Bovine digital dermatitis in the Brazilian Amazon biome and topical treatment with Copaifera reticulata oil

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ABSTRACT.- Bomjardim H.A., Oliveira M.C., Brito M.F., Oliveira C.M.C., Monteiro B.M., Silveira N.S.S. & Barbosa J.D. 2020. Bovine digital dermatitis in the Brazilian Amazon biome and topical treatment with Copaifera reticulata oil. Pesquisa Veterinária Brasileira 40(11):842-851. Graduate Program in Animal Science, Instituto de Medicina Veterinária, Campus de Castanhal, Universidade Federal do Pará, Rodovia BR-316 Km 61, Castanhal, PA 68741-740, Brazil. E-mail: henriquebomjardim@unifesspa.edu.br

Bovine Digital Dermatitis (BDD) was studied in crossbred dairy cows grazing in Rondon do Pará, in the state of Pará, as well as in Açaílandia and Cidelândia, in the state of Maranhão, Brazilian Amazon biome. The digits inspection from the dairy cows during milking was performed in ten farms comprising four visits (August and November 2016; April and July 2017). The cows were kept all year in pastures, and were mechanically milked on concrete floors and the animals were protected against the rains in eight farms, maintaining a daily cleaning, however, it could not be found a concrete floor in pre- or post-milking to ensure milking parlor on three farms. Manual milking on no concrete floors was performed in two farms. No preventive measures against hoof lesions were adopted. The BDD prevalence was 1.3% (22/1664), and no statistical difference among rainy or no rainy season was obtained (p = 0.72). The BDD lesions were classified according to “M system” (M0 = no lesion, M1 = active ulceration <2cm, M2 = active ulceration >2cm, M3 = healing stage, M4 = chronic stage, M4.1 = M4 with active ulceration). Regarding the 22 BDD lesions observed, 22.7% (5/22) were M1 stage, 36.4% (8/22) M2, 22.7% (5/22) M3, 13.6% (3/22) M4 and 4.5% (1/22) M1. Hypertrophic hairs at the edges of the lesions caused by fly larvae of genus Cochliomyia spp. as well as alterations on the hoof were also observed. Topical treatment was performed in six BDD lesions with a raw extract from trees of the genus Copaifera reticulata (Copaiba oil) and compared with the treatment of salicylic acid paste in five BDD lesions. The lesions were protected with a bandage for seven days and followed weekly until recovery. The complete therapeutic responses were 83.4% (5/6) and 75% (3/4), respectively, with an average time of seven weeks. The BDD in the Amazon biome occurs in low prevalence, not seasonal, and presents macro and microscopic features similar to BDD lesions from dairy cattle kept in free-stall housing. The treatment with copaiba oil showed similar results to the treatment of salicylic acid paste and can be used in control measures to BDD in the Amazon biome.

INDEXTERMS: Bovine digital dermatitis, Amazon region, Brazil, topical treatment, Copaifera reticulata, copaiba oil, Mortellaro’s disease, grazing system.
as the prevalence of DDB, it is used as a natural treatment for foot diseases in cattle, especially in the Amazon region (Cruz et al. 2005, Silveira et al. 2018).

INTRODUCTION

Bovine digital dermatitis (BDD) is a contagious inflammatory lesion of the skin of bovine digits (Chell & Mortellaro 1974). It has a multifactorial etiology with the interaction between environment, host, and bacterial agents, especially spirochetes of the genus *Treponema* sp. (Blowey & Sharp 1988, Rodríguez-Lainz et al. 1996, Scholey et al. 2010, Krull et al. 2014). It is a disease that may be considered endemic when dairy cows are kept in free-stall stables (Wilson-Welder et al. 2015) and being of low prevalence in beef cattle and crossbred dairy cows kept in a pasture (Wells et al. 1999, Brown et al. 2000, Silveira et al. 2009, 2018, Sullivan et al. 2013).

