BF-1410
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RESEARCH ARTICLE Indian Journal of Animal Research

Evaluating the Efficiency of Newly Formulated Pomade® and Ceftiofur Hydrochloride for Treating Foot Rot in Dairy Cattle

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10.18805/IJAR.BF-1410

ABSTRACT

Background: Foot rot is an important contagious disease that causes economic loss in dairy cattle. Even though many antibiotic treatments have been tried on foot rot, very few information about new topical treatment method or product have been reported about the disease. The main objective of this paper is to evaluate an alternative new treatment for foot root disease in dairy cattle.

Methods: Forty-one swap samples were collected from cattle's feet which were further investigated under microscopic examination and polymerase chain reaction (PCR) for *Dichelobacter nodosus* and *Fusobacterium necrophorum*. Newly formulated pomade® and ceftiofur hydrochloride (Eficur®) applications were used in foot rot cases.

Result: Polymerized chain reaction revealed *D. nodosus* in all the samples were as *F. necrophorum* was seen only in 22 (53.66%) samples. In this study, *D. nodosus* was considered as the primary agent involved in foot disease of cattle and *F. necrophorum* along with other bacterias were considered to be associated in the infection. The foot rot wounds formed in all cases (100%) in which *D. nodosus*, *F. necrophorum* and other bacteria were detected were healed along with tissue regeneration. As a result of treatment applications, a success rate of 93.33% was obtained in lameness resulting due to medium foot rot cases and 45.45% in severe acute lameness. The lameness recovery rate was found to be 80.48%.

Key words: Ceftiofur hydrochloride, Dairy cattle, Foot rot, Pomade, Treatment.

INTRODUCTION

Foot rot disease has a wide geographical distribution around the world (Haggman *et al.,* 2015). Foot rot has been reported in dairy and breeder cattle kept under various climate zones and breeding conditions (Terrell *et al.,* 2014). Although *F. necrophorum* is considered as the main factor of foot rot disease, *D. nodosus* and *F. necrophorum*, especially with a synergic effect, are together responsible for the occurrence and development of the disease (Zhou *et al.,* 2009). *F. necrophorum* is normally the microflora of animal and human gastrointestinal system. The exotoxin of this bacteria is the primary toxin of leucotoxin (*iktA*) ruminant leucocytes which is an important virulent-factor (Nagaraja *et al,.* 2005). As for *D. nodosus*, it is a bacteria usually seen in epidermal tissue of cattle feet (*iktA*). The toxin of *F. necrophorum* has a synergic effect on these two bacteria, improving the reproducing activity of *D. nodosus* and causes lesions in the feet. Besides, other bacterial factors were also present in the formation of the disease (Nagaraja *et al.,* 2005). Many preparatory factors take part in development of the disease in cattle (Osova *et al.,* 2017). For treatment of the disease, systemically used antimicrobials and topical applications prepared from various compounds are commonly used (Cook and Cutler, 1995; Sano *et al.,* 2007; Van Metre, 2017). Prompt treatment with parenteral antibiotics and local care of the foot lesion shorten recovery time to 2 to 4 days (Radostits *et al.,* 2000). Present study was aimed to evaluate the clinical efficiency of newly formulated Pomade®, together with Ceftiofur hydrochloride (Eficur®) for the treatment of foot rot in cattle.

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How to cite this article: Yiğitarslan, K., Kale, M., Öztürk, D. and Mamak, N. (2021). Evaluating the Efficiency of Newly Formulated Pomade® and Ceftiofur Hydrochloride for Treating Foot Rot in Dairy Cattle. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1410.

Submitted: 19-07-2021 **Accepted:** 08-10-2021 **Online:**

MATERIALS AND METHODS

Clinical symptoms and sample collections

The study was conducted (January 2018-April 2021) in forty-one dairy cows which showed clinical symptoms, such as pain, lameness, swelling and erythema in their feet. The degree of lameness was detected in cattle on the basis of clinical lameness. Degree of lameness was classified as light (+), medium (++) and severe (+++). Two swap samples (amies agar gel medium transport swabs, cultiplast, LP ItalianaSPA, Italy) were taken from plantar interdigital hoof area of each sick animal. Before taking the samples, the section was washed with warm water, cleaned mechanically

using Batticon® (10% iodine + 10 g polivinilpirolidon, Turkey) cotton. One of the swaps was used for microscopic examination while the second one for PCR diagnostics.

