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Outbreak of *Dermatophilus congolensis* in Grazing Beef Cattle in Northeastern Mexico: First Report

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Abstract

Dermatophilus congolensis is a bacterium that causes exudative dermatitis with scab formation in bovines. Humidity and ticks are predisposing factors. This study describes skin lesions in 27 bovines from a Simbrah herd of grazing livestock of 180 females (15%), aged 18 to 30 months old, in Aldama, Tamaulipas, Mexico. Lesions were distributed in the lower extremities, the belly and the neck and consisted of thick grayish to dark brown scabs with exudates and blood. Scabs were collected from four of the most severely affected animals, placed in sterile tubes and then transported in refrigeration to the laboratory. Samples were processed for isolate *D. congolensis*. The four samples revealed long branched filaments made up of coccoid cells arranged in parallel rows, one after the other, which is a characteristic of *D. congolensis*. In this outbreak, morbidity was 15% (27/180) and mortality was 22% (6/27). The PCR using ESP1 and ESP2 primers amplified a product between 400 and 500 bp, and the 16 s sequence was 100% identical to *D. congolensis* gene for 16S rRNA, strain: NBRC 105199 Sequence ID: dbj|AB550800.1|. The observation of lesions, clinical signs, identification and sequence led to this first report of bovine dermatophilosis in Mexico.

Keywords: *Dermatophilus congolensis;* Bovines; Tamaulipas; Mexico

Introduction

Dermatophilus congolensis bacterium is an actinomycete that forms branched filaments which first divide into transverse planes and then into multiple longitudinal planes to constitute packages of ovoid cells or resistant spores. Due to the action of humidity and carbon dioxide on the skin, these cells or spores are then transformed into mobile spores or infecting zoospores [1].

In bovines, *D. congolensis* causes an acute or chronic disease of the epidermis, characterized by inflammatory lesions that leave exudative and bleeding scabs, affecting mostly ruminants [2]. Animals are infected by mobile zoospores and the main routes of transmission are tick bites and contact with contaminated fomites and exudates or scabs from infected animals. Ticks of the genus *Ambyomma* may spread or trigger the disease.

The disease has worldwide distribution, prevailing in tropical areas and associated with humid environments and other factors. The first case was reported in Congo in 1915 and it has been isolated since then in animal infections chiefly in Africa: Kenya, Ethiopia, Tanzania, Nigeria, South Africa, Asia: Turkey, India, China, and Central and South America: Argentina, Uruguay, and Brazil, but also in Australia, the United States: New York, Kentucky, Florida, Texas, Canada, and Europe: France, Spain, Germany, as a chronic endemic disease and, more rarely, as an acute and epidemic infection. It is most commonly associated with goats, sheep, cows and horses but also affects a worldwide variety of domestic and wild animals, such as marine mammals, cats, antelope, buffalo and deer [2-10].

The purpose of this study was to describe, by means of clinical observations and bacteriological analysis, the outbreak of a disease that was causing skin lesions in grazing bovines in the state of Tamaulipas, Mexico.

Material and Methods

In July 2013 we received a clinical report about dermatological lesions in a ranch that breeds Simbrah grazing bovines, whose population consists of 180 female bovines between 18 and 30 months of age, housed in a pasture of African Star Grass (*Cynodon plestostachium*). The ranch is located in the municipality of Aldama, state of Tamaulipas, Mexico, 23° 18.47´N, 97° 51.11´WO. Climate is dry tropical, with temperatures ranging from 3° to 41°C, mean temperature of 24.5°C, and an annual rainfall of 800 mm. Prior to the observation of the cases, there had been a considerable infestation of ticks of the genuses *Boophilus* and *Amblyomma*. Aspersion baths with amidine given to the whole bovine population led to the observation of the distinctive skin lesions, caused by the detachment of ticks. Cases of bovine papillomavirus appeared at the same time in 30% of the animals.