This disease was firstly described in Italy in 1974, and in the following years also in other European countries and North America, however, nowadays, the BDD is a worldwide disease (Blowey & Sharp 1988, Murray et al. 1996, Rodríguez-Lainz et al. 1999, Manske et al. 2002a, Sogstad et al. 2005). Among foot disorders, it stands out as the primary infectious disease related to overuse or inappropriate use of antibiotic therapy, as well as the presence of residues in milk or meat, and the high costs and toxicity in the use of formaldehyde or copper sulfate (Moore et al. 2001, Holzhauer et al. 2011, Evans et al. 2016, Yamamoto et al. 2018). As alternative treatments, there are raising incentives to invest in non-antibiotic products, i.e., having good efficacy, low cost, and low toxicity, which do not generate residues in the milk and are relatively easy to use.

Among the alternatives for using antibiotics, the salicylic acid (2-hydroxybenzoic), protected by bandages, has been shown efficacy similar to the treatment with oxytetracycline in dairy cows (Schultz & Capion 2013). This salicylic acid has antimicrobial, keratinolytic, and keratoplastic effects; i.e., in addition to "fighting" bacteria, it can dissolve the superficial layer of the epidermis and act in deeper layers and promote tissue repair (Lodén et al. 1995, Monte et al. 2014). Salicylic acid, seeing that it is a non-antibiotic product, and shall be allowed a 24-hour grace period for meat and milk, may present positive results for the treatment of BDD (Schultz & Capion 2013).

In Brazil, a natural substance extracted from the trunk of the genus *Copaifera*, commonly known as copaba oil, is used in traditional folk medicine to treat various diseases in humans and animals (Pieri et al. 2009). In the Amazon region, this oil is well known and also available at low cost in open markets and markets in general. Copaiba oil is a substance rich in diterpenic acids and sesquiterpene hydrocarbons that have, among other properties, antimicrobial activities, anti-inflammatory effects, analgesic, and healing actions (Cascon & Gilbert 2000, Carvalho et al. 2005, Pacheco et al. 2006, Veiga Júnior et al. 2007, Santos et al. 2008, Barbosa et al. 2019). This plant may be used due their properties, and it could be a useful and ecologically sustainable alternative in treating BDD in the Brazilian Amazon region.

In this region, where animals are allowed to graze throughout the year, and therefore there is a dairy cattle breed predilections, seeing that these animals are more resistant to high temperatures and humidity. On the other hand, the BDD’s epidemiological situation is not well studied, and alternative treatments for using of antibiotics should be proposed. Therefore, the objective of this study was aimed to investigate the BDD in crossbred dairy cows reared under pasture in the Brazilian Amazon biome, as well as testing the topical treatment for skin lesions with the crude extract of *Copaifera reticulata* and comparing the data with the treatment with salicylic acid paste.
MATERIALS AND METHODS

Study area and period. The BDD was evaluated in crossbred dairy cows in 10 properties located in the municipalities of Rondon do Pará/PA, Açailândia/MA and Cidelândia/MA, in the Brazilian Amazon biome. The climate of this region is related to the category of super humid equatorial, type Am, according to the Köppen classification, also being at the limit of transition to type Aw. It has average annual temperatures of 26°C (maximum of 32°C and minimum of 22°C). The relative humidity is high, between climate oscillations in the rainy season (from November to May) and the dry season (from June to October), from 100 to 52%, and an average of 78%. The index of annual rainfall has an average of 2,000 mm (Rondon do Pará 2018). Four visits were made in 2016 and 2017 – the first visit was in August 2016, the second one in November 2016, the third in April 2017, and the forth in July 2017.

Previously, and when visiting the properties, the cumulative rainfall indexes of the region were obtained from the database of the “Instituto Nacional de Meteorologia” (INMET) of the meteorological station in the municipality of Rondon do Pará, from January 2016 to July 2017 (INMET 2018), in order to assess possible seasonality in the occurrence of the disease.

The study was carried out according to the ethical principles of animal experimentation recommended by the “Colégio Brasileiro de Experimentação Animal” (COBEA).

Studied farms. The farms were selected owing to their prior knowledge regarding foot disorders as well as the producers’ acceptance to participate in the study. The dairy cows were kept on pasture, in which the vegetation consists of Urochloa brizantha (synonym Brachiaria) (about 95%) and grasses of the genus Panicum spp. cv Mombaça and being added commercial mineral supplementation. Supplementary cornmeal concentrate and soybean meal, corn silage, or sugar cane was offered to the animals in 50% (5/10) of the properties, mainly in the dry period. In 80% (8/10) of the properties, milking was mechanized and carried out in rooms with concrete floors and the animals were protected against rain, including pre- and/or post-milking rooms on concrete floors or beaten floors. In 20% (2/10) of the properties, milking was manual and carried out in a place with a beaten floor. In the properties in which mechanical milking was carried out, and the milking rooms was cleaned daily and at the end of each milking session. This cleaning could be done by scraping the organic matter and washing with water. All properties did not adopt measures to prevent foot infections or control the animals that entry into the herd (Table 1).