Microscopic examination

One swaps obtained was used for microscopic examination. Three preparates were prepared from each swap. Preparates were stained by gram staining method and examined under X100 objective (Nikon, Japan) at the Microbiology Labs.

DNA extraction and PCR

DNA extraction was processed by freezing and boiling according to Osova *et al.* (2018). The detection of *D. nodosus* and *F. necrophorum* were used as primers for 16SrRNA and leucotoxin gene region (*IktA*) (Sentegen Biotech, Ankara, Turkey), respectively (Table 1). PCR was performed in 20µl reaction mixture (10µl Master Mix (Fermentas)(2X) (Thermo Fischer Scientific, Brno, Czech Republic), 7µl ddH_2O (Thermo Fischer Scientific, Brno, Czech Republic), 1µl (10 µM Primer F), 1µl (10 µM Primer R), 1µl DNA) for each sample. The amplification was performed at 95°C, for 3 min for predenaturation, 35 cycles, 30s for denaturation, 61°C, 30s for annealing, 72°C, 30s for elongation and final elongation at 72°C, for 10 min. PCR products were electrophoresed in 1.5% agarose gel at 100 V for 45 min, stained with ethidium bromide (0.5 µg/ml) and PCR products were photographed (EDAS 290, Eastman Kodak Company, Rochester, NY, USA) under UV light. DNA extraction and PCR procedures were done at the Microbiology and Virology Labs.

Application of the Pomade® and additional treatments

The pomade® was prepared with a single formulation that could treat symptoms of dermatitis, hyperplasia, foot founder, ulcer, decay, apsis, fracture, lump, bleeding, rash, necrosis, pain and lameness that developed depending on several microorganisms on skin and hoof of animals (bacteria, fungus and papillomavirus). The formulation contained rifampicin which prevented mRNA transcription by inhibiting RNA polymerase, allylamine, aluminium subacetate, alcohol, boric acid, lanolin, talcum powder and zinc. The pomade® might be used for foot (skin and hoofs) infections of animals developed due to invasion of some microorganisms (bacteria, fungus, yeast and papillomavirus). It is an odorous, reliable and effective with no side effects having a texture of fine powder. An application for making the medicine Pomade patent has already been initiated to Turkish Patent and Trademark Office in National Patent field with the number 2019/08799. Pomade was prepared at the Virology Labs.

Animals with light (+), medium (++) and severe (+++) lameness levels having wounds and bleeding in their plantar interdigital hoof zones were clinically recorded. Foot rot area was cleaned and washed mechanically with warm water and finally with Batticon®. 5 grams of the Pomade was applied on this area by the help of a spatula. Later, the part affected with foot rot was covered using American cloth firstly and then the foot was wrapped with sticking rubber foot bandage. Finally, bituminous foot bandage was applied. The bandaged foot was not opened for three days. It was opened on days 4 and 7, for application of Pomade and rebandaged. On day 11, the foot bandage was opened and wound area was examined by hand palpation and macroscopically. Together with pomade® application, an antibiotic ceftiofur hydrochloride (Eficur®) was administered subcutaneously for ten days (1 mg ceftiofur/kg b.w./day). During the treatment, all animals were cared in a clean separate paddock along with supports (dry fodder and soft stalk).

RESULTS AND DISCUSSION

Microscopic examination

In bacterioscopy, *D. nodosus*, *F. necrophorum* and other Gram positive and Gram negative bacteria were seen in slides, but bacterial identification was not performed for other bacteria in this study.

PCR results

In this study, 16SrRNA species specific gene for *D. nododus* was detected in all 41 swap samples by PCR (Table 2). The presence of *IktA*, specific gene for *F. necrophorum* was determined in 22 samples (Table 2).

Treatment results

All the animals used in the study had lameness. At the end of treatment applications lasting ten days (newly formulated pomade® and ceftiofur hydrochloride, Eficur®), the success rate was 93.33% for light and medium level lameness animals and 45.45% for severe acute ones (Table 3). During the controls on the 11th day of the treatment, recovery and tissue regeneration were seen in all animals having wounds which developed depending on foot rot caused by *D. nodosus*, *F. necrophorum* and other bacteria. (Fig 1-4) The total lameness recovery rate was stated as 80.48%.

