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CASE REPORT
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Stephanofilariasis in Holstein Cows - Diagnostic Approach

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ABSTRACT

***Background*:** Stephanofilariasis is a parasitic disease caused by the nematode *Stephanofilaria* spp., considered a zoonotic disease, that affects several species, mainly dairy cattle. The condition causes chronic ulcerative dermatitis, due to the mechanical action of the parasite on the hair follicles and dermal papillae. The recommended diagnostic method is histopathological examination, but alternative methods have been studied for the detection of the agent since it does not demonstrate good results. There is no specific treatment, however, the use of organophosphates is recommended. The objective of this work is to report an outbreak of stephanofilariasis with unusual lesions in cattle from a dairy cattle farm in the city of Boa Vista do Buricá, RS, Brazil, as well as to demonstrate an alternative method for the diagnosis of the disease.
***Cases*:** We prospectively evaluated 15 Holstein cows, aged between 1 and 10-year-old whose data and materials for diagnosis, were provided by the property owner. The outbreak occurred in the summer, in a period of greater rainfall, affecting cows of different ages and totaling 48% of the herd. These showed ulcerative lesions on the skin of the groin, limbs and interdigital regions, and less frequently in the udder. The samples were obtained through tissue biopsy and scraped from the lesions, being packed in 10% formaldehyde for fixation. Afterward, the formaldehyde was centrifuged at 800 *g* for 5 min, which consisted of analyzing the sediment through optical microscopy and without staining, aiming at the direct search of the agent. The tissues, on the other hand, underwent routine processing and stained with Hematoxylin and Eosin. Histopathological analysis did not reveal *Stephanofilaria* sp. subjects in morphological analysis and revealed acanthosis, spongiosis, ulcers and fibrosis in the analyzed tissue. The examination of direct research with formaldehyde proved to be efficient, and fast. In the direct examination of the agent, structures similar to filarial and compatible with *Stephanofilaria* sp. in all samples. Thus, the present study demonstrated that filariasis can present in the form of outbreaks and with unusual injuries. The examination of direct research with formaldehyde, proved to be efficient and fast.
***Discussion*:** Semi-confined and confined animals may have a higher occurrence of strephanofilariasis, since the parasite needs humid and warm environments to proliferate and that, the proliferation of stephanophilariasis vectors is intensified in environments with low sunlight, high temperatures, high humidity, and low cleaning frequency. The city of Boa Vista do Buricá, where the outbreak occurred, reached a minimum average temperature of 18.5ºC and an average of 29ºC, with an average rainfall of 120.5 mm. Cutaneous lesions have been seen in animals from 1 to 10 years of age and no racial predisposition, age, or blackout stage is reported. The histological diagnosis, although mentioned as a form of diagnosis, has low efficacy in finding parasites in the tissue, is also used as a diagnosis, direct examination of the agent with saline solution, and impression of the lesion stained by the Romanoswsky method. However, in this report, formaldehyde proved to be a new option, as efficiently as saline.

Keywords: filariasis, flies, nematode, dairy cattle, zoonotic disease, direct examination, lactation ulcer.

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INTRODUCTION

Stephanofilariasis is a disease caused by the nematode *Stephanofilaria* spp. and, its occurrence is associated with transmission through hematophagous flies such as *Musca planiceps*, *Haematobia irritans*, and *Stomoxys calcitrans*, and not hematophagous such as *Musca conducens* [3]. It can affect several species, such as cattle, mainly of dairy lineage, in addition to buffaloes, rhinos, and elephants. The microfilariae of *Stephanofilaria* spp. they have also been identified in the blood of domestic canids [11], and it is considered a zoonosis, since it can cause ulcerative nodular dermatitis (indolent varicose ulcer) in humans [10]. The objective of this work is to report an outbreak of stephanofilariasis with unusual lesions in cattle from a dairy cattle farm in the city of Boa Vista do Buricá, RS, Brazil.

CASES

We prospectively evaluated 15 Holstein cows, aged between 1 and 10 years, on a property located in the city of Boa Vista do Buricá in Rio Grande do Sul. The samples were received at the Veterinary Pathology service. The exam was authorized by the producer by signing the Free and Informed Term of the Federal University of Fronteira Sul, Realeza campus.