Animals. The crossbreeding had herds that were composed from the Holstein breed’s crosses between the Zebu breeds (Gir, Guzerá, Tapubá, and Nelore) in different blood grades, from three to 12 years of age and at different stages of the lactation period.

Diagnosis. The BDD diagnosis was performed during the milking of dairy cows by inspecting all bovine distal limbs with a flashlight. When the organic matter adhered to the distal limbs, they were superficially scraped (manual milking) or cleaned with water jets (mechanical milking).

The bovine distal limbs of 1,664 dairy cows were inspected. Concerning the four visits in order to test all dairy cows, it was not possible to identify each animal, and some bovine distal limbs were inspected more than once. Indeed, some cows in lactation moved to the dry cow group after the first visit, and others belonging to the dry cow group moved to the lactating cow group since there were cows in different third lactation.

At the end of milking, it was performed the scores of claudication symptoms of dairy cows identified with BDD, on a scale from 0 (without claudication) to 5 (severe claudication), according to Flower & Weary (2006). Afterward, physical restraint was performed with ropes and kept in the lateral position for cleaning, inspection, and treatment. In dairy cows highly agitated, 2% xylazine at a dose of 0.1mg/kg body weight was previously administered intramuscularly. The distal extremities were then washed with water, and the lesions measured in centimeters in the vertical and horizontal directions. The anatomical location and macroscopic characteristics were described, as well as the photographic record.

Classification. The lesions were classified into four stages, according to their macro and microscopic characteristics, such as, M1 (first stage of ulcer, <2cm in diameter), M2 (stage of painful ulcer, >2cm), M3 (healing stage, covered in a crust), M4 (chronic stage with hyperkeratotic surface), M4.1 (stage M4 with ulcerated area), according to the score of Döpfer et al. (1997) and Berry et al. (2012).

Table 1. Characteristics of the 10 properties studied regarding the size of the dairy cows lots, supplemented with concentrate and corn or sugarcane silage, manual or mechanical milking and the type of flooring material of the milking pens in the Brazilian biome

| Municipality          | Property | Dairy Cows* | Concentrate | Silage** | Milking*** | Concrete flooring |
|-----------------------|----------|-------------|-------------|----------|------------|------------------|
|                       |          | kg/animal/day | Offer period | Animals | kg/animal | Pre-milking | Milking | Post-milking |
| Rondon do Pará/PA     | I        | 70          | 3           | Dry      | All        | ME          | X       | X         |
|                       | II       | 55          | 4           | Dry and rainy | 20 cows | X           | ME       | X         | X         |
|                       | III      | 50          | 4.5         | Dry and rainy | 20 cows | X           | ME       | X         | x         |
|                       | IV       | 20          | 2           | Dry      | All        | ME          | -       | -         |
|                       | V        | 25          | -           | -        | -          | ME          | -       | -         |
|                       | VI       | 15          | 4           | Dry and rainy | -      | X           | ME       | X         | X         |
| Açailândia/MA         | VII      | 60          | -           | -        | -          | MAM         | -       | -         |
|                       | VIII     | 40          | -           | -        | -          | MAM         | -       | -         |
|                       | IX       | 35          | 6           | Dry and rainy | 14 cows | -           | ME       | X         | X         |
| Cidelândia/MA        | X        | 60          | -           | -        | -          | X           | ME      | -         |

*Average dairy cows between the four visits made, ** about 10 to 15kg/animal/day of corn silage was offered; on property II, cows received about 25 to 30kg/animal/day of corn or sugarcane, *** ME = mechanical milking, MAM = manual milking; PA = Pará, MA = Maranhão.
Treatment. Topical treatment was tested, with approximately 7ml of crude extract of *Copaifera reticulata*, in six BDD lesions of six cattle. After the biopsy, histopathological study and molecular biology were performed, described by Bomjardim et al. (2020). The extract used was obtained by drilling the tree trunk in August 2016 in Rondon do Pará. The species was determined by the “Empresa Brasileira de Pesquisa Agropecuária” (Embrapa) Amazônia Oriental. For comparing the two groups, topical treatment with 10g of a commercial paste based on 660mg/g salicylic acid (Novaderma®, WDT, Garbsen, Germany) was also carried out on five BDD lesions of five cattle. Both products were applied with a bandage composed of gauze, orthopedic cotton, crepon bandage, and adhesive tape. The bandage was removed after seven days, and a macroscopic description of the lesions was performed. All treated cattle were followed up, and the lesions were described weekly until healing.