The first clinical symptoms observed in foot rot was sudden lameness caused by extreme pain, acute swelling, redness in interdigital tissues and coronary bands, malodorous and necrotic lesions in interdigital spaces along with loss of appetite (Biggs *et al.,* 2019). Many researchers have stated that hoof diseases and lameness are seen more on feet rather than

Table 1: Primer sequences, target gene and size of the products used in PCR.

Primer	Target gene	Sequence (5′-3′)	PCR product
D. nodosus	16SrRNA	5′-GCTAAGGAAAAAGCACCGGC-3′	295 bp
		3′-GTTTGCTACCCACGCTTTCG-5′	
F. necrophorum	*IktA*	5′-TTTTGGAGTCGGAGTCGCAG-3′	362 bp
		3′-CTCCGGCTGCAAGAATTCCA-3′	

Table 2: *D. nodosus* and *F. necrophorum* PCR results in the samples.

Bacteria	+	%
D. nodosus	19	46.34
D. nodosus +F. necrophorum	22	53.66
Total	41	100

Table 3: Recovery rates according to lameness levels.

Lameness levels	Number of cases	Number of recoveries and %
Light (+)	15	14 (93.33%)
Medium (++)	15	14 (93.33%)
Severe (+++)	11	5 (45.45%)
Total	41	33 (80.48%)

forefeet (Neveux *et al.,* 2006). In the present study, foot rot cases and lameness were found more on feet. In various studies, this condition was stated to have been caused by more weight bearing on hind feet (Yayla *et al.,* 2012).

Foot rot is a contagious disease and *F. necrophorum* and *D. nodosus* are the main causative factors. These bacteria might be present in stools of healthy animals. *D. nodosus* and *F. necrophorum* were stated to be present normally on the skin located in interdigital spaces of cattle feet (Osova *et al.,* 2018). The discharge from the feet of infected animals and stools might cause contamination of the environment. In foot rot cases, *Porphyromonas levii, P. asaccha-rolytica, Prevotella intermedia and P. melaninogenica, Staphylococcus aureus, Escherichia coli* and *Trueperella pyogenes* are other bacteria that can be isolated (Kontturi *et al.,* 2019). As a result of injuries on the skin, these bacteria reach subcutaneous tissues and replicate swiftly. They penetrate in deeper tissues by means of their exotoxins and cause the disease (Nagaraja *et al.,* 2005; Biggs *et al.,* 2019). Shivasharanappa *et al.* (2014) stated that the foot rot factor in sheep was *D. nodosus* and it have caused infection by itself in cases where *D. nodosus* was not detected while Knappe-Poindecker *et al.,* (2015) was of the opinion that foot rot factor was *D. nodosus* in sheep which might play a role in contaminating cattle. In this study, all swap samples taken from feet depicted *D. nodosus*, *F. necrophorum* and other bacteria under microscope. In another study (Osava *et al.,* 2018; Kontturi *et al.,* 2019), revealed that *D. nodosus* and *F. necrophorum* were the main organism producing foot rot, however, other Gram negative and Gram positive bacteria were isolated. However in our study, other bacteria were not isolated or identified. Using PCR, *D. nodosus* was found in all samples and *D. nodosus* and *F. necrophorum* were found together in 53.66% cases. In a cattle business in Finland, *F. necrophorum* was considered as the main factor in foot rot cases, nevertheless, both bacteria were generally detected together (Kontturi *et al.,* 2019). Kontturi *et al.* (2019) similarly stated that occurrence of mixed bacteria *i.e. D. nodosus* + *F. necrophorum* was as high 82.4% and medium 52.6% at

Fig 1: Case 1, Right footpad, before treatment, (0 Day), Burdur.

Fig 2: Case 1, Right footpad, after treatment, (11th Day), Burdur.

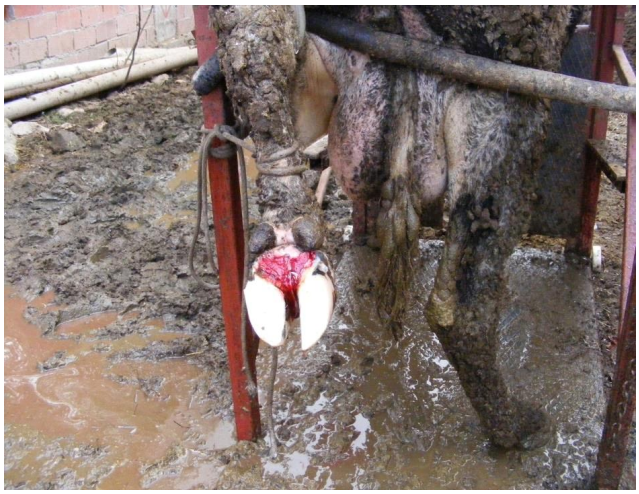

Fig 3: Case 2, Left footpad, before treatment, (0. Day), Burdur.