The cows came from a property where the producer, under the guidance of a veterinarian and due to a history of skin wounds with an undefined diagnosis, sent material to the pathology laboratory. These were selected based on the clinical lesions presented and the characteristics of the infection. Ulcerative and exudative elliptical lesions were collected from the udder, groin and interdigital regions, respectively, from 3 animals, whose samples were subjected to histopathology and direct parasite research.

Epidemiological information, such as property history and clinical data, was provided by the Veterinarian as the technician responsible and with the producer. Climatological data were obtained by the Rio Grandense do Arroz Institute (IRGA), which have climatological averages for the region.

In order to diagnose stephanofilariasis in animals, samples of tissue and skin scraping, groin, udder and interdigital lesions were obtained from different animals. The tissue fragments were acquired through incisional biopsy, with a representative area of the lesion and preservation of whole tissue in the same sample. For its removal procedure, local asepsis based on alkyl dimethyl benzyl ammonium chloride and polyiexiethylenonylphenyleter[1] was performed, infiltrative local anesthesia with 2% lidocaine[2] at a dose of 1 mL per lesion. The incision was made with excision of cutaneous fragments approximately 2 cm in diameter and depth of 1 cm, using a scalpel. All material was fixed in 10% formaldehyde and sent to the Veterinary Pathology Laboratory of UFFS-Campus Realeza. The preparation of the conventional histological slides was processed according to Nunes & Cinsa [13] with modifications and hematoxylin and eosin staining.

Due to the difficulty in visualizing the parasite previously described in the literature in histological samples, it was decided to perform a new form of diagnosis. Briefly, the formaldehyde present in the tissue samples, was collected and subjected to centrifugation. The centrifugation procedure followed the adaptation of that described by Miyakawa *et al*. [8] previously. Thus, 10 mL of formaldehyde were separated from the samples, the contents were subjected to centrifugation at 600 *g* for 5 min, then the sediment was removed and observed under an optical microscope with the aid of a glass slide and coverslip, without the use of dyes.

The outbreak of the disease occurred in a semi-intensive dairy farm with a herd of 23 cows and 8 heifers, with ages varying between 1 and 10 years. In the present study, ulcerative skin lesions were observed in 48.3% (15/31) of the herd. Although the occurrence of lesions in the udder region and in dairy cows are reported as the main lesions [4], in the present study, the lesions were located 66.6% (10/13) in pelvic limbs close to the hoof, 13.3 % (2/13) in the cranial region to the udder, 13.3% (2/13) in the interdigital region and 6.6% (1/31) in the groin.

The lesions were ulcerative, with well-defined, circular, crusted edges and with purulent exudate (Figure 1), in addition, some animals started with edema in the affected region and 1 animal developed myiasis, there were cases of mastitis. According to the veterinarian's report, lesions with similar characteristics have appeared with great frequency in recent years on the property, but never affecting so many animals.

Likewise, it was observed that the animals in the present study showed a decrease in production of approximately 4 L / cow / day. In the lesions submitted to histopathological examination, it was not possible to observe the presence of parasitic structures, however, mild orthokeratotic hyperkeratosis, diffuse papillary

acanthosis, keratin pearls (Figure 2), prominent intercellular connection (spongiosis) and ulcers were found. Diffusely in the superficial dermis, there was an intense eosinophilic inflammatory infiltrate and moderate perivascular lymphocytic infiltrate, proliferation of fibrous connective tissue in the dermis. The morphological diagnosis was determined as diffuse eosinophilic lymphocytic inflammation.

In direct analyzes with formaldehyde sediment, parasites were observed in 100% of the samples. The adult forms of the parasite (Figure 3), were intact, being possible to observe numerous specimens per sample. With direct examination, characteristics compatible with *Stephanofilaria* spp. were observed, such as size and morphological characteristics, for example, the presence of spines on the head and spike.

The treatment was established by the property's veterinarian, based on cleaning the wounds with iodine[3] and copper sulfate pentahydrate[3] (concentrations not provided) at the beginning of the lesions, when aggravated, trichlorfon ointment and zinc oxide with cresyl acid[3] [Unguento Chemitec® - BID, topic] were used. In addition, oxytetracycline, penicillin with streptomycin[4] [Oxi Vansil LA® - 20 mg/kg, SID, i.m] and ivermectin[5] [Supremo® - 1 mL for every 50 kg, i.m]. Despite the improvement, the animals did not show complete remission of the lesions.