During the months of June and July there was an unusual rainfall of 300 mm, distributed in four events. Out of 180 animals, 27 (15%) presented exudative skin lesions with painful thick grayish to dark brown bleeding scabs (Figure 1), mainly on the extremities, although

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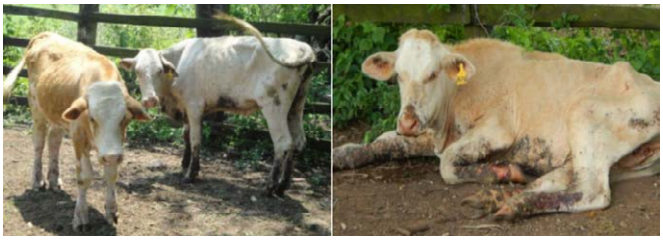

Figure 1: Skin lesions in the extremities.

on the belly, neck, back and head as well. Six of the 27 animals were severely affected and endured fever, weakness, prostration and death. Scabs from the extremities of four of these six animals were collected and placed in 50 mL Falcon tubes. The exudate was collected with swabs in test tubes in Stuart transportation medium. Both samples were refrigerated at 4°C.

It should be mentioned that there were other isolated cases with similar signs and lesions at other ranches within an 80-km radius from the ranch where isolates were obtained. Animals were treated with intramuscular penicillin and dexamethasone, each twice a day for a week, without any improvement.

Direct analysis was carried out in the Mycology Laboratory of the Microbiology and Immunology Department of the School of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico (UNAM). Scabs were placed on a microscope slide, a sterile physiological saline solution was added, and the sample was left to stand for 5 minutes. Afterwards the scabs were macerated with a scalpel, fixed with methanol and stained with Giemsa during 30 minutes.

Isolation was carried out by processing the samples using Haalstra's method [11].which required grounding a small quantity of scabs and placing them in a vial, with 2 mL of sterile distilled water, and leaving them to stand for 3 to 5 hours at room temperature. The vial was then carefully placed in an anaerobiosis jar at room temperature for 15 minutes to promote the migration to the surface of mobile zoospores by means of chemotactic attraction. The next step was taking 20 µl to be grown in pure culture in a blood agar plate. The inoculated plates were incubated during four days at 37°C and at an atmosphere of 5-10% CO_2. Identification was carried out based on the macroscopic and microscopic characteristics of the colonies and hemolytic zones. The catalase test was also performed.

DNA extraction was carried out applying the method described by Martínez et al. [12]. For the identification of this specie was performed a PCR with the following primers: ESP1 F (5´-cctcagcagaaaattcacca-3´), ESP1 R (5´-cgtacattcccggaatcttc-3´). DNA was amplified in 50 µL mix reaction which contained, 30ng of extracted DNA, 1 units of DreamTaq, 5 µL of DreamTaq Buffer 10X, 400 µM of all four dNTP´s, 0.2 µM of each primer and Q.S. to 50 µL of water of injections. PCR conditions were as follows: 94°C at 5 min, 35 cycles of 94°C at 30 seconds, 62°C at 30 seconds and 72°C at 45 seconds, finalized by a 9 minutes step at 72°C.

16s gene PCR for sequencing with 16s F (5´-acatgcaagtcgaacgatga-3´) 16s R (5´-agcctcgcaccctacgtatt-3´) primers, with the same concentrations of reagents, temperature and times of the previous PCR. In order to compare the sequence with the database BlastX (http://www.ncbi.nlm.nih.gov/BLAST) to obtain a high identity with a 16s sequence of a *Dermatophilus congolensis* previously entered into this database [13].

Results

The 27 animals with lesions were separated from the herd and given treatment with 3% iodine solution, once a week, without showing any improvement. Once the laboratory results established that *D. congolensis* was the etiologic agent, a four-week treatment was initiated two weeks later which consisted of long acting oxytetracycline at 1mL/10 kg of weight (20 mg/kg) every four days, while continuing with the 3% iodine aspersion every other day. Six weeks went by since the moment when the problem was detected until the end of the treatment regimen. Six animals died during this period. Samples were collected from four of them for bacteriological analysis. Eight animals recovered and 13 showed significant improvement, and no new cases have been reported in the herd since.