Statistical analysis. The binomial variables were presented in percentage (%) for the frequency of occurrence. The comparison between the variables according to the visits (1, 2, 3, and 4) was carried out by analysis of variance (ANOVA), using the GLASMIX procedure of SAS® version 9.3 (SAS/STAT, SAS Institute Inc., Cary, NC, USA).

The statistical models were based on the linear effects in classification method regarding variable visit and farms (I to X). The binary response variables consisted of the occurrences of BDD and lesion stages (dist = binomial).

Correlation analyzes were performed using the SAS CORR RANK procedure. The continuous response variables consisted of the occurrences of BDD and lesion stages.

Prevalence

According to the four visits in 10 properties, the BDD was diagnosed in 90% (9/10) of the properties, being detected in, at least, one of the four visits made, with a prevalence of 1.3% (22/1,664). Between visits, there was no significant difference in the occurrence of BDD and stage correlations were observed between the occurrence of BDD and the rainfall index (r=0.02, p=0.38) (Fig.1).

Clinical characteristics

The BDD was observed mainly in the plantar region and affected the commissure of the inter digital space and/or in the coronary bands, but also in the palmar region, as well as in the medial portion of the quartile and cranial of the commissure of inter digital space. By evaluating these animals, the claudication was a discrete clinical sign and scores ranged from 1 to 2, including the absent one (Table 3).

The lesions were diagnosed in the first stage of ulcer (M1 and M2), healing stage (M3), chronic stage (M4), and chronic stage with an area of ulceration (M4.1). Regarding the forms of ulcers, the lesion sizes ranged from 1.0 to 5.5cm, being spherical to oval in shape, with concave, alopecic, moist, reddish surfaces, as well as with a granular surface, being painful to touch the high edges. The healing lesion sizes ranged from 0.3 to 4.5cm and covered with grayish crusty material. In three

RESULTS

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**Table 2. Quantities of dairy cows inspected by property and number of animals with bovine digital dermatitis (BDD), as well as the percentage in the four visits carried out from August 2016 to July 2017**

| Property | 1st - Aug/16 | 2nd - Nov/16 | 3rd - Apr/17 | 4th - Jul/17 |
|----------|--------------|--------------|--------------|--------------|
|          | n | BDD | % | n | BDD | % | n | BDD | % | n | BDD | % |
| I        | 81 | 2  | 2.5 | 73 | -  | -  | 55 | 1  | 1.8 | 61 | -  | -  |
| II       | 78 | 1  | 1.3 | 74 | -  | -  | 41 | -  | -  | 30 | 1  | 3.3 |
| III      | 52 | 1  | 1.9 | 53 | 1  | 1.9 | 42 | -  | -  | 61 | 1  | 1.6 |
| IV       | 22 | -  | -   | 19 | 1  | 5.3 | 10 | -  | -  | 23 | -  | -   |
| V        | 27 | 1  | 3.7 | 29 | 2  | 6.9 | 22 | 1  | 4.6 | 19 | 1  | 5.3 |
| VI       | 14 | 1  | 7.1 | 15 | -  | -   | 12 | -  | -  | 18 | -  | -   |
| VII      | 69 | 1  | 1.5 | 69 | -  | -   | 43 | -  | -  | 50 | -  | -   |
| VIII     | 49 | 1  | 2.0 | 31 | 1  | 3.2 | 33 | -  | -  | -  | *  | *   |
| IX       | 23 | -  | -   | 28 | -  | -   | 34 | -  | -  | 53 | -  | -   |
| X        | 61 | -  | -   | 47 | -  | -   | 61 | 1  | 1.6 | 82 | 3  | 3.7 |
| Total    | 476| 8  | 1.7*| 438| 5  | 1.1*| 353| 3  | 0.9*| 397| 6  | 1.5*|

* Property not evaluated in the period, as the cows were not milked; The letters present on the same line do not differ statistically (p>0.05).
cows, there was a history of topical treatment of lesions with unspecified antibiotics.

