Research in Veterinary Science 115 (2017) 478–483

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves

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ARTICLE INFO

Keywords:
Oregano essential oil
Calves
Diarrhea
Treatment
Prevention

ABSTRACT

The objective of this study was to evaluate under field conditions, whether daily administration of oregano essential oil is effective in preventing and/or diminishing the severity of neonatal diarrhea syndrome in calves aged less than 15 days. Ninety-one newborn calves from three dairy farms were assigned into two groups; "Eco" group (n = 46) calves were drenched with Greek oregano (*Origanum vulgare* ssp. *Hirtum*) essential oil (ECODIAR® liquid 5%) at the dose of 12.5 mg/kg body weight once per day until the age of 10 days. "Conts" group (n = 45) calves were left untreated and served as controls. All animals were monitored daily for the incidence of diarrhea until the age of 15 days and their fecal score was recorded. Fecal samples were collected on days 3, 6 and 10 for microbiological and parasitological evaluation. Average fecal score throughout the experiment, incidence of diarrhea, duration and severity of diarrhea episodes were significantly lower in Eco group compared to the controls. Daily administration of oregano essential oil in calves for the first 10 days of their life effectively diminishes the severity of naturally acquired diarrhea under field conditions and, under certain hygiene practices, possess a preventive effect against neonatal diarrhea syndrome.

1. Introduction

Neonatal calf diarrhea is the most common cause of illness and mortality in calves and a major cause of economic loss to cattle herds (Barragry, 1997; De la Fuente et al., 1999). Economic losses, on top to mortality, is associated with treatment, veterinary and labor costs as well as with reduced growth rates of the affected calves (Anderson et al., 2003; Ok et al., 2009). The most important infectious agents associated with the disease are enterotoxic *Escherichia coli* K99/F5 (ETEC), *Rotavirus A* (RVA), *Bovine coronavirus* (BCoV), and *Cryptosporidium* spp. with RVA and *Cryptosporidium* spp. being most frequently identified in fecal samples from young calves (Gulliksen et al., 2009; Bartels et al., 2010; Silverlås et al., 2010). Co-infections with more than one of these pathogens are frequently detected in diarrheic calves in clinical practice and are associated with increased morbidity and mortality rates (Blanchard, 2012).

It is well documented that diarrheic calves, regardless of the causative agent or agents of diarrhea, often have small intestinal overgrowth of *E. coli* bacteria. This overgrowth increases the severity of diarrhea, retards the recovery and increases the risk of bacteremia and death (Constable, 2004). In respect to this, oral administration of antibacterial agents that inhibit coliform bacterial overgrowth in calves with diarrhea might have beneficial effects on the disease outcome by decreasing the duration and the severity of the disease and by preventing the development of bacteremia.

Oregano essential oil could be used as such an antibacterial agent providing an antibiotic alternative for this purpose. It has been proven in many experiments *in vitro* that oregano essential oil and its main constituents, carvacrol and thymol, have strong antibacterial activity against gram negative bacteria and especially *E. coli* (Elgayyar et al., 2001; Si et al., 2008; Nazzaro et al., 2013). Apart from its antibacterial properties, oregano essential oil was also found to have antiviral (Pilau et al., 2011) and anticryptosporidial (Gaur et al., 2016) effects *in vitro*. Based on these properties it could be hypothesized that the administration of oregano essential oil in newborn calves might have beneficial effects not only in treating but also in preventing diarrhea syndrome. In

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http://dx.doi.org/10.1016/j.rvsc.2017.07.029
Received 1 May 2017; Received in revised form 26 July 2017; Accepted 27 July 2017

the available literature there is not any relevant controlled research evaluating potential prophylactic or treatment effects of oregano essential oil on diarrhea in calves aged < 15 days. In the only available study it was observed that the administration of oregano leaves had comparable effects with neomycin on the duration of diarrhea in diarrheic calves aged 7 to 20 days without however using control group (Bampidis et al., 2006).

The objective of the present study was to evaluate under field conditions whether daily administration of oregano essential oil is effective in preventing and/or diminishing the severity of neonatal diarrhea syndrome in calves aged < 15 days.

2. Materials and methods

2.1. Animals and experimental design

Prior to the onset of the experiment the minimum required total sample size was calculated using General Linear Multivariate Model with Wilks Likelihood Ratioprocedure at the GLIMMPSE software (http://glimmpse.samplesizeshop.org/). The desired power was set at 0.8, the type I error rate at 0.05, the desired detectable difference of days with diarrhea at 2 days with standard deviation 1; the means scale factor was set at 0.5 and the variability scale factor at 2. The results of the analysis revealed that a minimum sample size of 66 calves (33 per group) was required (Power = 0.808).

Ninety-one newborn Holstein calves (48 females and 43 males) from three dairy farms were finally used in the study. They were all born to dams that were vaccinated against *Rotavirus*, *Coronavirus* and *E. coli* F5 (K99)/F41 antigens (Lactovac C; Zoetis, Hellas) one month prior to the expected day of calving. The calves were randomly alternately assigned into one of two groups according to their birth date in each farm until the total number of 30 calves per farm was reached. In Farm 1 however 31 calves were finally used due to a twin birth at the last calving. The experiment was run in all 3 farms simultaneously from September 10th 2016 to November 12th 2016. The animals of Eco group (n = 46) were orally drenched with Greek oregano (*Origanum vulgare* ssp. *hirtum*) essential oil (ECODIAR® liquid 5%; Ecopharm Hellas S.A., Kilkis, Greece) at the dose of 12.5 mg/kg body weight once per day for the first 10 days of their life whereas those of Conts group (n = 45) were left untreated and served as controls.