With respect to the bacteriological analysis, the four samples stained with Giemsa revealed long branched filaments, made up of coccoid cells arranged in parallel rows, one after the other, which are characteristic of *Dermatophilus congolensis* (Figure 2) and of diagnostic value [14]. The sample in blood agar revealed rough, dry, whitish-gray beta-hemolytic colonies (Figure 3). Gram stain revealed the characteristic morphology of actinomycetes (Figure 4) and the presence of beta-hemolysis. With the positive catalase reaction, it was established that these were isolates of *D. congolensis*.

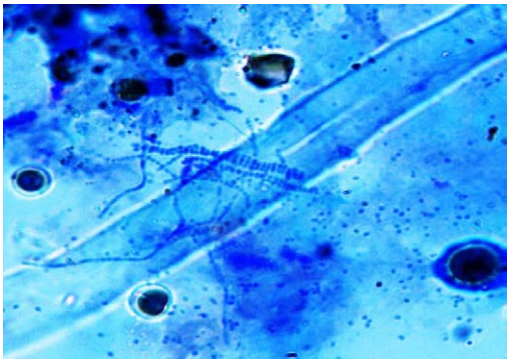

Figure 2: Scabs from clinical samples, stained with Giemsa, showing long branched septate filaments made up of coccoid cells arranged in consecutive parallel rows.

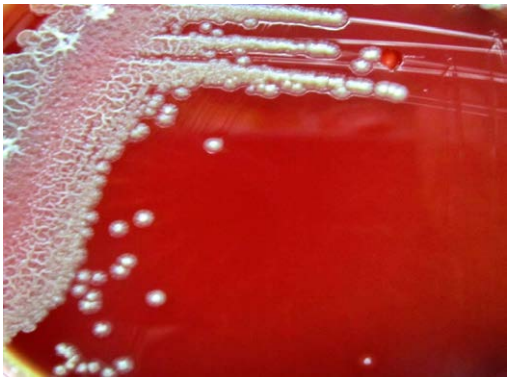

Figure 3: Growth of *Dermatophilus congolensis* in blood agar. The arrows point at the colonies.

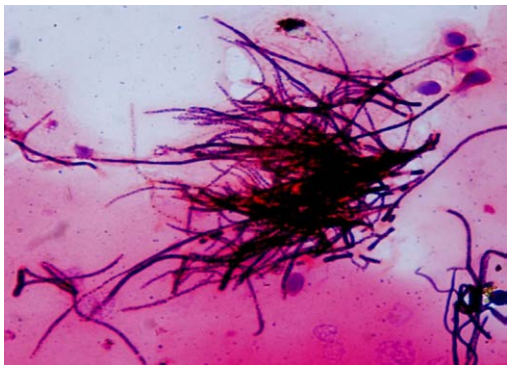

Figure 4: Chains of *Dermatophilus congolensis* with coccoid shapes in Gram stain, from the culture in blood agar.

The PCR using ESP1 and ESP2 primers amplified a product between 400 and 500 bp, indicating the identification of *Dermatophilus congolensis* (Figure 5). The 16 s sequence was 100% identical to *Dermatophilus congolensis* gene for 16S rRNA, partial sequence, strain: NBRC 105199 Sequence ID: dbj|AB550800.1|.

Discussion

The diagnosis of an outbreak by *D. congolensis* was made, based on the clinical signs and the lesions of the bovines, as well as through its isolation and identification in the laboratory. The exudative skin lesions seen with the thick scabs characteristic of dermatophilosis matched those described by other authors [15]. However, differences were found in the evolution of the lesion distribution in the body of the bovines.

