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RARE AND OPPORTUNISTIC MYCOSIS BY *Nigrospora* spp. IN A CALF

(MICOSE RARA E OPORTUNISTA POR Nigrospora spp. EM BEZERRA)

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RESUMO

Os fungos estão presentes em diferentes ambientes, onde homens e animais estão expostos, como no solo, plantas e no ar. No entanto, fungos ambientais podem se tornar oportunistas e causar graves lesões de pele, oculares, pulmonares, micetomas, dentre outras, visto que as micoses oportunistas estão associadas às condições imunológicas do hospedeiro. O fungo do gênero *Nigrospora* é raramente considerado um patógeno oportunista ou agente primário de micoses. O objetivo deste relato de caso é descrever uma rara micose oportunista de pele, causada por *Nigrospora* em um bezerro de seis meses, fêmea, com escore de condição corporal ruim devido à desnutrição. Amostras de pele foram inoculadas em placas de Petri contendo ágar Sabouraud e após sete dias de incubação a 25(±2) ºC, lâminas de cultura examinadas em microscopia ótica com azul de lactofenol permitiram a identificação de estruturas conidiais de *Nigrospora* spp. Com a aplicação tópica semanal de solução a 1% de iodóforo, o animal apresentou a completa remissão dos sintomas após oito semanas.
Palavras-chave: micose; fungo; desnutrição; *Nigrospora*; bovino

ABSTRACT

The fungi are present in different environments, where humans and animals are exposed, such as in soil, plants and air. However, environmental fungi may become opportunistic and cause serious injury in skin, eyes, lungs, mycetomas, among other, since opportunistic mycosis are associated with the immunological conditions of host. The fungus of the genus *Nigrospora* is rarely considered an opportunistic pathogen or primary agent of mycoses. The objective of this case report was to describe a rare case of opportunistic skin mycosis caused by *Nigrospora* in a six-month-old female calf with bad body condition score due to malnutrition. Skin samples were inoculated in Petri dishes containing Sabouraud Dextrose agar and after seven days in incubation at 25(±2) ºC, slides of culture examinated under optical microscopy with lactophenol cotton blue allowed the identification of conidial structures of *Nigrospora* spp. After the treatment with weekly application with 1% iodine solution, the animal presented complete remission of symptoms after eight weeks.
Keywords: mycosis; fungus; malnutrition; *Nigrospora* spp.; cattle

INTRODUCTION

Dematiaceous fungi are a heterogeneous black fungi group present in diverse environments worldwide. Many species in this group are known to cause allergic reactions and potentially fatal diseases in humans and animals, especially in tropical and subtropical climates (YEW et al., 2014). *Nigrospora* species are a filamentous melanized (dematiaceous) group of fungi that belong to the phylum Ascomycota, widespread in the environment and abundant in regions with a tropical or subtropical climate, more commonly occurring outdoors than indoors and readily isolated from soil, rural indoor cattle shed and decaying plants (DE HOOG et al., 2000).The generic name *Nigrospora* was first introduced by Zimmerman (1902) for *N. panici,* which was isolated as an endophyte from leaves of *Panicum amphibium* in Java, Indonesia (WANG *et al.*, 2017).

The presence of pathogenic fungi for animals and plants is detached as an important study of epidemiology and prophylaxis of mycoses, with special importance of the knowledge about the habitat or the different reservoir of these agents. Usually these fungi do not determine mycotic infections, however, the spores or even vegetative forms (in favourable conditions) may germinate, grow and cause various disturbances in the tegument or in the respiratory tree of humans and animals, often serious infections (SIQUEIRA *et al.*, 1990).

Widespread in the environment *Nigrospora* spp. is only rarely encountered as a true pathogen. Thus far, literature includes only five cases of human infections caused by this violently spore discharging fungus (WEBSTER, 1952), which includes infections of the skin, nail and eye in both immunocompetent and immunocompromised patients (BHARATHI *et al.*, 2011, FAN *et al.*, 2009, MOTSWALEDi and PILLAY, 2019). Ananya et al. (2014) reported another incidence of this fungus exhibiting its infrequent pathogenic role. One case was described in a 45-year-old immunocompetent woman from a rural area in south India presented to the ophthalmology outpatient department in a Hospital in India with a history of injury of the right eye. The patient suffered a traumatic inoculation of the spores on being hit with a cow's tail most likely contaminated with soil and vegetative matter which are constant habitats of this widely distributed fungus.