The experiment started at the day of calving (day 1) and lasted until day 15. Each calf was separated from its dam after calving, weighed, its navel was disinfected using an antibiotic spray (TERRAMYCIN™ AEROSOL SPRAY; Zoetis, Hellas) and was offered its first colostrum meal. The calves of Eco group were orally drenched immediately afterwards with the respective amount of oregano essential oil that was diluted with normal saline up to the volume of 60 ml with the aid of a feeding syringe of equal volume. The following days and until day 10, oregano essential oil was administered in the calves of this group at the same way after the morning feeding. The same procedures were followed in all farms.

Feces were scored every day throughout the experiment after the morning feeding by the same person who was blinded to group allocation using a three point scale with 1 = normal, 2 = intermediate and 3 = watery and the score was recorded. Calves with fecal scores ≥ 2 were considered diarrheic. Based on these records, number of days with diarrhea (fecal score ≥ 2) was determined and diarrhea index (DI) was calculated: DI = number of days with diarrhea × average fecal score on these days.

Fecal samples were collected on days 3, 6 and 10 of the experiment for microbiological and parasitological evaluation. The samples were obtained directly from the rectum using sterile latex gloves, were separated into 3 aliquots in sterile containers and were transferred refrigerated to the laboratories for analysis. On the same days, the calves of Eco group were weighed and the daily dosage of oregano essential oil was modified accordingly. On day 3 a blood sample was also collected *via* jugular vein-puncture (21 G) into evacuated glass tubes from all calves and transferred refrigerated in the laboratory for the evaluation of passive immunity transfer.

All calves were routinely clinically examined by the same person who was blinded to group allocation at the fecal sampling days (days 3, 6 and 10) and at the end of the experiment (day 15). In cases of diarrhea the animals were clinically evaluated daily until recovery. Diarrheic calves in all farms continued milk feeding and were receiving an extra meal per day with oral electrolytes (Diaproof K®; Virbac, Hellas). In cases of dehydration or inappetence the calves were receiving Lactated Ringers solution intravenously and, if blood serum glucose concentration was lower than 3 mmol/l (as determined on farm with a handheld glucose meter; FreeStyle Precision, Abbott, UK), a dextrose 10% solution was also administered intravenously. Calves with inappetence, hypoglycemia, depression and fever or hypothermia were also treated with antibiotics, based on antimicrobial susceptibility tests the last six months (Farm 2: enrofloxacin 5 mg/kg SC, Baytril®, Bayer Animal Health GmbH, Germany; Farms 1 and 3: ceftiofour 2.2 mg/kg SC, Excenel® RTU, Zoetis, Hellas) and non-steroid anti-inflammatory drugs (carprofen 1.4 mg/kg SC, Rimadyl® Cattle, Zoetis, Hellas). In calves of Eco group that were still diarrheic after day 10, administration of oregano essential oil continued until recovery.

2.2. Calf housing and feeding management

Newborn calves in Farm 1, were housed in individual pens in a separate building. Windows (1 m by 0,5 m) served as ventilation inlets, situated 1 m above the pens and oil heaters were used for heat supplementation when ambient temperature was below 4 °C. Each individual pen had a concrete floor area of 2 m^2, with visual access to neighboring pens. Long stem wheat straw bedding was layed at a density of 70 kg/m^3. Wet bedding was removed and replaced twice a week. All rails, gates, partitions, walls, feeders and floors were cleaned on a weekly basis and after each calf was removed from the building. A pressure water system and a broad spectrum disinfectant were used and adequate time for drying was provided. Farm 2 housed newborn calves outdoors, in individual polyethylene hutches with an outside run for the calves to move around. Hutches were situated on free draining concrete and were placed 0,3 m apart, enabling visual contact between calves. Each individual hatch had a concrete floor area of 2.4 m^2. Newborn calves in Farm 3 were open housed in group pens partially enclosed under a metallic shed. Each group pen had a concrete floor area of 12 m^2 for approximately 15 calves.

Clean, dry straw bedding which was disposed of after each batch of calves, was used in all Farms. Concrete feed troughs on the external side of the pens and water bowls mounted inside the pens were used in Farm 1. Feed and water buckets were secured outside individual pens and hutches in Farms 2 and 3, respectively.

Newborn calves were bottle-fed fresh colostrum from their respective mothers in all Farms. Each calf was fed 2 l of colostrum within 2 h after birth and another 2 l after 6 h. At days two and three of their lives, all calves were fed colostrum/milk from their respective dams, at 10% of their bodyweight. At four days old and afterwards, calves in all Farms consumed milk replacer meal. The nutrient analysis of the milk replacer used is presented in Table 1. Milk replacer was offered at 39 °C, the optimal drinking temperature, twice a day. Calves were fed 10% of their bodyweight in milk replacer. Along with that, all Farms offered fresh water, which was available at all times and was replaced daily. The same calf starter (Table 2) was also offered starting at day five in all Farms. Approximately 250 g of calf starter per day were offered in a shallow bucket. The amount increased gradually, as calves started consuming all of the feed. All buckets were emptied and refreshed every 12 h with clean feed.