In this outbreak, lesions began in the lower part of the extremities, probably due to the fact that these anatomical areas were exposed to prolonged humidity during the rains that preceded the outbreak. Only two animals whose symptoms had worsened showed lesions on the belly, neck, back and head. This is in contrast to reports by other authors who have described primary lesions in the neck, head and belly of the animals. In the case of an outbreak in Brazil in eight Brahman calves and five Nelore ones, of 1-12 months of age, raised under an intensive regimen, lesions were restricted to the head, ears and neck [16] In an outbreak in Argentina that affected calves between 5 days and 2 months of age, lesions began in the face and continued to the back and hind legs [15].

Rainfall in the months of June and July in Aldama, Tamaulipas were a predisposing factor for the infection. This risk factor has been well documented and described by other authors, who have mentioned that high rainfall and, therefore, humidity favor the presence of dermatophilosis in bovines [1].

Morbidity in Aldama was 15% higher than the 5.2, 11.4 y 7.9% values that have been reported by other authors [3,10,17]. The 22% mortality registered in this outbreak (6/27) is not common [15,18]. Mentioned similar percentages associated with an outbreak in Argentina where animals with severe signs were systemically affected and had consequently secondary complications that led to the death of some of them. Maillard et al. [19] mention that bovine dermatophilosis in tropical areas, may cause severe productive losses, and reach a mortality of 15%.

Morbidity and mortality in the outbreak in Tamaulipas were influenced by the elevated susceptibility of the animals, caused by a massive tick infestation and the high level of humidity registered prior to the outbreak. We attribute the mortality to the lack of knowledge about the etiology of the disease it and the administration of the wrong treatment. This was confirmed once the etiologic agent was discovered and adequate treatment was given, which put a stop to the problems and loss of the animals [20].

In this region of Tamaulipas State, the presence of a massive infestation of ticks of the *Boophilus* and *Amblyomma* genuses is a endemic problem. This factor prior to the outbreak demonstrates the role of ticks in transmitting the infection or predisposing animals to it. This risk factor associated with the lesions caused by the tick and the immunomodulating effects of the saliva secreted in the bite have been demonstrated by other authors [4,21]. Ndhlovu and Masika determinated in Zimbabwe the risk factors associated with clinical dermatophilosis and concluded that management practices aimed at movement and tick control would help reduce the prevalence of clinical dermatophilosis in cattle herds [22] *D. congolensis* bacterium is considered to be part of group 1 of exotic diseases not found nationwide in Mexico [23]. On the other hand, reports of isolates of *D. congolensis* in caprines in the state of Yucatan [24], in a horse in Mexico City [25] and sheep in the State of Mexico [26] have not included bovines, thus making this the first report about dermatophilosis in cattle.

In Mexico there is total ignorance about this disease among field veterinarians and laboratory technicians. This has led to inadequate diagnoses of the disease and mistaken treatments. Regarding the isolation of the microorganism, one of the greatest challenges was the fact that clinical samples are usually obtained from highly contaminated anatomical areas, with the bacteria in the culture media masking the growth of *Dermatophilus*. In order to prevent its contamination and/or development in a pure culture, several strategies were needed. have successfully used several media, such as nutritive agar, blood agar, MacConkey agar and Salmonella-Shigella agar for this purpose [27,28].

In this study, blood agar was used for the initial isolation

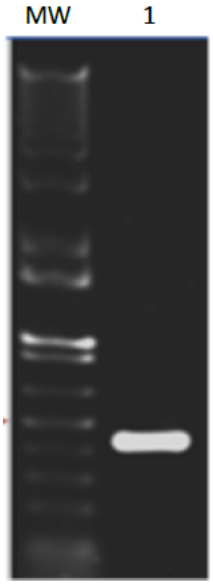

Figure 5: PCR amplification of *D. congolensis* using ESP1 and ESP2 primers. MW, 1 kb Plus DNA Ladder (Invitrogen). Lane 1, a band between 400 and 500 bp.off