Fungal pathogens were isolated and identified from sputa of 100 HIV positive patients admitted at a hospital, in India, during the period of 2 months from June 2010 to July 2010. Out of total 96 isolates *Candida* spp. was the major group comprising 55 in number (57%) but *Nigrospora* spp. were isolated in one sample (1%) (BHARATHI and RANI, 2011). A study in Malaysia of dematiaceous fungi using seventy-five strains isolated from various human clinical specimens identified two *Nigrospora oryzae* that showed potential multidrug resistance (YEW *et al.*, 2014).

In a study on the fungal microbiota of 133 healthy and asymptomatic equines (devoid of cutaneous lesions), was isolated *Nigrospora* spp. in 0.7% of total animals (ISHIKAWA *et al.*, 1996). A total of 34 fungal genera were collected from beef carcasses in a study of Ismail et al. (1995). The authors recorded *Nigrospora* spp. on inner surface of shoulder in beef carcasses and not on walls, floors and water may be derived from the hides. This should be taken into consideration in further studies on these flora as sources of contamination.

The frequency or occurrence of the spores of *Nigrospora* species at every month of the year in the United States revealed that *Nigrospora* spores are typically present outdoors throughout the whole year at a consistent but low density but tend to be higher from August to October (DELGADO, 2006). Siqueira et al. (1990) performed a survey to correlation the presence of various fungi on external integument of healthy bovine and four forage plants, according to climatic variations. The authors reported the low frequency (4.18%) of *Nigrospora* spp. in calves in all season during one year in Brazil. This report aims to describe a case of rare and opportunistic mycosis caused by *Nigrospora* spp. in the low-weight and immunosuppressed bovine.

CASE REPORT

In a technical visit of the veterinarian of Itapeva Rural Development Office (CATI) at a dairy farm, located in the city of Buri (latitude 23º 47' 51" S and longitude 48º 35' 34" W) belonging to the state of São Paulo, the producer complained about a female calf of Holstein Black and White breed, with six months old, that had lesions with crusts, distributed across several areas of the body (Figure 1). According to the owner, the calf was raised in the pasture and received a small amount of corn silage in the winter. The owner did not carry out a preventive health control program for internal and external parasites. The animal was the only of herd that presented the lesions, that were distributed in the region of the croup, thighs, back, neck, shoulder blades and presenting low body score condition.

Figure 1. Holstein Black and White calf

The clinical examination showed the presence of crusts on the skin that, when highlighted, were left to show areas of smooth and moist skin. Other areas where crusts were more adhered, presented bleeding after removal (Figure 2).

Figure 2. Lesions distributed on skin

Fifty specimens (scales, crusts, hair and nails) were collected from the animal and sent to the microbiology laboratory at room temperature (25 °C ± 2), to the mycological analysis. The procedures of isolation fungi from samples were in accordance with good laboratory practice and the material was manipulated in a laminar flow cabinet, according to the laboratory guide for the routine isolation and identification of common fungi designed by Pitt and Hocking (2009).

The samples were inoculated in Petri dishes containing Sabouraud Dextrose Agar and incubated for 7 days in a standard Biochemical Oxygen Demand (B.O.D.) incubator at 25±2ºC for proper growth of fungal colonies to render identification. The number of colonies recorded was then expressed as number of colony-forming units (CFU) sampled.

The colonies were woolly in texture, with the color turning from white to grey to black, producing a reverse pigmentation which darkened from pale to dark brown and finally to black. A slide of UFC removed from culture and examination in a microscopy with lactophenol cotton blue. The examination of slides in optical microscope revealed branching septate hyphae, with the conidiophores exhibiting a single conidium at their inflated apex. The short conidiophores that swell and then taper to the point of conidia formation (Figure 3 a and b).

