Microbial Pathogenesis 114 (2018) 95–98

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

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

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

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

ARTICLE INFO

Keywords:
Diarrhea
Homeopathic
Treatment
Calves
Intestinal health
E. coli

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®) 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®. 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 *Escherichia coli* and *Giardia duodenalis* were identified as the responsible organisms. The *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® controls diarrhea with 50% of efficacy, and reduces antibiotic utilization.

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 hyper secretion, 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.

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

https://doi.org/10.1016/j.micpath.2017.11.041
Received 12 October 2017; Received in revised form 22 November 2017; Accepted 23 November 2017
Available online 24 November 2017
0882-4010/

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 animas 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 quality colostrum (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 TM®, 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

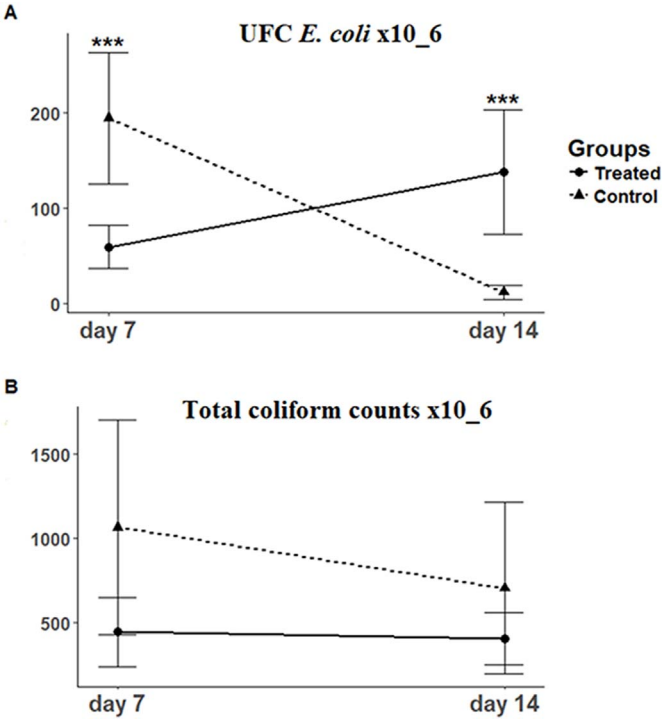

Fig. 1. A. Distribution by group of *Escherichia coli* CFUs and B. total coliforms. Mean and standard deviation are shown. ***$P < 0.001$.

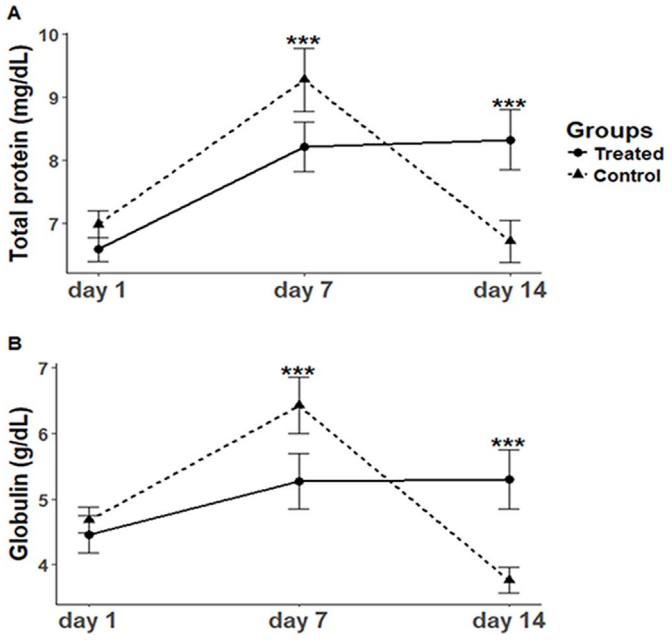

Fig. 2. Distribution by group of total protein and globulin levels. Mean and standard deviation are shown. ***P < 0.001.

