animals with similar symptoms [14,15].

Serum levels of total protein and globulin remain within normal limits for calves from birth to 45 days of life [16–18]. Concentration of both factors increases after ingestion of colostrum, which is rich in protein [16]. In our study, total protein levels increased by day of life 14 in treated, but not in control animals. The control group had higher globulin levels on day of life 7, compared with the treatment group, possibly due to the inflammatory response *E. coli* infection. However, globulin levels in the control group decreased on day 14 of life, possibly as a consequence of severe diarrhea, that can lead to poor absorption of nutrients and/or exhaustion of the immune system. It is important to emphasize that infection caused by ETEC causes intestinal villus atrophy, consequently impairing nutrient absorption [12]. This effect may have been mitigated in the control group *E. coli* infection was treated with antibiotics. We believe that the homeopathic product contributed to protection of the intestinal epithelium, and consequently prevented diarrhea. This treatment for diarrhea has been used in humans for more than 20 years [19,20], but its use in animals is contingent on published studies demonstrating benefit. Thus, it is important that the scientific community investigate the effectiveness of these products.

Serum glucose and triglycerides levels were greater in the treatment group, suggesting that the homeopathic product improved nutrient absorption. In the control group, impairment of nutrient absorption caused by diarrhea may explain the lower serum levels of glucose and triglycerides [21,22]. Newborn calves have limited energy reserves, therefore any condition that impairs the energy ingestion can alter glycemic status [23]. These data reinforce the notion that the homeopathic product contributes to improvement of intestinal health, favoring nutrient absorption. The other metabolic markers did not differ between groups. This may be because occurred because lipid metabolism and protein catabolism can take place via alternative pathways that do not depend solely on dietary intake.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>Mean (standard deviation)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>1</td>
<td>2.13 (0.34)</td>
<td>0.25</td>
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<tr>
<td></td>
<td>7</td>
<td>2.94 (0.45)</td>
<td>0.65</td>
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<tr>
<td></td>
<td>14</td>
<td>3.02 (0.68)</td>
<td>0.86</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
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<tr>
<td></td>
<td>7</td>
<td>51.70 (14.46)</td>
<td>0.84</td>
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<tr>
<td></td>
<td>14</td>
<td>48.28 (16.01)</td>
<td>0.38</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
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<td>30.84 (8.16)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>28.10 (5.3)</td>
<td>0.71</td>
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<td></td>
<td>14</td>
<td>29.5 (7.11)</td>
<td>0.80</td>
</tr>
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</table>
5. Conclusion

The homeopathic product Dia 100° was 50% effective in controlling bovine neonatal diarrhea, and reduced the use of antibiotics (60% of calves compared control). In addition, the product reduces the number of pathogenic bacteria in fecal samples, contributes to improvement of intestinal health, and favors nutrient absorption.

Compliance with ethical standards

Experimental protocol was approved by the Animal Welfare Committee of the State University of Santa Catarina (UDESC), under number: 3354300517.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References


