Aspects concerning the diagnosis by direct microscopic examination of the samples in cats

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ABSTRACT. Dermatophytosis is a medical term used to designate a fungal infection of the superficial layers of the skin, nails and hair. The etiological agents of the dermatophytosis in animals are the fungi of the Trichophyton and Microsporum genera.

In felines, superficial mycosis is produced by Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes and, more rarely, by other genera (M. persicolor, T. verrucosum, T. terrestre). During the last years, there were no more cases of favus reported in felines. A proper therapy and the prevention of dermatophytosis are based on a correct diagnosis of the disease. There are multiple methods and techniques that may be used for making a diagnosis of such diseases: Clinical Examination, Fluorescence Test, Microscopical Examination of Pathological Samples. Examination of Pathological Samples by Seeding on Selective Culture Media, Histopathologic Examination, Biological Testing, Biochemical Examination, Haematological Examination and Leucocytogram.

Introduction

Dermatophytosis is a medical term used to designate a fungal infection of the superficial layers of the skin, nails and hair. The etiological agents of the dermatophytosis in animals are the fungi of the Trichophyton and Microsporum genera.

In felines, superficial mycosis is produced by Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes and, more rarely, by other genera (M. persicolor, T. verrucosum, T. terrestre). During the last years, there were no more cases of favus reported in felines.

Dermatophytes are keratinophilic and grow on the superficial layers of the skin and obtain nutrients from the keratin they decompose. In extremely rare cases, especially in immunosuppressed individuals, they may affect inner layers of the skin or even viscera.

The study of dermatophytosis in felines is extremely important, given both their implications in the pathology of this species as well as their high contagiousness. Cats are the main natural reservoir of Microsporum canis. This disease is easily transmitted to animal populations as well as to humans. Recently, in our country, too, dermatophytosis with fungal etiology involving etiologic agents hosted by animals are increasingly frequent (Fig. 1).
The pathology of the skin in felines is extremely varied and complex. The determination of the etiology may be sometimes difficult.

The diagnosis of dermatophytosis should be made efficient by developing, implementing and monitoring a strict programme supporting the veterinarian practitioner to establish a control schedule according to the etiology and the epidemiology of each individual case.

A proper therapy and the prevention of dermatophytosis are based on a correct diagnosis of the disease. There are multiple methods and techniques that may be used for making a diagnosis of such diseases: Clinical Examination, Fluorescence Test, and Microscopical Examination of Pathological Samples. Examination of Pathological Samples by Seeding on Selective Culture Media, Histopathologic Examination, Biological Testing, Biochemical Examination, Haematological Examination and Leucocytogram.

**Instruments and method**

Microscopic examination of the samples (hair scales) has as objective identification of the spores and/or hyphae. This kind of examination state the presence or the absence of the infection with dermatophytes and sometimes the species of dermatophytes involved in the pathogenetic process.

Microscopic examination requires the preparation of the animal and equipment before the samples are collected.

Depressed areas were first disinfected with alcohol 70°.

Samples collecting was done by using tweezers and scalpel. Hair (broken, frayed, twisted or hair with healthy appearance) was wrested with tweezers from the edges and centre of the lesions. The scales were collected with a scalpel after the lesions scracing.

For the microscopic examination were used microscope slides, cover slips, microscope and clearing agents as chloral lactophenol or lactophenol Amann, NaOH (10%), Na SO₄ (10%).

The hair and small flakes of skin were placed on a microscope slides and covered with a drop of clearing agent.

High quality of the preparations are obtained by using chloral lactophenol as a clarifying agent and allowing the blades with the pathological material put on the clarifying agent blade and covered with a blade to stand for 24 hours.

Meantime the dissociant has enough time to break the tissue and release the spores more evident. The microscopic imagines of the samples examined immediately after preparations are not clear and generate many
errors on diagnosis. We do not prefer NaOH as clearing agent as it is an aggressive substance for the hair; this substance destroy the hair, and its fragments could form mass which can be confounded with mass of arthrospores.

