2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(3): 375-379, September 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.36380/scil.2020.wvj46

Comparison of Probiotic *Lactobacillus acidophilus* and Oxytetracycline for the Treatment of Early Stage Interdigital Necrobacillosis in Dairy Cows

Zhanara Kenesovna Tulemissova[1], Merej Ajbynovich Torehanov[1], Raya Zhaksygulovna Myktybayeva[2], Assem Serikovna Ibazhanova[1*], Damir Mikdatovich Khussainov[1], Zhanat Mukhametkaliyevna Batanova[1], and Symbat Suttibayevna Usmangaliyeva[1]

[1]*Department of Biological Safety, Kazakh National Agrarian University, 050000 Almaty, Kazakhstan*
[2]*Department of Microbiology and Virology, Kazakh National Agrarian University, 050000 Almaty, Kazakhstan*

*Corresponding author's Email: assemibazhanova@yahoo.com; ORCID: 0000-0002-2833-1413

ORIGINAL ARTICLE
pii: S232245682000046-10
Received: 20 Jul 2020
Accepted: 30 Aug 2020

ABSTRACT

The objective of this multilocation field trial was to compare the effectiveness of the topical application of a powdered probiotic strain and intramuscular injection of oxytetracycline for the treatment of interdigital necrobacillosis in dairy cows. The current study was conducted from April 2018 to May 2020 on 230 dairy cows with early-stage interdigital necrobacillosis diagnosed by the research veterinarians on 6 farms situated in the Almaty region of Kazakhstan. The animals were recruited randomly to one of the following groups. A trial group in which the affected hoof of a cow was cleaned, coated with powder containing 10^6 colony-forming units of *Lactobacillus acidophilus* per gram, and then bandaged. Cows in the positive control group were subjected to a single intramuscular injection of oxytetracycline at the dosage of 1.0 milligram per kilogram of bodyweight which is considered the routine treatment for interdigital necrobacillosis in the feedlots. Both procedures were executed every 72 hours during a period of two weeks with the subsequent two-week follow-up period. The treatment was discontinued when a cow was assessed as cured. Both groups were monitored daily for their limb condition and the degree of lameness during 28 days. Those cows having no evidence of lameness and lesions attributed to interdigital necrobacillosis with no disease recurrence recorded within the observation period, were considered cured. On day 15, the overall cure rates for *Lactobacillus acidophilus* 015k-1 strain and oxytetracycline were 80.87% and 83.48%, respectively. The overall odds ratio for the cure rate in the probiotic group versus oxytetracycline was 0.837. However, on day 28, all cows in both groups were found to be clinically free from the disease, and cure rates were 100%. No recurrence was recorded in any of the cases. It was concluded that the topical administration of the probiotic powder to dairy cows with early-stage interdigital necrobacillosis can result in cure rates nearly as high as those for intramuscular oxytetracycline within a period of 28 days. This is the first report on the treatment effect of *Lactobacillus acidophilus* locally applied to cattle with early-stage interdigital necrobacillosis.

Keywords: Dairy cows, Foot rot, Interdigital necrobacillosis, *Lactobacillus acidophilus*, Oxytetracycline, Probiotic, Topical administration

INTRODUCTION

Interdigital necrobacillosis (IN) is an infectious podal disease caused mainly by *Fusobacterium necrophorum* and *Dichelobacter nodosus*. These Gram-negative aerotolerant anaerobic bacteria symbiotically produce several toxins and extracellular enzymes involved in the degradation of immunologically competent cells of host, bacterial proliferation, and dermotoxic activity (Nagaraja, 2016; Osová et al., 2018; Carvallo et al., 2020). *D. nodosus* inhabits the epidermis covering the limbs of cattle, whereas *F. necrophorum* is normally present in the digestive tract of healthy cattle, but once penetrated into other tissues, it can turn into a necrosis-inducing opportunistic pathogen (Francis et al., 2019). The common clinical signs of IN include lameness (most frequently unilateral), fever, reduced feed intake, edema, and erythema in the interdigital space and the coronary band (Van Metre, 2017).

Despite the array of steps towards effective treatments for IN the disease is still distributed in many countries (Renault et al., 2018; Dendani-Chadi et al., 2020; Silva et al., 2020). In the past few decades, the efficacy of vaccination against IN has been called into question on account of a number of issues, such as serogroup conversion of the infectious agents or diet-dependent effect (Checkley et al., 2005; Markey et al., 2013). A recent study on sheep with footrot has disclosed that a whole-cell *D. nodosus* vaccine and a recombinant fimbrial vaccine could reduce the disease with approximately twice less efficaciousness as opposed to foot bathing regimens (Allworth and Egerton, 2018).