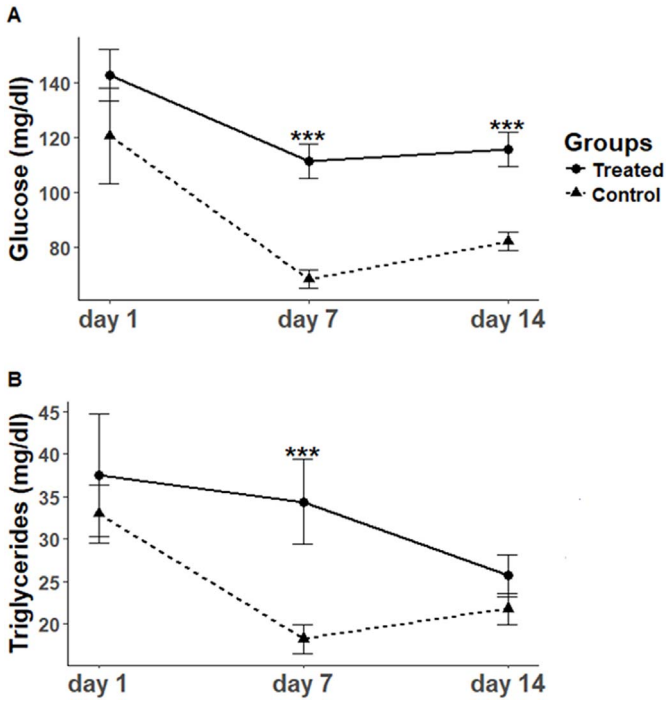

Fig. 3. Distribution by group of total glucose and triglycerides levels. Mean and standard deviation are shown. ***P < 0.001.

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 o ur 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, Podophylum 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 the 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].

Table 1
Distribution of metabolic variables for treated and control groups. Mean values with standard deviation are shown.

Variable	Day	Mean (standard deviation)		
		Control	Treatment	P value
Albumin (mg/dL)	1	2.13 (0.34)	2.31 (0.34)	0.25
	7	2.94 (0.45)	2.85 (0.37)	0.65
	14	3.02 (0.68)	2.96 (0.90)	0.86
Cholesterol (mg/dL)	1	28.40 (8.64)	27.9 (12.81)	0.92
	7	51.70 (14.46)	53.0 (13.43)	0.84
	14	48.28 (16.01)	61.90 (37.7)	0.38
Urea (mg/dL)	1	30.84 (8.16)	37.2 (16.16)	0.27
	7	28.10 (5.3)	27.0 (7.13)	0.71
	14	29.5 (7.11)	30.40 (6.25)	0.80

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. Is important 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, 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.

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.

Acknowledgment

Real H for making the products available for testing.