DISCUSSION

It is known that semi-confined and confined animals may have a higher occurrence of stephanofilariasis, since the parasite needs moist and warm environments to proliferate [3,14]. The city of Boa Vista do Buricá during the period of this outbreak reached an average minimum temperature of 18.5°C and an average maximum of 29°C. The average rainfall for this period was 120.5 mm [6]. The proliferation of diptera, vectors of strephanofilariasis, is intensified in environments with low sunlight, high temperatures, high humidity and low cleaning frequency [3,17]. Results were obtained, with a greater occurrence of the disease between the months of December to March, a period of high rainfall [7].

There is no racial predisposition, age or lactation stage [3]. This disease has a greater occurrence from the age of 4 years, and usually does not present lesions from the age of 10, as well as a greater predilection for females than for males [2]. The large number of

Figure 1. Stephanofilariasis in a cow. Ulcerative lesions found in the animals on the property. A- Crusted lesion from the ventral skull to the udder, in the midline region. B- Injury in the groin area. C- Large circular lesion in the pelvic limb. D- Interdigital hoof injury.

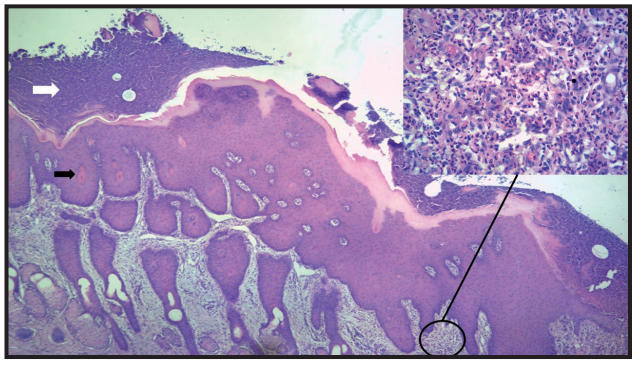

Figure 2. Stephanofilariasis: epidermis and superficial dermis. Observe papillary acanthosis, keratin pearls (black arrow), crusted formation (white arrow) and eosinophilic infiltrate (highest increase). [HE; 10x].

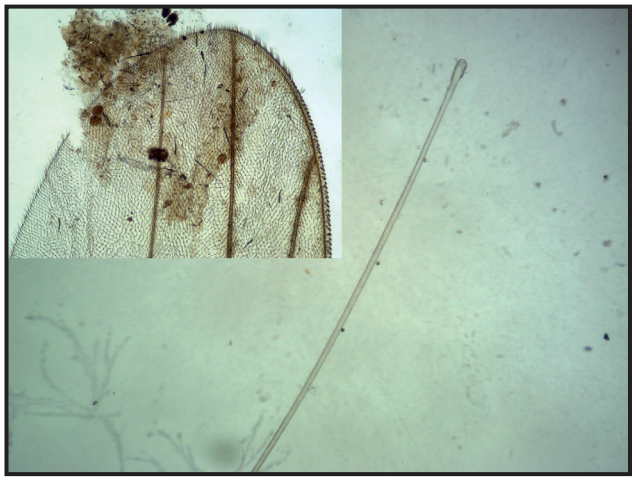

Figure 3. Direct examination for research of the agent in 10% formaldehyde solution. Adult form of *Stephanofilaria* sp. In the insert, it is possible to observe the parasite head in higher magnification.

lesion distribution sites contradicts the evidence found in the work of Miyakawa *et al*. [7] who found lesions only in the udder region, and a single animal with a lesion in the flank fold. Lesions at the base of the tail, thigh and insertion of the udder, associated only with lactating cows were already reported [12]. On the other hand, there is a study that found a higher prevalence of injuries in the neck region [2]. There are rare reports of the occurrence of lesions of *Stephanofilaria* spp. in cattle members. In India, Singh *et al*. [17] observed injuries in paradigms, hull, caused by *Stephanofilaria assamensis*. It can be inferred that in Brazil some cases are diagnosed as bacterial pododermatitis.

The infected animals have pruritus, which can vary from mild to intense, causing constant licking and pain, the sum of these signs results in decreased milk production [15]. The decrease in milk production is a consequence of hoof injuries, which in turn cause pain and reluctance to access the water and food troughs. In addition, lesions can become a gateway for other microorganisms such as bacteria, especially in the case of semi-intensive and intensive breeding systems, as there is a large accumulation of organic matter, thus aggravating changes [5]. Opportunistic infections, such as bacterial infections, can be decisive in decreasing production, dissipating cross-infections, in the development of mastitis and the occurrence of myiasis, through the contact of animals with flies [14].