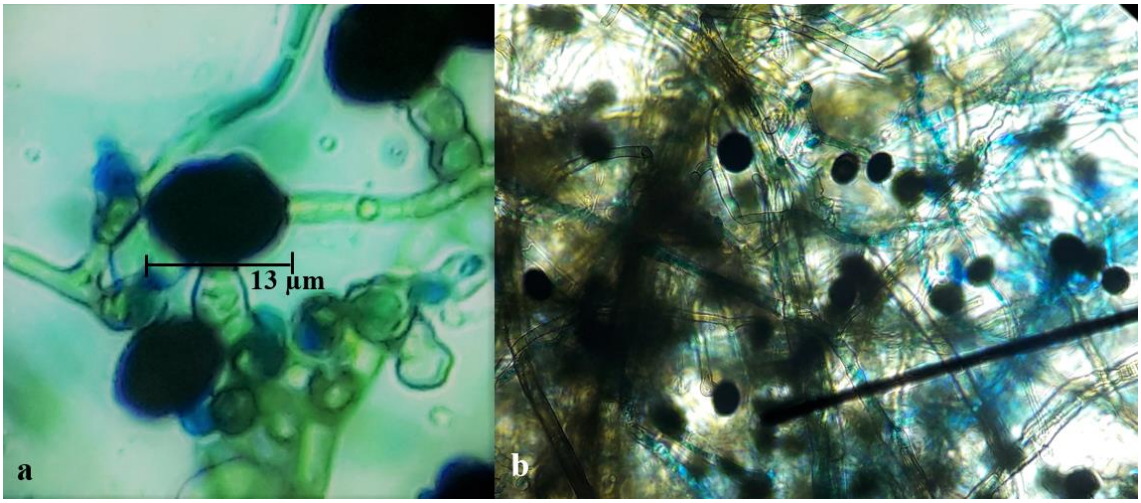

Figure 3. Black conidia size (a), hyphae and spores (b) of *Nigrospora* spp. isolated from crusts and skin samples

The conidia or spores are large, unicellular, black, ovoid in shape, with the older spores showing a horizontal flattening. The description was found to fit the characteristic "black spores" of *Nigrospora* spp., according to the typical textbook description with conidia ranging from young ovoid to older flattened (ABDEL-LATIF *et al.*, 2015; DELGADO, 2006).

In culture, the color of the colony darkens in proportion with the increasing amount of sporulation during incubation (LARONE, 1995). A full-grown culture was black on both the obverse and reverse sides. The asexual spores (conidia) were found to be typical of the textbook description with conidia ranging from young ovoid to older flattened (DELGADO, 2006). Macroscopically, colonies were compact and wooly, white at first but black areas appear due to the production of black globose conidia (ABDEL-LATIF *et al.*, 2015).

According to Ananya et al. (2014), the teleomorphic stage (sexual phase) of *Nigrospora* are included in Khuskia, another genus of the phylum Ascomycota. The fungal isolate has been deposited at The Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, The Netherlands, and can be accessed with the accession number CBS 137557. Although *Nigrospora sphaerica* grew very rapidly in culture of the clinical specimen, sporulation took nearly five weeks which would warrant prolonged incubation and usage of multiple techniques like slide culture and banana peel culture for the purpose of accurate speciation of those pathogenic fungi showing no or delayed sporulation (ANANYA *et al.*, 2014).

In this case the treatment was done by removing the crusts and spraying the animal with an iodophor solution (1:100), once a week until the disappearance of the lesions, after 8

weeks. Thirty days after the beginning of treatment, the animal already presented evolution of the condition with a decrease in the number of crusts by the body and growth of new hairs in the affected areas (Figure 4).

Figure 4. The decrease in the number of crusts in skin area

The dietary adjustment was corn silage supplementation (5 kg/animal) and commercial calf feed (1 kg/day), being fed separately from the other calves. The vermifuge used was levamisole hydrochloride 7.5%. The owner was instructed regarding the improvement in the nutritional management of the animals and the sanitary control program, to prevent the recurrence of the mycosis. After 50 days of treatment initiation, the animal was almost cured.

DISCUSSION

Mould spores are in the soil and in plant debris lying ready to infect the growing plant in the field. Fungi can infect dairy cattle causing a disease referred to as mycosis and is most likely when cows may be immune suppressed during stressful periods. A mycosis can occur in various locations such as the lungs, mammary gland, uterus, or intestine. An intestinal infection may result in hemorrhagic bowel. Mold spore counts and mould identification can be helpful to diagnosis (WHITLOW and HAGLER, 2017).