In the chronic stage (stage M4), the lesion sizes ranged from 2.0 to 9.2 cm, consisting of irregular shapes with a convex surface, yellowish-white to blackish-brown hyperkeratotic projections, and painless to touch. In two cows, the lesion extended to the coronary bands, and the commissure of the interdigital space assumed the shape of the letter “W”. In an M4 stage, the area of ulceration was reddish, moist, and painful to touch (M4.1).

It was also observed hypertrophy at the edges of the lesions, changes in the corneal tissue of the digits, such as retinal detachment, accumulation of putrid organic matter in the cornea crack formed by the retinal detachment, double sole, and larvae of Cochliomyia spp. (Table 3).

**Treatment**

A cure rate of 83.4% (5/6) was obtained after topical treatment with copaiba oil and 75% (3/4) after topical treatment with salicylic acid paste. It was necessary to have follow-up of the animals until complete skin recovery in an average time of seven weeks (Table 4). After removing the bandage, in seven days, the lesions treated with copaiba oil presented an area of reduced ulcer, as well as in the active lesions and the hyperkeratotic lesions in the chronic stage, covered by whitish crusty material on the lesion and presence of characteristic odor of copaiba oil (Fig.2). A second topical application of copaiba oil with a bandage was carried out on a bovine for seven days. When the bandage was removed, the lesion had a reddish, moist area, painful to the touch, and a putrid odor.

In the treatment with salicylic acid paste, the lesions, when removing the bandage, were covered with a yellowish to whitish crusty material with a characteristic acid odor (Fig.3). In a bovine, the skin adjacent to the lesion was necrotic.

Before topical application of the products and bandages, the manual removal of fly larvae from the lesions was performed with anatomical forceps. By removing the bandage, after seven days, excision of the cracks formed in the corneal tissue, and the double sole was performed with the aid of “rinetas”. After removing the bandage until the normal skin recovered, the presence of eggs or fly larvae was not observed.

**Table 3. Classification of scores* regarding in bovine digital dermatitis (BDD), clinical signs, lesion location, macroscopic characterization and other changes in the corneal tissue in crossbred dairy cows raised on pasture in the Brazilian Amazon biome**

| Stage of BDD | Bovine Member** | Degree of lameness | Anatomical region*** | Diameter (cm) | HH | Fly larvae | Alterations in corneal tissue |
|--------------|-----------------|--------------------|----------------------|---------------|----|------------|-----------------------------|
| M1           |                 |                    |                      |               |    |            | D   | OM | DS |
| 01           | LP              | 0                  | IC, PL              | 1.0x2.0       | -  | -          | -   | -  | -  |
| 02           | LP              | 0                  | RMQ, PL            | 1.6x1.5       | -  | -          | -   | -  | -  |
| 03           | LP              | 0                  | CB/LD, PL         | 1.5x1.7       | x  | -          | -   | -  | -  |
| 04           | LP              | 0                  | IC, PL            | 1.3x2.0       | -  | x          | -   | -  | -  |
| 05           | LP              | 0                  | RMQ, PL          | 1.2x2.4       | x  | -          | -   | -  | -  |
| M2           |                 |                    |                      |               |    |            | D   | OM | DS |
| 06           | LP              | 0                  | IC, PL            | 2.0x2.0       | x  | -          | -   | -  | -  |
| 07           | RP              | 1                  | CB/LD, ET, PL     | 3.2x1.5       | -  | x          | x   | x  | -  |
| 08           | RP              | 1                  | IC, CB/MD, PL    | 2.5x2.5       | -  | x          | x   | x  | -  |
| 09           | RP              | 2                  | CB/LD, ET, PL     | 2.5x4.0       | -  | x          | x   | x  | -  |
| 10           | LP              | 2                  | CB/LD, ET, PL     | 3.7x2.4       | -  | x          | x   | x  | -  |
| 11           | RT              | 1                  | IC, C            | 4.5x3.6       | x  | -          | x   | -  | -  |
| 12           | RP              | 1                  | IC, CB/LD e MD, PL| 4.3x5.5       | x  | x          | x   | -  | -  |
| 13           | LP              | 2                  | IC, CB/LD e MD, ET/MD, PL | 5.0x5.0 | x  | x          | x   | x  | x  |
| M3           |                 |                    |                      |               |    |            | D   | OM | DS |
| 14           | LP              | 0                  | CB/LD, PL         | 0.3x0.4       | -  | -          | x   | -  | -  |
| 15           | LP              | 0                  | IC, PL            | 0.6x0.9       | -  | -          | x   | x  | -  |
| 16           | RP              | 0                  | IC, PL            | 1.3x2.0       | -  | -          | x   | -  | -  |
| 17           | LP              | 0                  | IC, PL            | 1.9x4.8       | -  | -          | x   | -  | -  |
| 18           | LP              | 1                  | CB/LD, ET, PL     | 4.5x3.7       | x  | -          | x   | -  | -  |
| M4           |                 |                    |                      |               |    |            | D   | OM | DS |
| 19           | LP              | 0                  | CB/LD e MD, PL    | 2.8x2.0       | x  | -          | -   | -  | -  |
| 20           | LP              | 1                  | IC, CB/LD e MD, PL| 6.4x5.5       | -  | x          | x   | -  | -  |
| 21           | LP              | 0                  | IC, CB/LD e MD, PL| 9.2x7x4       | x  | x          | x   | x  | -  |
| M4.1         |                 |                    |                      |               |    |            | D   | OM | DS |
| 22           | RT              | 2                  | CB/LD e MD, PA    | 6.0x5.0       | x  | x          | x   | x  | -  |