The diagnosis by direct microscopical examination was made on 64 cats of different ages and on both sexes.

Results and debates

An infection with *Microsporum gypseum* in a cat was diagnosed based on the big spores (5-8µm) displayed in chains along the hairs (Fig. 2-4).

Simultaneously with the identification of the spores (2-3µm) grouped in specific peripilar hair casts, we diagnosed an infection with *Microsporum canis* (Fig. 3).

Figura 2
Large spores (5-8µm) of the *Microsporum gypseum* displayed in cluster and parallel chains along the hair (authentic).

Figura 3
Coat of small spores along a certain of the hair length. Specific aspect on *Microsporum canis*’s infection. Together with the affected hair free arthrospores are noticed (x40) (authentic).
Figura 4
Coat of small spores, lying in a thin sheath around the hair. Speciphye aspect on Microsporum canis infection. (x40) (authentic).

As it could be observed in the photographic material the arthrospores are disposed along of the hair or a certain part of the hair.

Small spores of *Trichophyton mentagrophytes* (3-5µm) were also displayed in chains in the preparation obtained from cat hairs. A drop of methylen blue added in the microscopic samples generated a clearer picture on the arthrospores (Fig. 5).

Most of the pictures previously are showing the arthrospores peripilar. The following imagines show the spores penetrating the hair. Inside of the hair there are new chains of arthrospores. This process is more often present in Microsporum canis infection. The result of this phenomenon is that the hair becomes fragile and rise higher than about 3 mm above the skin surface (Fig. 6).

Figura 5
Chains of small spores dispused in parallel along the hair - colored with methilen blu (x90) (authentic).
Figura 6
The spores penetrates the hair. Inside of the hair there are new chains of arthrospores and hyphae. Infected hair with Microsporum canis is very damaged (x40) (authentic).

In scales the arthrospores are arranged as spherules (Fig. 7, 8).

Figura 7
Clusters of spores in skin scales (x40) (authentic).

Figura 8
Clusters of spores in skin scales next to a non-infected hair (authentic).
The diagnosis of dermatophytosis by microscopic examination is difficult especially for the veterinarians who are not so experienced because the hyphae and the spores that could be often confound with cotton fibres, mosaic fungus as a result of using NaOH 10% as clearing agent and false spores as a result of the pigments accumulation in a crust (Fig. 9 - 10).

![Figura 9](image)

Figura 9
Artefacts – Mosaic fungus as a result of using NaOH 10% as clearing agent (authentic).

![Figura 10](image)

Figura 10
False spores as a result of the pigments accumulation in a crust (authentic).

Even if, in principal, the direct microscopic examination of the pathological samples is satisfactory for the determination of the dermatophyt etiology of the lesions and if the microscopic samples are clear, we recommend cultural examination in order to confirm the positivity of the infection.

**Conclusions**

1. For the diagnosis of the dermatophytosis by direct microscopic examination is necessary to identify the arthrospores, their dimension and disposal along the hair.

2. We diagnosed an infection with *Microsporum canis* simultaneously with the identification of the spores (2-3µm) grouped in specific peripilar hair casts.

3. Infection with *Microsporum gypseum* in a cat was diagnosed based on the big spores (5-8µm) displayed in chains along the hairs.
4. Small spores of *Trichophyton mentagrophytes* (3-5µm) were also displayed in chains in the preparation obtained from cat hairs.

5. In Microsporum canis infection the spores penetrating the hair. Inside of the hair there are new chains of arthrospores, the spores penetrating the hair. The result of this phenomenon is that the hair becomes fragile and rise higher than about 3 mm above the skin surface.

6. In scales the arthrospores are arranged as oodies.

7. Qualitative preparations are obtained by using chloral lactophenol as a clarifying agent and allowing the blades with the pathological material put on the clarifying agent blade and covered with a blade to stand for 24 hours.

8. The hyphae and the spores that could be often confound with cotton fibres, mosaic fungus as a results of using NaOH 10% as clearing agent and false spores as a result of the pigments accumulation in a crust.

**Bibliography**