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Generalized Dermatophytosis of Combined Etiology in a Circus Tiger (*Panthera Tigris Altaica*)

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ABSTRACT

There have been few reports on the generalized tinea caused by mixed infection and its treatment. The article describes a case of combined tiger dermatophytosis caused by *Microsporum canis* and opportunistic skin mycosis associated with mixed aspergillosis infection. The infected hair was affected by fungal elements, confirmed by Wood's lamp and cultural and morphological studies. Cultural and morphological diagnostics were carried out by inoculation of biomaterial on differential Sabouraud media with cycloheximide. The causative agent of classic dermatomycosis *Microsporum canis* and the causative agents of opportunistic mycoses fungi of the genus Aspergillus were isolated and identified from the affected foci on the skin of a tiger cub. Dermatosis of combined etiology was diagnosed. The preparations were selected and treated for the generalized mycosis of the tiger cub. Itraconazole was effective for treatment. There have been few reports on the generalized tinea caused by mixed infection and its treatment. The article describes a case of combined tiger dermatophytosis caused by *Microsporum canis* and opportunistic skin mycosis associated with mixed aspergillosis infection. The infected hair was affected by fungal elements, confirmed by Wood's lamp and cultural and morphological studies. Cultural and morphological diagnostics were carried out by inoculation of biomaterial on differential Sabouraud media with cycloheximide. The causative agent of classic dermatomycosis *Microsporum canis* and the causative agents of opportunistic mycoses fungi of the genus Aspergillus were isolated and identified from the affected foci on the skin of a tiger cub. Dermatosis of combined etiology was diagnosed. The preparations were selected and treated for the generalized mycosis of the tiger cub. Itraconazole was effective for treatment.

Key words: *Panthera tigris altaica*, *Microsporum canis*, *Aspergillus*, tinea of carnivores, *ITS2*.

INTRODUCTION

The isolation of *M. canis* from wild mammals is mentioned quite often (Omar et al. 2020). Based on the biology of the pathogen, it is logical that *M. canis* is more common among carnivores. There is a description of asymptomatic and clinically expressed cases of dermatophytosis in several species of wild canids: wild fox, red fox, gray wolf and maned wolf (Malmasi et al. 2009; Pereira et al. 2018). Cases of dermatophytosis in wild felines are much more common. At the same time, mention is made of the identification of the tinea caused by *Microsporum canis* from wild or circus felines such as tigers, lions, panthers, jaguars and others (Bentubo et al. 2006).

The detection of ringworm caused by *m. Canis* and *M. gypseum* in lions (*Panther leo*) with the emergence of a small epidemic focus or lack thereof was reported (Bentubo et al. 2006). A case of dermatophytosis was described in wild felines (*Puma concolor*) in Florida, USA (1999). Of the three samples taken from the cougar, one was infected with *M. gypseum*, and the other two were infected with *Trichophyton mentagrophytes* (Rotstein et al. 1999).

M. canis, isolated from wild felines, can be identified as the only agent of infection or in combination with *T. mentagrophytes* (Rotstein et al. 1999; Mihaylov et al. 2016). Often, other pathogens of dermatomycosis, for example, *M. gypseum*, are detected from wild animals. Analysis of the detection of dermatophytes in the wool samples of wild felines: ocelot (*Felis pardalis*), lion (*Panthera leo*), and tiger (*Panthera tigris*) made it possible to isolate *M. gypseum* from ocelot (Albano et al. 2013). Geophilic dermatomycete *M. gypseum* was isolated from two apparently healthy lionesses (Bentubo et al. 2006).

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The approach to the treatment of wild animals with tinea is no less relevant. The selection and use of medicinal products for wild animals is a problem, therefore, it is necessary to search for safe and promising remedies and methods of treatment and rapid restoration of the animal's coat. This is very important for animal reserves, circuses and zoos (Sykes et al. 2005). The objective of this study was to investigate the fungal composition and diversity of parasitic strains including opportunistic types and successful treatment.

MATERIALS AND METHODS

The research protocol was discussed and approved at a meeting of the local ethical committee of the S. Seifullin KATU belonging to the Ministry of Education and Science of the Republic of Kazakhstan, dated May 13, 2019.