* According to Döpfer et al. (1997) and Berry et al. (2012); ** Affected member: RT = right-sided thoracic, LT = left-sided thoracic, RP = right-sided pelvic, LP = left-sided pelvic; *** Affected region: IC = Commissure of the interdigital space, CB = coronary band, PA = palmar, C = cranial, LD = lateral digit, MD = medial digit, ET = extent of injury to the talon, MD = medial region of the quartile; HH = hypertrophic hair, D = retinal detachment, OM = organic matter in dark color with putrid odor, DS = double sole.
**DISCUSSION**

The BDD is widespread among cattle herds from the studied properties, with a prevalence of 1.3% (22/1,664). This result was superior to that one observed by Silveira et al. (2009) in 2008, being performed in the same study region, in which an occurrence of 0.48% of BDD was observed (6/1,236). However, the study conducted by Silveira et al. (2009) paid special attention to claudicating animals. Non-claudicating animals, suffering from initial lesions, as observed in this study, were not detected, which could increase the prevalence rate of BDD in 2008. However, this higher prevalence of BDD in this region may be related to the absence of prophylactic and therapeutic measures for the contagious disease of the foot.

The low prevalence of BDD in cows destined for dairy production, in the Amazon biome, observed in this study and also observed by Machado et al. (2008) and Silveira et al. (2009) was similar to the prevalence of the disease in beef cattle from the same biome, as reported by Silveira et al. (2018), in dairy cows reared under a semi-intensive manner in the Brazilian Cerrado (Leão et al. 2009, Tomasella et al. 2014) and the Atlantic Forest (Souza et al. 2007). However, the prevalence of BDD was at a lowest level, i.e., a minimum of 30%, observed in dairy cows intensively reared and housed in a free-stall system in Brazil (Cruz et al. 2001, Souza et al. 2015), in Canada (Cramer et al. 2008), the Netherlands (Somers et al. 2005), Denmark (Klitgaard et al. 2008), Germany (Nordhoff et al. 2008) and Japan (Yano et al. 2010). These studies emphasize the close relationship between BDD and the type of breeding animals.