References

[1] A. Volpato, A.A. Tonin, G. Machado, L.M. Stefani, G. Campigotto, G.M. Galli, A.S. Da Silva, Gastrointestinal protozoa in dairy calves: identification of risk factors for infection, Rev. MZV Cordobá 22 (2017) 5910–5924.
[2] F.J. Benesi, Síndrome diarréia dos bezerros, Rev. CRMV-ES 2 (1999) 10–13.
[3] D.M. Foster, G.W. Smith, Pathophysiology of diarrhea in calves, Vet. Clin. North Am. Food Anim. Pract. 25 (2009) 13–36.
[4] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment, Chemosphere 65 (2006) 725–759.
[5] M.C. Arenales, A. Moraes, F. Moraes, Evaluation of the Use of Homeopathic Products for the Control of Parasites and Weight in Indian Cattle (Nelore), World Buiatrics Congress, Brazil, 2006.
[6] V.M. Arruda, M.C. Cupertino, S.P. Lisboa, V.W.D. Casali, Homeopatia tri-una na agronomia: as propostas de Roberto Costa e algumas relações com os agrossistemas, Fitotecnia – UFV, Viçosa, 2005, p. 119.
[7] T.F. Fagundes, L.G.P. Vidal, P.A.M. Alves, W.S. Tassanari, S.M.O. Coelho, R.C.A.A. Menezes, A.H. Fonseca, M.J.S. Pereira, Análise descritiva da diarreia em uma coorte de bezerras criadas em sistema de casinhas até cem dias de idade, Município de Piraí, Estado do Rio de Janeiro, Brasil, Semina Ciênc. Agr. 35 (2014) 1895–1912.
[8] S.G. Monteiro, Parasitologia na Medicina Veterinária vol. 1, Roca, São Paulo, 2010, p. 368.
[9] D.R. Snodgrass, H.R. Terzolo, D. Sherwood, I. Campbell, J.D. Menzies, B.A. Synge, A etiology of diarrhoea in young calves, Vet. Rec. 119 (1986) 31–34.
[10] L.F.D. Schuch, Diarreia em bezerros, in: F. Riet-Correa, A.L. Schild, R.A.A. Lemos, J.R.J. Borges (Eds.), Doenças de ruminantes e equídeos, vol. 3, 2007, pp. 496–508.
[11] A.A.R. Lemos, A.C. Coelho, Enfermidades caracterizadas por diarreias, Principais enfermidades em bovinos de corte do Mato Grosso do Sul, UFMS, Campo Grande, 2008, pp. 265–294.
[12] Y. Cho, K.J. Yoon, An overview of calf diarrhea - infectious etiology, diagnosis, and intervention, J. Vet. Sci. 15 (2014) 1–17.
[13] C.M. Real, Homeopatia populacional: Fundamentos Ruptura de um Paradigma, Hora Vet. 164 (2008) 13–20.
[14] L.C.C. Da Costa Filho, V.L.D. Queiroz, M.F.D.A. de Souza, C.E.S.N. Zúccari, E.V. da Costa, Homeopatia aplicada à reprodução animal, Arq. Ciênc. Vet. Zool. UNIPAR 17 (2015) 63–68.
[15] M.Z. Teixeira, C.H. Guedes, P.V. Barreto, M.A. Martins, The placebo effect and homeopathy, Homeopathy 99 (2010) 119–129.
[16] M.C. Reis, J.N. Costa, A.P.C. Peixoto, Efeito da idade e da suplementação oral com o acetato de DL-alfa-tocoferol sobre os níveis séricos de vitamina E sobre o proteinograma de bezerros, Rev. Bras. Saúde Prod. Anim. 8 (2007) 152–161.
[17] J.A.G. Hill, D.W. Costa, M.E.F. Castro, W. Hartmann, F.J. Benesi, Proteína total, proteinograma eletro forético e gama glutamiltransferase de bezerras com 30 horas de vida no Município de Campo Largo, Paraná, Rev. Acadêmica 5 (2007) 295–301.
[18] F.A. Paiva, J.A. Negrão, A.R. Bueno, A. Saran-Netto, C.G. Lima, Efeito do manejo de fornecimento de colostro na imunidade passiva, cortisol e metabólitos plasmáticos de bezerros Holandeses, Arq. Bras. Med. Vet. Zootec. 58 (2006) 739–743.
[19] J. Jacobs, L.M. Jimenez, S.S. Gloyd, Treatment of acute childhood diarrhea with homeopathic medicine: a randomized double-blind controlled study in Nicaragua, Pediatrics 93 (1994) 719–725.
[20] J. Jacobs, L.M. Jimenez, S. Malthouse, Homeopathic treatment of acute childhood diarrhea: results from a clinical trial in Nepal, J. Alt. Compl. Med. 6 (2000) 131–140.
[21] L.D. Lewis, R.W. Phillips, Diarrheic induced changes in intracellular and extracellular ion concentrations in neonatal calves, Ann. Rech. Vétér 4 (1973) 99–111.
[22] F.M. Trefz, I. Lorenz, P.D. Constable, Effects of profound acidemia on the dynamic glucose and insulin response and plasma potassium and phosphorus concentrations during an intravenous glucose tolerance test in neonatal calves, J. Dairy Sci. (2017) S0022–0302(17)30824-X.
[23] G.P. Carlson, Clinical chemistry tests, in: B.P. Smith (Ed.), Large Animal Internal Medicine, Mosby Elseivier, St. Louis, 2009, pp. 375–397.