Biomaterials from a tiger cub (born in 2019, May 15) with clinical signs of skin lesions were carried out according to the generally accepted method: samples of the affected hair and scales of the affected skin were taken with tweezers at the border with healthy skin (Fig. 1A). The anesthesia protocol included the use of anesthetics containing 1.0mg/kg xylazine and 10.0mg/kg ketamine. Blood samples were taken from the femoral artery. Biomaterial samples were delivered to the laboratory within 2 hours.

Blood serum was used to stage an agglutination reaction with a previously prepared *M. canis* antigen (Karkenova et al. 2014). Serum and antigen were mixed in a ratio of 1:2 (50μL) on a glass slide, mixed and incubated at room temperature for 5min. The result was considered visually. A positive reaction was characterized by the presence of flocculent sediment. Biomaterial samples (hair, crusts, blood) were placed on dishes containing Sabouraud chloramphenicol dextrose agar. Incubated at 28°C for 18-25 days until the formation of characteristic colonies. Fungi were identified by their macro- and microscopic morphological characteristics. Phenotypic identification also was performed using the determinant of microorganisms (Sutton et al. 2001).

Genomic DNA was extracted from *M. canis* using liquid nitrogen and phenol-chloroform extraction method. The *ITS* region on rDNA was amplified by using specific primers *ITS4* (5'-TCCTCCGCTTATTGATATGC-3') and *ITS5* (5'-GGAAGTAAAAGTCGTAACAAGG-3') and received the PCR product with size of 526bp. The PCR reaction was done under the following conditions: an initial denaturation set up at 94°C for 5min was followed by 35 cycles of denaturation at 95°C for 30s, annealing at 52°C for 40s and extension at 72°C for 50s, with a final extension step of 72°C for 7min. The sequencing was done by using BigDye® Terminator v3.1 Cycle Sequencing Kit.

The treatment regimen was selected considering the anamnesis, the development of clinical signs of the disease and the results of identification of the pathogen. A fungicidal drug of systemic action - Orungamin (Ozone LLC, Zhigulevsk, RF) was proposed as an antifungal agent. The drug is taken within 14 days, 1 capsule containing 100mg of active substance itraconazole. To reduce itching and alleviate the general condition of the animal, hydrocortisone is recommended once 5mL (25mg/mL) intramuscularly.

Statistical Analysis

Statistical analysis was performed in Microsoft Office to determine the significance of the differences between the various indicators.

RESULTS

An external examination of the animal was carried out, which showed that the tiger cub (7-month-old, male) weighing about 70kg, emaciated, aggressive, restless, nervous, constantly itching. On the head, paws and tail, areas with typical foci of ringworm in the form of seals, dry crusts, more characteristic of trichophytosis, were identified (Fig. 1A). NaOH microscopy of the affected hair showed the presence of spores randomly located inside the hair and disintegrating mycelium on the hair surface.

Express diagnostics for tinea of carnivores in the reaction of droplet agglutination with an antigen from *M. canis* within 5s confirmed the presence of specific antibodies to the causative agent in the blood serum. In a drop of serum in the presence of electrolytes, the corpuscular antigen and antibodies were bound, which was accompanied by the appearance of agglutinate in the form of a noticeable fine precipitate (Fig. 2).

By culture studies, typical fluffy grayish-white colonies of the *M. canis* pathogen and colonies of molds were obtained from all biomaterial samples from the body of a tiger cub (Fig. 3A). Also, when inoculating blood serum and blood clots of a tiger cub on nutrient media, it was possible to isolate a pathogen circulating in the animal's blood, there was no growth of opportunistic fungi (Fig. 3B). According to the cultural and morphological characteristics, one of the pathogens was confirmed as *M. canis* (Fig. 3A1 and 3B). Macro morphological characteristics, microscopy and molecular identification of the colonies of molds showed that, they were mixed fungi of the genus *Aspergillus*.