### Table 4. Topical treatment with salicylic acid paste and crude extract of *Copaifera reticulata* (Copaiba oil) from stages M1, M2, M4 and M4.1 of bovine digital dermatitis (BDD) in the Brazilian Amazon biome

| Topical treatment                                      | Bovine | BDD | Macroscopic characteristic of lesions by removing bandage | Healing process (weeks) | Result |
|--------------------------------------------------------|--------|-----|----------------------------------------------------------|-------------------------|--------|
| Salicylic acid paste (Novaderma®)                       | 6      | M2  | Surface covered by salicylic acid paste                  | 8                       | Treated|
|                                                        | 7      | M2  | Covered by flaky white material                          | 3                       | *      |
|                                                        | 8      | M2  | Off-white yellow surface                                 | 10                      | NT **  |
|                                                        | 12     | M2  | Yellow surface and skin adjacent to the necrotic lesion   | 10                      | Treated|
|                                                        | 19     | M4  | Brownish-yellow surface                                  | 4                       | Treated|
| Crude extract of *Copaifera reticulata*                 | 1      | M1  | Covered by flaky white material                          | 9                       | Treated|
|                                                        | 10     | M2  | Wet and reddish surface                                  | 8                       | Treated|
|                                                        | 13     | M2  | Reddish, granular surface and covered with yellowish crusty material | 9                       | NT **  |
|                                                        | 20     | M4  | Covered by flaky white material                          | 10                      | Treated|
|                                                        | 21     | M4  | Covered by flaky white material                          | 7                       | Treated|
|                                                        | 22     | M4.1| Circumscribed area with a wet and reddish surface, surrounded by yellowish material | 5                       | Treated|

* Treatment accompanied by only 21 days, as the animal died on the property of unclear cause, ** NT = non treated.
Regarding the extensive breeding system, cattle are kept in extensive pasture areas, areas of lower humidity. The bovine digits are less exposed to feces, and there is less contact between animals, as the concentration of animals per area is low. Breeding conditions opposite to what is observed in stable dairy cows, where digits are exposed continuously to feces and urine, which promote the skin of bovine digits to high humidity, mostly in stables where feces are not scraping frequently, as pointed out by Somers et al. (2005).

The prevalence of BDD in the properties in this study was constant over this time study, with no statistical correlation between the rainfall rate and the occurrence of the disease (r=0.02, p=0.38), which indicates that the disease can occur at any time of year in the Amazon biome, regardless of whether rains or not (Fig.1). However, a close relationship between a high prevalence of foot infections and poor hygiene conditions is pointed out by Van Metre (2017) or problems with muddy pens by Rodríguez-Lainz et al. (1996). For this reason, we believe that the rainy season may have a significant influence on the occurrence of BDD in the Amazon biome. In this season, the animals remain in pens from two to four hours, i.e., before, during, or after milking with muddy pens, feces, and urine, as reported by Silveira et al. (2009). The influence of the rainy season and the BDD occurrence is shown in Figure 1, where there is an increase in the disease occurrence from December to May.

Relun et al. (2013b) and Krull et al. (2016) observed an average of 140 days (5 months) for normal digit skin to present an ulcerated lesion in stable dairy cows. This time of development of the lesions may explain the increase in the prevalence of BDD, although not statistically significant, after the rainy season.

According to Sullivan et al. (2013), lameness is the first clinical sign observed in BDD. In this study, lameness was a clinical sign absent in the initial lesions (M1), in those in the healing stage (M3), and in the chronic (M4) and mild stages, grades form 1 to 2, in active lesions. For Frankena et al. (2009), the degree of claudication in cattle increases in parallel with the stage or severity of BDD lesions, when deeper and more extensive structures of the skin and or structures of the corneal tissue are reached. Thus, it is necessary to inspect the distal extremities during milking periodically. According to Orsel et al. (2017), the bovine digital space must be inspected during milking, i.e., being an easy practice to be performed and allowed the identification of BDD lesions in the initial stages, for immediate treatment, and monitoring of the herd.

The BDD lesions in the present study occurred preferentially on the skin of the plantar region of the caudal commissure and coronary bands, the preferred location for the formation of the lesions, as was also reported by Read & Walker (1998) and Cruz et al. (2001). Sogstad et al. (2005) related this preference of lesions to the limbs with more contact with feces and urine. In beef cattle, foot lesions preferentially occur in the pelvic limbs. These are also due to abrasions on the bovine digital skin with the non-castrated cattle, as observed by Silveira et al. (2018). Despite this preference, the thoracic digits and the pelvic digits of dorsal region should also be inspected.