and purification of the actinomycete. It was observed that the microaerophilic conditions helped purify the microorganism, as described by several authors [9,29,30], who also used Haalstra's method [11]. They showed that even though *D. congolensis* is able to develop in aerobic or anaerobic conditions, it thrives under microaerophilic conditions to 5-10% CO_2. Because of this and based on our own experience, we can confirm that Haalstra's method [11] is still the most adequate one to isolate *Dermatophilus*, since anaerobic conditions probably restrict the growth of other bacteria present in the clinical samples. Furthermore, it has been demonstrated in experiments with isolated strains from bovines that *D. congolensis* is inhibited *in vitro* by the presence of normal skin bacterial flora, such as *Bacillus* spp. Difficulties to isolate and identify *D. congolensis* have been hence the reasons for the lack of reports of more cases of dermatophilosis [13].

In conclusion, the presence of dermatophilosis in grazing bovines from Aldama, Tamaulipas, Mexico), was established. Being this the first report about the presence of *D. congolensis* in bovines, we consider that training programs for veterinarians and laboratory staff should be implemented to attain correct diagnoses and successful treatments.

Competing Interests

The authors declare that they have no competing interests.

References

1. Zaria LT (1993) Dermatophilus congolensis infection (Dermatophilosis) in animals and man! An update. Comp Immunol Microbiol Infect Dis 16: 179-222.
2. Burd EM, Juzych LA, Rudrik JT, Habib F (2007) Pustular dermatitis caused by Dermatophilus congolensis. J Clin Microbiol 45: 1655-1658.
3. Abdel-Halim MM, El-Fayomy MM, El-Sayed MM, Abdel-Samee AA (1995) Dermatophilosis in farm animals in Egypt. Clinical studies, diagnosis and treatment. J Egypt Vet Med Ass 55: 325-337.
4. Berhanu D, Woldemeskel M (1999) Bovine dermatophilosis and its influencing factors in central Ethiopia. Zentralbl Veterinarmed A 46: 593-597.
5. Higgins R, Paul M, Malo R, Picard B, Paquette JG, et al. (1979) [Dermatophilus congolensis infection in a dairy cow in Quebec (author's transl)]. Can Vet J 20: 279-283.
6. Medway W (1980) Some bacterial and mycotic diseases of marine mammals. J Am Vet Med Assoc 177: 831-834.
7. Nemeth NM, Ruder MG, Gerhold RW, Brown JD, Munk BA, et al. (2013) Experimental Infection of Bar-Headed Geese (Anser indicus) and Ruddy Shelducks (Tadorna ferruginea) With a Clade 2.3.2 H5N1 Highly Pathogenic Avian Influenza Virus. Vet Pathol 50: 961-970.
8. Otero M, Sánchez RO, Fazzio LE, Travería GE, Romero JR, et al. (2003) Ocurrencia de de dermatofilosis caprina en la provincia de Buenos Aires. Analecta Vet 23: 15-18.
9. Shaibu SJ, Kazeem HM, Abdullahi US, Fatihu MY (2011) Phenotypic and genotypic characterization of isolates of Dermatophilus congolensis from cattle, sheep and goats in Jos, Nigeria, African. J Microbiol Research 5: 467-474.
10. Sharma DR, Kwatra MS, Saini SS, Dhillon SS, Gill BS, et al. (1992) Epidemiological studies on dermatophilosis outbreaks in Punjab, Indian. J Comp Microbiol Immunol and Infect. Diseases 13: 5-9.
11. Haalstra RT (1965) Isolation of Dermatophilus congolensis from skin lesions in the diagnosis of streptothricosis, Vet Rec 77: 824-834.
