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Short Communication

Genomic Detection of Bovine Digital Dermatitis Treponemes in Sole Ulcers

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Abstract

Objective- Evaluation of possible contamination of sole ulcers with digital dermatitis *Treponema* spp.

Design- Clinical detection

Animals- Thirty-three cows with sole ulcer lesion.

Procedures- Hoof trimmings were done by professional veterinarian hoof trimmer on a regular basis. Sole ulcer and digital dermatitis recorded as a wounded lesion in zone 4 and 10 of the hooves during hoof trimming and recorded in special sheets. A total of thirty-three tissue samples from sole ulcers in zone 4 of digits were taken and the 16S rRNA gene was followed by PCR assay.

Results- Annual incidence of clinical digital dermatitis recorded as 7.1 percent as the second prevalent digital lesion in the farm following sole ulcer. *Treponema* spp. was found in 42 percent of the samples, none of the positive samples show concurrent lesion of digital dermatitis and sole ulcer.

Conclusion and Clinical Relevance- Since no concurrent lesions were found in sole ulcer samples, finding *Treponema* spp. in tissues may be a result of the possible pathogenic presence of the bacteria in this region. Multifactorial causes of sole ulcer make different clinical presentations for the lesion and a microbial cause for the sole ulcer that led to non-healing ulcers were reported.

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1. Introduction

Sole ulcer (SU) is one of the most common lesions of bovine hooves, which causes drastic economic losses (from 232 to 982 \$/case) to dairy industry.¹ longer calving to first service interval (4.83 and 8.0 days longer due to mild and severe lesions of SU), lower milk yield (1.47 to 2.66 kg/day of energy-corrected milk),²⁻⁴ reducing animal welfare by changing cow's time budget, severe pain and hyperalgesia^{5,6} and high herd level prevalence (e.g. 92% of dairy herds in Alberta, Canada and 85% in Netherlands) make sole ulcer as an important concern.^{7,8}

High herd level prevalence of bovine digital dermatitis (BDD) (e.g. 90.6% of dairy herds in Netherlands, 70% in United Kingdom, and 63.8% in New Zealand), makes the disease an important concern although the cow level prevalence has variation among different regions (e.g. 1.2% in New Zealand and 21.2% in Netherlands).^{9,10} BDD was first reported in Iran in 1991,¹¹ but cow or herd level prevalence of the disease is not available in Iran, however annual incidence of BDD was reported as 11.66% in a study of four large farms in Iran.¹² These data can be noticed as infectious claw disorders, specially BDD are common in dairy farms. Although there are some studies in Iran, which show the prevalence or incidence of SU, but these studies are limited to specific areas or slaughterhouse studies, not the whole country. However the annual incidence of SU in some dairy farms in Iran was reported 23.11%.^{13,14}

Weakening of the suspensory apparatus of the third phalanx considered as the main pathogenesis of the lesions. Increased metalloproteinase enzymes and peripartum hormonal activity, cow comfort disturbances such as prolonged standing on hard uneven walking surfaces or changes in cow's time budget, horn overgrowth, and claw conformation known as main risk factors of the disease.^{15,16} There are some ideas about concurrent incidence of claw horn lesions (such as toe necrosis) in the herd with high prevalence of BDD.¹⁷ Better healing response of non-

healing sole ulcer disorders by using a topical solution containing antibiotics (cephalosporin) and copper sulfate maybe considered as a reason for involving a bacterial pathogen in the process.¹⁸

Several studies are conducted to find the precise etiology of BDD, but it is still not completely clear. BDD appears to be a polymicrobial disease, with multiple different species of bacteria being isolated from or detected in BDD lesions, specifically *Treponema* spp., seem to be the most critical bacteria in clinical BDD. However, difficulties in *Treponema* culture and the relatively high percentage of bacteria, in BDD lesions mean that culture-independent methods are more useful in studying the etiology of BDD.^{19,20} Finding treponemes in some bovine foot lesions except traditional location of BDD and similar appearance of the lesions in some sole ulcer and white line disease to BDD lesions besides refractory response to traditional treatment of these lesions make the idea of possible pathogenic role of the BDD causing treponemes in sole ulcer or white line disease lesions.²¹

This current study was designed to detect the main pathogenic bacteria of BDD, *Treponema* spp., in SU that may affect the considerations for treatment and control the hoof lesions in the herd. Any genomic footprint of *Treponema* spp. was followed in SU lesions.

2. Materials and Methods

Cows were selected from a dairy farm with 1600 dairy cows located in central part of Iran in a high altitude (Shahrekord) region (average annual milk production: 35 lit/day/cow). Cows were housed in free stall barns, milked three times a day and fed by total mixed ration. Hoof care programs (regular hoof trimming, locomotion scoring, hoof bathing, digital lesions diagnosis and treatment) were done by a skilled veterinarian.

This study was done on January to March 2012. Sole ulcers were diagnosed in hoof trimming chute and one cubic centimeter biopsy was taken from each SU in zone 4

of the hoof. All samples were taken from the center of the lesions and after washing by distilled water stored in sterile containers on ice and finally stored at -80° C. DNA was extracted from tissue samples by Cinnapure DNA extraction kit (Sinaclon, Tehran, Iran), according to the manufacturer's instructions. Following PCR tests, 6 μ l of the amplified products were loaded on a 1.3% agarose gel, and visualized by staining with ethidium bromide and compared with DNA marker (100 base pair ladders, SMOBIO, Hsinchu, Taiwan).