Regarding probiotics, the experimental evidence demonstrates the capability of different *Lactobacillus acidophilus* strains to suppress *Listeria*, *Shigella*, *Salmonella* Typhimurium, *Escherichia coli*, and other pathogens through a variety

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of mechanisms, such as membrane depolarization and intracellular acidification (Liévin-Le Moal and Servin, 2014). An *in vitro* study has also revealed the antagonistic activity of *L. acidophilus* 015k-1 strain against *F. necrophorum* strains isolated from limbs of cattle (Myktybaeva et al., 2020).

Therefore, the purpose of this multilocation field trial was to compare the effectiveness of the topical application of a powdered *L. acidophilus* 015k-1 strain and intramuscular injection of oxytetracycline for the treatment of IN in dairy cows.

MATERIALS AND METHODS

Ethical approval

All procedures involving the cattle were performed in conformity with Directive 2010/63/EU and were approved by the ethics committee of Kazakh National Agrarian University (Almaty, Kazakhstan).

Study design

This research was conducted on six farms with a total of 1291 cows, located in the Almaty region of Kazakhstan from April 2018 to May 2020. Cattle were housed in open dirt feedlot pens. In each case, research veterinarians performed clinical examination of the cattle. Cows exhibiting early signs of IN represented by lesions graded as 1 or 2 in accordance with a standard scoring system (Jelinek et al., 2001) and a mild degree of lameness according to Jaeger (2016), were allocated randomly to one of two groups. The trial group consisted of cows for which the affected hoof was cleaned, coated with probiotic powder (Lactobacterin-TK2®, Akyntaj LLP, Kazakhstan) containing 10^6 colony-forming units of *L. acidophilus* 015k-1 per gram, and then bandaged (Figure 1). On the other hand, cows in the control group were subjected to a single intramuscular injection of Nitox 200® (oxytetracycline; Nita-farm, Russia) at the dosage of 1.0 milligram per kilogram of bodyweight which is considered the routine treatment for IN in the feedlots. Both procedures were performed every 72 hours during a period of two weeks with the subsequent two-week follow-up period. In case a cow was assessed as cured during this period, its treatment was discontinued. Each cow was ear-tagged. Both groups were monitored daily by licensed veterinarians for the condition of the limb and the degree of lameness during 28 days. Those cows having no evidence of lameness and lesions attributed to IN, with no disease recurrence recorded within the observation period, were considered cured.

Statistical analysis

In order to evaluate the association between variables, two-sided Fisher's exact test was used in the current study. For each of the herds pulled, the odds ratios of clinical cure of cows treated with *L. acidophilus* 015k-1 versus oxytetracycline were computed along with the confidence interval estimated by the Baptista-Pike method. All calculations were carried out in GraphPad Prism version 8.3.0 for Windows (GraphPad Software, San Diego, California, USA).

Figure 1. The procedure of applying *Lactobacillus acidophilus* 015k-1 to the foot of a cow with interdigital necrobacillosis

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RESULTS

Among the animals examined, 230 of them were diagnosed with early-stage IN (Table 1).

Cure rates for both treated groups on day 15 of treatment are shown in Table 2.

On day 15, the overall cure rates for *L. acidophilus* 015k-1 and oxytetracycline were 80.87% and 83.48%, respectively. The overall odds ratio for the cure rate in the *L. acidophilus* group versus oxytetracycline was 0.837. This means that the odds of cure after two weeks of the topical application of *L. acidophilus* are 83.7% of the odds of cure after a two-week-long intramuscular injection of oxytetracycline (Ranganathan et al., 2015). However, on day 28, all cows in both groups were found to be clinically free from IN, and consequently, the cure rates were 100%. For this reason, the data are not displayed in a tabular form. There was no recurrence of IN during the observation period.

Table 1. Prevalence of early-stage interdigital necrobacillosis in dairy cows included in the study on livestock farms located in the Almaty region of Kazakhstan

Farm name	Trial period	Cows (total)	Cows with early-stage IN (included in probiotic-treated group[1])	Cows with early-stage IN (included in antibiotic-treated group[2])
Darnis	April – May 2018	262	19	19
Bajserke – Agro	September – October 2018	536	27	27
Almaty	March – April 2019	118	16	16
Anar – K	May 2019	68	13	13
Nurzhan	September – October 2019	75	12	12
Samsybaev M	April – May 2020	232	28	28
Total	April 2018 – May 2020	1291	115	115

IN: interdigital necrobacillosis [1] topical administration of probiotic powder containing 10^6 colony-forming units of *Lactobacillus acidophilus* 015k-1 per gram. [2]single intramuscular injection of oxytetracycline at the dosage of 1 mg/kg of body weight.