12. Martinez AG, Santillan MA, Guzman CC, Diaz AE (2012) Development of an enzime-linked immunoabsorbent assay (ELISA) for the diagnosis of bovine paratuberculosis. Rev Mex Cienc Pec 3:1-18.
13. García-Sánchez A, Cerrato R, Larrasa J, Ambrose NC, Parra A, et al. (2004) Identification of an alkaline ceramidase gene from Dermatophilus congolensis. Vet Microbiol 99: 67-74.
14. OIE (2008) Dermatofilosis. Manual de la OIE sobre animales terrestres Capítulo 2.4.10.
15. Cattáneo M, Bermúdez J, Apolo A, Bermúdez I, Heras G, et al. (2009) Brote de dermatofilosis en terneros. Analecta Vet 29: 32-34.
16. Cunha PHJ, Keller SA, Oliveira FJP, Badial RP, Oliveira AP, et al. (2010) Dermatofilose em bovinos criados em regime de confinamento. Vet. e Zootec 17: 224-228.
17. Kassaye E, Moser I, Woldemeskel M (2003) Epidemiological study on clinical bovine dermatophilosis in northern Ethiopia. Dtsch Tierarztl Wochenschr 110: 422-425.
18. Cattáneo M, Apolo A, Bermúdez J (2005) Dermatofilosis en bovinos. Rev Argentina de Zoonosis 2: 122-125.
19. Maillard JC, Berthier D, Chantal I, Thevenon S, Sidibé I, et al. (2003) Selection assisted by a BoLA-DR/DQ haplotype against susceptibility to bovine dermatophilosis. Genet Sel Evol 35 Suppl 1: S193-200.
20. Awad WS, Nadra-Elwgoud M, Abdou JI, El-Sayed AA (2008) Diagnosis and treatment of bovine, ovine and equine dermatophilosis. J of Applied Sci Res 4: 367-374.
21. Ambrose N, Lloyd D, Maillard JC (1999) Immune responses to Dermatophilus congolensis infections. Parasitol Today 15: 295-300.
22. Ndhlovu DN, Masika PJ (2014) Risk factors associated with clinical dermatophilosis in smallholder sector cattle herds of Zimbabwe at the Amblyomma variegatum and Amblyomma hebraeum interface. Trop Anim Health Prod.
23. SAGARPA (2007) Acuerdo mediante el cual se enlistan las enfermedades y plagas de los animales, exóticas y endémicas de notificación obligatoria en los Estados Unidos Mexicanos, Diario Oficial, jueves 20 de septiembre de.
24. Torres AJF, Pérez OC (1993) Reporte de caso de dermatofilosis (Dermatophilus congolensis) en cabras y su tratamiento con oxitetraciclina en el estado de Yucatán, México. Reunión Nacional de Investigación Pecuaria. Jalisco Guadalajara, Jal. 27-30.
25. Merino MM, Callejas GG (1988) Aislamiento del actinomiceto Dermatophilus congolensis a partir de un brote de dermatofilosis en equinos en México. Vet Méx 19: 245-247.
26. Cruz A, Cervantes-Olivares RA, Tortora-Pérez JL (1990) Caracterización microbiológica y serológica de Dermatophilus congolensis, a partir de un aislamiento en infección simultánea con el virus de ectima contagioso (ORF), Reunión Nacional de Investigación Pecuaria 1990, Villahermosa, Tabasco. 45.
27. Mannan MA, Khan MSR, Rahman MM, Begum F. Uddin MZ (2009) Isolation and identification of Dermatophilus bacteria from the skin lesions of cattle. Bangl J Vet Med 7: 342-347.
28. Nath BD, Ahasan SM, Rahman SM, FazlulHuque AKM (2010) Prevalence and therapeutic management of bovine dermatophilosis. Bangladesh Research Publications J. 4: 198-207.
29. Colles CM, Colles KM, Galpin JR (2010) Equine pastern dermatitis. Equine Vet Educ 22: 1-5.
30. Tresamol PV, Sassendranath MR (2013) Antibiogram of Dermatophilus congolensis isolates from cattle. Inter J of Livestock Research 3: 117-121.