The BDD lesions that formed or reached the cell crown led to the retinal detachment and the formation of a cornea crack. This change, already reported in dairy cows by Cruz et al. (2001) and in beef cattle by Sullivan et al. (2013), is probably related to the interruption or decrease in the supply of nutrients and oxygen to the precursor cells of the corneal tissue, located in the cell crown, due to the inflammatory response triggered. The accumulation of organic matter in these bovine dermatitis accompanied by cracked between the digits, associated with low oxygenation conditions, probably promotes an environment favorable to the colonization of anaerobic bacteria that lead to the destruction of healthy corneal tissue and the occurrence of other changes in the digits such as septic pododermatitis and double sole in cattle. Therefore, BDD can lead to the development of more secondary severe lesions in the digits of cattle. Cruz et al. (2001) also described angular deformations and increased sole thickness as changes secondary to BDD. In treating BDD lesions, it is necessary to remove these secondary changes in the corneal tissue. The development of larvae of flies of the genus Cochliomyia spp. in lesions of BDD, species of Diptera are more common in tropical areas, they can aggravate the lesions and lead cattle to a more severe clinical lameness, as they produce proteolytic enzymes that degrade healthy tissue.

Topical treatment with copaiba oil, protected by bandaging for seven days, proved to have similar therapeutic efficacy when compared to salicylic acid paste. Both showed similar results to the topical treatment with oxytetracycline reported by Manske et al. (2002b). When removing the bandage, both treatments showed improvement in the macroscopic aspects of the lesions, reducing putrid odor and painful to touch. However, among the treatments with copaiba oil, bovine 13 had the area of inflammation of the lesion reduced in the first bandage. However, it presented with large area of ulcers, very painful to touch, and with a putrid odor. The amount of time of contact of the product with the wound was probably not sufficient to treat the injury. In the treatment with salicylic acid paste, it was observed, in a bovine, that the tissue adjacent to the lesion was necrotic. Salicylic acid paste, when used in excess, can react with normal tissue and promote areas of necrosis. Therefore, using this product, an amount of acid sufficient to cover the lesion surface is recommended.

Copaiba oil reduced partial and total ulceration in lesions in the active stage, undid the hyperkeratotic surface, and promoted tissue repair in lesions in the chronic stage. This improvement in the macroscopic characteristic of the lesions, by reducing inflammation, in the keratolytic effect, and in tissue repair, may have resulted from the joint action formula of different compounds present in the copaiba oil described by Cascon & Gilbert (2000). According to Veiga Júnior et al. (2007), among these compounds, β-caryophyllene has a potent anti-inflammatory action, and the fractionated diterpenic acids of Copaifera reticulata (ent-kaurenioic acids, kovalenic, (13E)-ent-labd-7,13-dien-15-oic) have high antimicrobial activity, according to Barbosa et al. (2019). Copaiba oil was effective in treating human periodontitis, described by Bardaji et al. (2016) and in dogs, by Pieri et al. (2010).

In both treatments, one in each, lesion recurrence was observed. These results pointed out to two possibilities: i) the treatment effect was not significant, since bacteria are found in deep layers of the epidermis, as shown by Zinicola et al. (2015); ii) the treatments were effective, but after a period, the lesions were reinfected, as pointed out by Berry et al. (2010) in lesions of BDD showing clinical improvement at the beginning and then returns to the active stage. In the
case of ineffective treatments or recurrence of the lesions, it is recommended to perform a second bandage with the same product or alternate the tested topical products.

With the low prevalence of the disease in the region regarding this study and difficulties to reach properties, due to long distances, it was not possible to evaluate both treatments in a larger number of cattle and to compare it with a control group, only being compared with the bandage. According to Manske et al. (2002b), the bandage may promote a longer time of action of the product with the lesion by keeping the bovine digits clean, without contact with mud or feces. Previous studies by Thomsen et al. (2012) pointed out that cleaning the digits periodically, the BDD prevalence can decrease. The results obtained regarding the animals treated in this study were probably related to a joint action formula of the product and the application of the bandage.

CONCLUSIONS

The BDD is a disease of low prevalence and not seasonal occurrence in crossbred dairy cows raised on pasture in the Brazilian Amazon biome. It is widespread among herds and has macroscopic characteristics similar to the BDD observed in dairy cows from farms housed in a free-stall system.

Copaiba oil has shown positive results regarding the treatment of BDD and may be an alternative to disease control measures in the Brazilian Amazon biome.

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