As a distinguishing feature from other *Aspergillus* species, pigmentation of the mycelium was used for *A. ruber* (syn. *Eurotium rubrum*) (Fig. 3A2, *A. chevalieri* (Fig. 3A3), *A. niger* (Fig. 3A4), *A. cristatus* (Fig. 3A5). The morphology of the isolates shows the expected phenotypic traits that are consistent with previous descriptions of the species (Hubka et al. 2013). The presented results made it possible to clarify the preliminary diagnosis and diagnose the generalized form of *M. canis* in carnivores, accompanied by the circulation of the pathogen in the bloodstream, complicated by *Aspergillus* opportunistic mixed skin infection.

The PCR products of isolated fungal strains were subjected to sequence analysis. We confirmed the identification of our isolates to species level as *M. canis*, *A. ruber*, *A. shevalieri*, *A. niger* and *A. cristatus* by searching for nucleotides in the BLAST system. The nucleotide sequences of the *M. canis* isolated from blood and head wool were deposited in NCBI GenBank data base (MT490877.1 and MT490879.1).

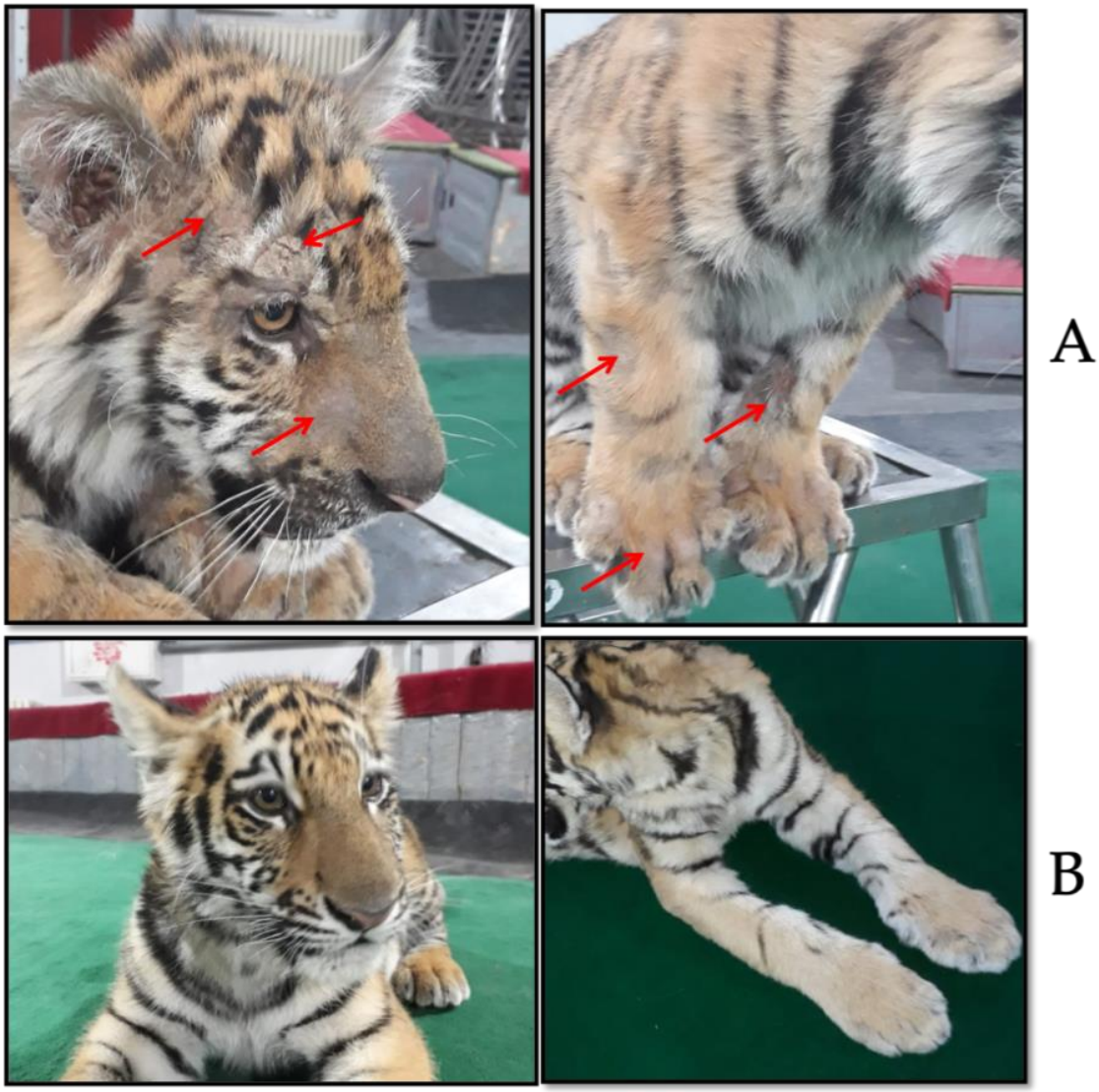

Fig. 1: Affected areas of the head and paws of a tiger cub with clinical signs of skin lesions before (A) and after treatment (B).

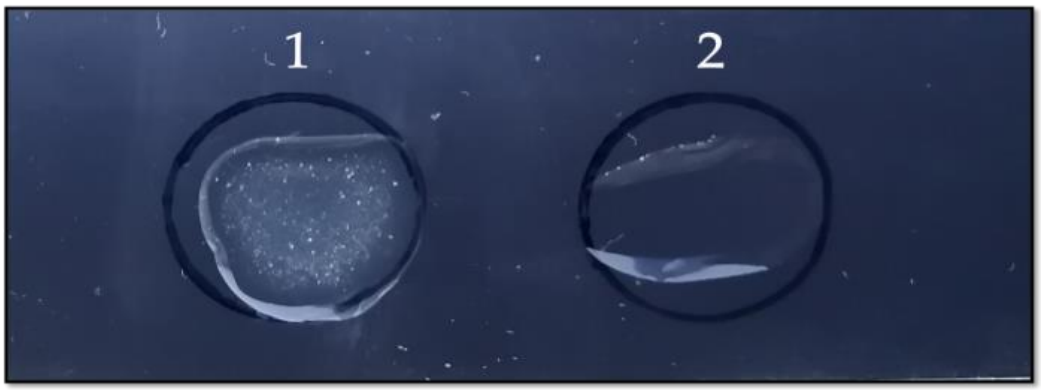

Fig. 2: The results of agglutination reactions confirm the presence of specific antibodies to the *M. canis* causative agent: (1) infected tiger cub serum, (2) negative control.

DISCUSSION

Our article describes a case of generalized microsporidia in a circus tiger cub (*Panthera tigris*) caused by *Microsporum canis*, complicated by *Aspergillus* opportunistic mixed skin infection. It has been proven that young animals are more susceptible to dermatophytes than adults. This is associated with a higher immunity in adults due to previous contact with the pathogen or with the immaturity of the immune system of young people (Pereira et al. 2018). Indeed, in the circus, where the sick tiger cub was, there were wild felines in neighboring cages, which did not undergo numerous transfers and remained healthy.