The bovine tegument can accommodate a great diversity of filamentous fungi, potentially capable of causing dermatoses. The analysis of soil indicated a microbiota of filamentous fungi classified in 30 genera, of which the most frequent were (in this order): *Nigrospora, Fusarium, Curvularia, Alternaria, Epicoccum, Paecilomyces* and *Trichoderma* (AMARAL *et al.*, 2011).

Dermatophytosis is the most commonly occurring highly infectious and contagious cutaneous mycosis of public health and economic significance. Pal (2017) described the etiologic significance of *Trichophton verrucosum* in bovine dermatophyosis as a global cutaneous mycosis of humans and a variety of animals. However, no one study described *Nigrospora* spp. as an agent of mycosis in bovine, as well as the treatment to this rare mycosis.

In addition, since several of these infections, such as disseminated mould infections, are rare compared to similar human infections and treatment protocols vary greatly, the adoption of a standard one is difficult. Advances in antifungal therapy during the last years characterizes human medicine. Unfortunately for the veterinary community, many of the newer drugs are too expensive to be adopted to treat animal mycoses. Some antimycotic drugs that have come off patent are currently more accessible but are still not an option for the often-necessary prolonged therapy courses (ELAD, 2018).

Apparently, the increased importance of human mycoses and the resulting publication of research and clinical data have had a bearing on veterinary medicine. Additional information stemming from this trend will, hopefully, improve the success rate in the treatment of animal mycoses. Further work on the development of cheap, safe and potent chemotherapeutic agents for the management of dermatophytosis should be conducted (PAL, 2017; ELAD, 2018).

According to Dias et al. (2013) the efficacy of topical agents in superficial mycoses depends not only on the type of lesion and the mechanism of action of the drug, but also on the viscosity, hydrophobicity and acidity of the formulation. Regardless of the type of formulation, topical agent penetration in hyperkeratotic lesions is often precarious. Products used for cutaneous application tend to be manufactured in creams or solutions and iodine or iodophors, e.g. povidone-iodine, have been widely used as an antiseptic for the prevention and treatment of mycoses. It is a highly efficient microbicide with a wide antimicrobial spectrum and its efficiency against clinically and epidemiologically significant new pathogens, such as methicillin-resistant *Staphylococcus aureus* and *Enterococcus* spp., that have antifungal activity (IP, 2011).

Pal (2017) recommended to *Trichophton verrucosum* dermatophytosis apply 2% solution of tincture iodine daily for 2 to 3 weeks on the lesions after the removal of crusts with disposable spatula, and also to properly destroy the crusted materials and wooden spatula by burning in order the prevent the spread of infection.

Diets should be formulated and fed to reduce nutritional stress (such as transitional diets) and to supply sufficient protein, energy, fiber, antioxidant nutrients, and buffers. While healthy cows with an active immune system are more resistant to mycotic infections (WHITLOW and HAGLER, 2017). The fungal infections depend on, exposure to sufficient inoculum size of organism and general resistance of the host. Immune deficiency predisposes to progression of infections by established pathogens.

CONCLUSION

Fungi are relatively uncommon causes of disease in immunocompetent animals. This report demonstrated that the dematiaceous fungi *Nigrospora* spp. are opportunistic moulds that caused infection after penetration of intact skin barriers, or other debilitating conditions exist in the host.

The confirmation test in laboratory of dermatophytosis in bovine is important to detect rare mycosis in cattle. The adopted protocol of treatment cleaning, removing the crusts and spraying the animal with an iodophor solution was successful after eight weeks in this rare mycosis caused by *Nigrospora* spp. in immunosuppressed bovine.

Nutrition is an important immune modulator that can influence the balance between health and disease. In addition, immune deficiency, caused by decreased food intake, can result in a higher incidence and duration of opportunistic infections. Vitamins, copper, beta-carotene, zinc, and selenium acquired in the diet are important to support immunity, involved in the formation of enzymes, energy production, proteins, cells, and hormones necessary for defense mechanisms. The balanced diet for the animal with the opportunistic fungal disease was important in the recovery of the calf health.

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