Fig 4: Case 2, Left footpad, after treatment, (11th Day), Burdur.

the end of PCR tests carried out for samples taken from interdigital phlegmon based on epidemics of Finnish dairy herds. In a study in Norway, in dairy cattle with hoof problems, *D. nodosus* prevalence was found to be 94.5% while it was 66% in the control group. In another study, the biggest problem in hoof disease was stated as *D. nodosus* bacteria (Knappe-Pointdecker *et al.,* 2013). In Eastern Slovakia, Osova *et al.,* (2018) found *D. nodosus* over 95% and *F. necrophorum* over 27% during bacterial planting in swap samples taken from feet of the healthy cows showing no lameness. They also stated that diagnosing *D. nodosus* by PCR was three times more susceptible compared to culture method. In contrast to our findings, Bennett *et al.* (2009) detected 53% *F. necrophorum* (*iktA* gene) and 5% *D. nodosus* (*fim A* gene) using PCR in swap samples taken from the feet of dairy cattle with lameness.

In foot rot treatment, systematic antimicrobial treatment applications are usually recommended during early stages of the disease (Cook and Cutler, 1995; Stokka *et al.,* 2001). We can identify the antibiotics used for foot rot treatment as ceftiofur, oxytetracycline, ampicilin, penicilin, sulphonamides strengthened by trimethoprim, florfenicol, spectinomycin, lincomycine, tulathromycin and tylosin (Osova *et al.,* 2017; Van Metre, 2017). In foot rot and case of outbreak of foot-hoof injuries, their progress and correspondingly leading to lameness, topical treatment should be started. Zinc sulphate, iodine formulations or peroxidase solutions are recommended to be used for destruction of *D. nodosus*, which is an anaerobic pathogen, with active oxygen. Local antibiotics (tetracycline) or regional intravenous long-acting antibiotics could also be administered (Osova *et al.,* 2017). In this study, rifampicin used in the pomade hindered RNA polymerase in bacteria and prevented mRNA synthesis and thus nucleic acid formation. This antibiotic was effective against many Gram positive and Gram negative bacteria *in vitro* (Suresh and Wadhwa, 2020). During the studies, use of the antibiotics topically was considered both to keep the infection under control and accelerated recovery from injuries as compared to control group (Saydam *et al.,* 2005). In this study, the antifungal effective agent used in the pomade was Naftifine hydrochloride which was obtained on the basis of antifungal medicine having azole structure with allyamine structure. The component was also proved to have antibacterial effects against Gram positive and Gram negative bacteria apart from its fungicidal activity (Şimşek and Şafak, 1996). Some excipient agents such as aluminium subacetate, boric acid and zinc oxide present in the pomade served as antiseptic, astringent, antiperspirant, desiccant and deodoriser. Other agents were as solvents, emulgators and desiccants (Pekcan, 2014). In present study, application of newly formulated pomade® and ceftiofur hydrochloride (Eficur®), during the controls on the 11th day in all foot rot cases resulted in 100% recovery with tissue regeneration. In addition, as a result of treatment applications for ten days, a success of 93.33% was achieved for light and medium level lameness and 45.45% for severe acute ones. The total lameness recovery rate was recorded as 80.48%. During treatment studies based on parenteral antibiotic applications carried out for foot rot cases, the success rates observed was 68% with oxytetracycline, 73-99% with ceftiofur sodium, 74% with tilmicosin and 99.5% with ceftiofur crystalline free acid (Sano *et al.,* 2007; Van Donkersgoed *et al.,* 2008). In a foot rot research proceeding using parenteral and topical antibiotic applications together, a success rate of 73% was achieved while it was 56% for cases with deep sepsis whose treatment was delayed (Cook and Cutler, 1995).

CONCLUSION

It can be concluded that foot rot cases in dairy cattle with mixed infections especially caused by *D. nodosus* and *F. necrophorum* and other bacteria following application of a newly formulated drug Pomade and Ceftiofur hydrochloride (Eficur®) proved to be effective for light, medium and severe acute lameness resulting in complete tissue regeneration and healing of foot rot wounds.

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