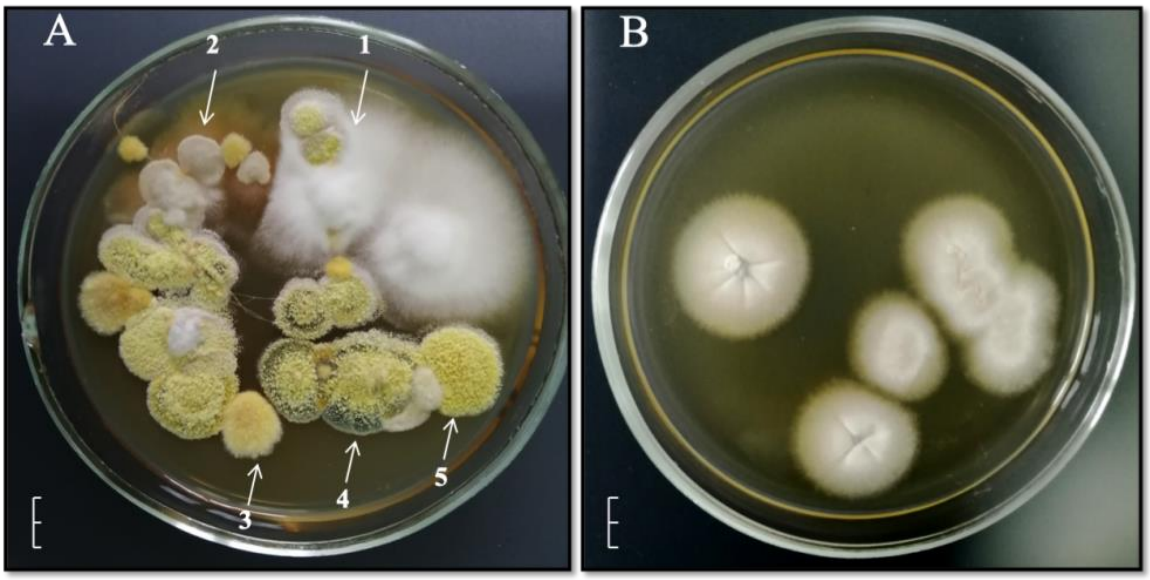

Fig. 3: Culture of micromycetes obtained during the primary isolation from the affected hair of a tiger cub (A), from blood clots (B); *M. canis* (1), *A. ruber* (2), *A. shevalieri* (3), *A. niger* (4), *A. cristatus* (5); bar=1cm.

To quickly identify the pathogen in biological material, we used the author's test system based on the agglutination reaction with the stained corpuscular *M. canis* antigen. This made it possible to confirm the presence of the microsporidia pathogen in the blood serum of the tiger cub. Also used the cultivation of fungi on Sabouraud dextrose agar. Microsporidia carnivores have been diagnosed. The circulation of the microsporidia pathogen in the blood of an animal indicates a generalized form of the disease. However, the lack of a therapeutic effect after the course of vaccination and the growth of opportunistic fungi on the surface of Sabouraud's dextrose agar suggested a mixed infection. Analysis of publications on the incidence of mixed infections in dermatophytosis in wild felines revealed that *M. canis* may occur in conjunction with uncharacteristic species of molds or yeasts from domestic and wild animals, including the *Aspergillus* genus (Dworecka-Kaszak et al. 2020; Seyedmousavi et al. 2018). According to Siqueira et al. (2018), the diversity of *Aspergillus* species in clinical specimens is constantly increasing. We also found that the tiger cub was defeated by the association of *M. canis* and *Aspergillus* spp.

As a result of genetic identification, the presence of the main pathogen of microsporidia in carnivorous *M. canis* was confirmed. Fungi of the genus *Aspergillus* were identified to the following species: *A. ruber* (syn. *Eurotium rubrum*), *A. chevalieri*, *A. cristatus*, *A. niger*. Despite the fact that systemic antifungal drugs pose a significant risk to the animal, we have chosen the tactics of treatment with the use of itraconazole, and it led to a complete recovery of the tiger cub (Fig. 1B). The quality of wool has improved, appetite has increased, physical activity has increased and the aggressiveness of the animal has decreased.

Conclusion

The causative agent of classic dermatomycosis *Microsporum canis* and the causative agents of opportunistic mycoses fungi of the genus *Aspergillus* were isolated and identified from the affected foci on the skin of a tiger cub. Cultural-morphological, microscopic, luminescent, serological diagnostics of carnivorous tinea and genetic identification of the causative agents of mycosis in tigers were carried out. The preparations were selected and the generalized mycosis of the tiger cub was treated.

Author's Contribution

Conceptualization: Kiyan V, Kukhar Y; Data curation: Kiyan V, Kukhar Y; Formal analysis: Kukhar Y; Funding acquisition: Kiyan V; Investigation: Smagulova A; Methodology: Kiyan V, Kukhar Y; Project administration: Kiyan V; Resources: Kiyan V, Kukhar Y; Software: Kukhar Y, Smagulova A; Supervision: Kiyan V; Validation: Kukhar Y; Visualization: Kukhar Y; Writing - original draft: Kukhar Y, Kiyan V; Writing - review and editing: Kiyan V.

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