No studies were found that identified the species and *Stephanofilaria* spp. that occurs in Brazil, thus, all existing classifications identify only the gender [8,15].

The histological diagnosis, although mentioned as a form of diagnosis, has low efficacy in finding parasites in the tissue [7]. Similar histopathological findings were also described by AFIP Wednesday Slide Conference [1], which also complements that adult parasites are present in hair follicles and cystic diverticula. Although recommended by some authors, the histopathological examination, over the years, has not proved to be effective in the research of *Stephanofilaria* spp. [1,7].

For diagnosis, direct examination of the agent with saline solution and imprint of the lesion stained with the Romanoswsky method are also used [8,15]. As the material was all submitted to a fixative solution, formaldehyde, it was decided to perform the same process mentioned by Miyakawa *et al*. [8] saline (0.9% NaCl), but with 10% formaldehyde. The direct search exam proved to be inexpensive, fast and effective in diagnosing the condition. It is recommended to use physiological saline solution for direct research, in a 1:10 ratio with a 3-hour rest followed by centrifugation. However, in the present report, formaldehyde proved to be a new option, with an efficiency similar to saline solution, which can facilitate mainly when parasitic structures are not observed in conventional histopathological examination. By of this the use of the material from the sample itself in the fixer is possible [8].

When harvesting the biopsy fragments and fixing in the formaldehyde, the parasites die and end up in the remaining formaldehyde in the flask. For this reason, it was not possible to find parasites in histopathological samples. The most used tests (direct examination in saline and imprint of the lesion) are obtained from the most superficial regions of the lesions, suggesting, therefore, that parasitism is more intense on the lesions surface [4]. Therefore, the low sensitivity of the histopathological examination is due to the fact that the parasites are diluted in formaldehyde [13]. Therefore, our results strongly suggest that the formalin from biopsy samples is useful in direct parasitological examination and identification of the parasite.

In general, there is no specific treatment for the disease. Pazinato *et al*. [15], in an outbreak used an artisanal mixture of 6% trichlorfon with petroleum jelly, obtaining 100% effectiveness in curing the disease. The applications were carried out twice a day for 7 days and associated with a daily application of chlorpyrifos, gentian violet and pine oil[3] (Lepecid BR Spray®). Similar results have also been reported using topical application of 6% trichlorfon in paste with petroleum jelly twice a day after milking, resulting in complete healing, but the healing time of the lesion varied by 39.1 ± 9.2 days [9]. Adherence capacity is also an important factor in the treatment, since the longer the drug remains in the lesion, the greater its action on the agent [16]. The lesions in uncharacteristic places of strephanofilariasis favored a late diagnosis, since, initially, there was no suspicion of the disease, thus leading to low treatment efficiency in terms of the outbreak occurrence.

The use of ivermectin was stressed, proving the effectiveness of the drug in paste 1% and 2% and its use "pour on", when associated with trichlorfon. Therefore, spraying with ivermectin can help treat this condition [9]. However, ivermectin has restrictions on its use, when

considering the sale of products of animal origin, since it can leave toxic residues in meat and milk. The maximum residue limit for ivermectin is 0.04 mg/kg, which can lead to considerable economic losses for the producer [16]. Tupinambás *et al*. [18], in a study of environmental control, substituted commercial products for clove essential oil, obtained control of the fly population in stalls.

Prophylactic methods must be recommended, they are mainly based on vector control. For this, it is necessary to always keep the environment clean, not to accumulate organic matter (urine and feces), especially in closed places [7]. Hygiene conditions in milking must also be recommended, since the disease can cause the development of mastitis, a disease that can be transmitted through milking. After the disease is installed in the herd, good hygiene practices should not be neglected on the spot, and when necessary, use drugs that can help control the vectors.

In the present study, the histopathological examination was not able to identify the parasites in the reported lesions, however, the direct parasitological examination of the formaldehyde packed with the samples proved to be effective in identifying the parasites. This research suggests that the centrifugation of the formaldehyde that was used to fix cutaneous lesions, is of great importance in the diagnosis of stephanofilariasis.

MANUFACTURERS

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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