A nested PCR assay was performed using universal primers based on Evans et al, 2009.²² Positive controls (10 BDD samples were taken, and positive PCR and sequencing results in pilot tests using current study primers used as positive samples)

3. Results

Occurrence of digital lesions recorded in Table 1. Digital dermatitis is the second prevalent disease in the herd following sole ulcer. Among 33 SU samples, 42% (14 out of 33 samples) were positive for *Treponema* spp., using PCR tests (Figure 1). None of the positive samples had concurrent clinical manifestation of BDD in the same digit, while 29% of lesions showed hoof lesions in other claw zones or digits.

4. Discussion

Genomic methods, as a precise and preferable way, for detecting such fastidious Spirochetes were used in

Table 1. Annual incidence of main hoof lesions (N/incidence).

Lesion	Spring	Summer	Fall	Winter	Whole year
Bovine digital dermatitis	34/2.1	34/2.1	15/0.9	30/1.9	113/7.1
Sole ulcer	39/2.4	49/3.1	44/2.8	37/2.3	169/10.6
White line disease	3/0.2	12/0.8	16/1	5/0.3	36/2.3
Interdigital necrobacillosis	28/1.6	10/0.6	9/0.6	42/2.6	89/5.6

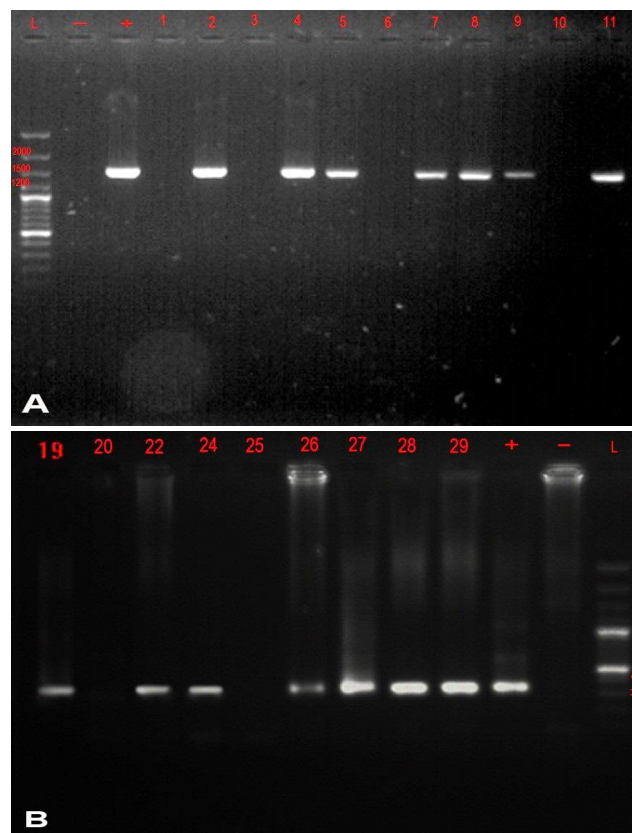


Figure 1. Nested PCR amplification products for detecting *Treponema* spp. following electrophoresis. **A.** PCR products of the first step amplification. L: 100 bp DNA laBDDer, +: *Treponema* spp. positive control, -: *Treponema* spp. negative control, 2,4,5,7,8,9,11: positive samples, 1,3,6,10: negative samples. **B.** PCR products of the second step amplification. L: 100 bp DNA laBDDer, +: *Treponema* spp. positive control, -: *Treponema* spp. negative control, 19,22,24,26,27,28,29: positive samples, 20,25: negative samples.

different studies²³⁻²⁵ Findings of this study were in agreement with Sykora *et al.*,²³ and Evans *et al.*²⁴ Evans detected *Treponema* medium-like, *Treponema phagedenis*-like and *Treponema denticola*-like phylotypes in non-healing claw horn lesions [84.2, 81.3, and 55.6 percent of samples of toe necrosis (n = 19), non-healing white line disease (n = 16), and non-healing sole ulcer (n = 9), respectively].²⁴ Sykora *et al.* found *Treponema* DNA in 42 non-healing white line disease and non-healing sole ulcer and 15 common white line disease.²³ Samples in this current study were taken regardless of its non-healing condition and show a prominent presence of treponemes in such lesions. The first hypothesis for presence of *Treponema* DNA in claw horn lesions is that

microorganism can invade the perioplic or laminar corium from a nearby BDD lesion and in the second hypothesis finding of the *Treponema* DNA maybe a result of secondary contamination of the SU lesions from herd environment or hoof trimming equipment.^{25,26} Results of the Table 1 showed that BDD is a common digital disease in this farm and its causative agent were spread in the farm. Since we couldn't find any concurrent BDD lesions in *Treponema* positive SU samples, it seems that the second hypothesis is more accurate in this farm. In this current study presence of treponemes were evaluated in SU, however other organism in addition to treponemes like *Fusobacterium* spp., *Fastidiosipila* spp., *Odoribacter* spp., *Filifactor* spp., Ruminococcaceae UCG-014 and other genera of the family Porphyromonadaceae were detected in SU significantly higher than healthy adjacent skin.²⁷ Findings of this study is just showing possible role of the treponemes in SU pathogenesis and treatment. Digital dermatitis associated toe ulcers/toe necrosis is well defined²⁸, and findings of this study and further investigation may find a digital dermatitis associated sole ulcer as well.

In conclusion, presence of *Treponema* spp. in sole ulcer samples regardless of concurrent lesion shows that correct control and prevention of BDD lesions in dairy farms is very important not only for controlling BDD but also as a treatment and control measure for sole ulcer to prevent its possible non-healing status.

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Conflict of Interests

All authors disclose any financial and personal relationships with other people or organizations that might inappropriately influence or bias this work.

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