Table 2. Cure rates for early-stage interdigital necrobacillosis in dairy cows included in the study on livestock farms located in the Almaty region of Kazakhstan on day 15 of treatment

Farm name	Probiotic-treated group[1]		Antibiotic-treated group[2]		*P*-value (Fisher's exact test)	OR	CI
	cured / treated		cured / treated				
	number	%	number	%			
Darnis	12 / 19	63.16	16 / 19	84.21	0.269	0.321	0.080 – 1.339
Bajserke – Agro	25 / 27	92.59	21 / 27	77.78	0.250	3.571	0.746 – 18.430
Almaty	9/ 16	56.25	14 / 16	87.50	0.113	0.184	0.035 – 1.000
Anar – K	10 / 13	76.92	13 / 13	100.00	0.220	0.000	0.000 – 1.065
Nurzhan	10 / 12	83.33	8 / 12	66.67	0.640	2.500	0.410 – 15.180
Samsybaev M	27 / 28	96.43	24 / 28	85.71	0.352	4.500	0.644 – 56.790
Total	93 / 115	80.87	96 / 115	83.48	0.731	0.837	0.423 – 1.616

OR: Odds ratio, CI: Confidence interval [1] topical administration of probiotic powder containing 10^6 colony-forming units of *Lactobacillus acidophilus* 015k-1 per gram. [2]single intramuscular injection of oxytetracycline at the dosage of 1 mg/kg of body weight. Treatments were repeated every 72 hours for two weeks but when a cow was assessed as cured, treatment was discontinued.

DISCUSSION

Considering that the initial point of foot rot is cutaneous dysbiosis, Ross et al. (2019) hypothesized that a probiotic culture with experimentally established efficacy against skin diseases might be a feasible topical treatment option. In line with the above, *L. acidophilus* has been widely proven to modulate epidermal conditions positively by means of cellular metabolites, antimicrobial peptides, and immune responses (Jeong et al., 2016; Lim et al., 2020). In contrast with antibiotics, the utilization of *L. acidophilus* 015k-1 does not entail the risk of antimicrobial resistance, and it requires no painful injections.

Unfortunately, we have failed to find a published study designed to evaluate the effect of any topical probiotic on foot lesions in livestock. In general, there has been a dearth of research addressing the topical application of a non-antibiotic approach for IN eradication. In a study conducted by Kaler et al. (2012), it was found that at the end of their 28-day-long trial the recovery from the disease was observed in only four out of the ten sheep with at least moderate interdigital phlegmon that had been administered locally with potassium permanganate solution, whilst recovery in a median time of 7 days was recorded for those 52 sheep treated with long-acting parenteral oxytetracycline and enrofloxacin.

Furthermore, Van Metre (2017) in his narrative review, has drawn a skeptical conclusion about the full range of

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transdermal substances utilized as active agents against claw disorders. Nonetheless, Persson et al. (2019) stated that dairy cows with early detected foot rot which were subjected to 100% salicylic acid powder into the interdigital space of the feet showed improvement in their general condition, lameness, body temperature, and coronary circumference after five days since administration. Given the difference in the duration of these trials, it is not possible to compare these findings with the obtained results of the current research.

To the best knowledge of the authors, the current study is the first report on the treatment effect of *L. acidophilus* topically administered to cattle with early-stage IN. The limitation of this research is in the absence of laboratory diagnosis. Meanwhile, it should be noted that the diagnosis of IN on grounds of clinical signs is used in contemporary veterinary practice for a variety of reasons (Osová et al., 2017; Põder, 2018; Kontturi, et al., 2020).

CONCLUSION

In conclusion, it can be stated that the cutaneous administration of *L. acidophilus* 015k-1 to dairy cows with early-stage IN every 72 hours for a period of up to two weeks can result in cure rates nearly as high as those for oxytetracycline intramuscularly injected using the same scheme. In the present case, the full recovery from the disease could be observed in both treatments at the end of the subsequent two-week follow-up period.

DECLARATIONS

Authors' contribution

All authors contributed equally to this work.

Competing interests

The authors declared no conflict of interest.

Acknowledgments

Authors wish to express their thanks to Sergey Sergeevich Kozhevnikov for translating this manuscript into the English language.

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