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Research Article

Identification of Fungi from Police Dogs in Borri, Khartoum, Sudan

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Abstract

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm.

Dogs can suffer a dermatophyte infection at any age, but a ringworm infection is more frequent in the young. *Microsporum canis* is considered highly contagious and potentially pathogenic for people. Cats are considered the reservoirs of *M.canis*. Isolation and identification of fungi in German Shepherd dogs have not been reported, yet. In this study (81) hair samples, (2) skin scrapings were collected from apparently healthy and clinically infected dogs of German-Shepherd dogs breed, of both sexes and different ages. The skin samples were taken from two dogs with cutaneous lesions. Determinations of dermatophytes, as well as the possible involvement of other fungi in dermatomycosis in dogs were studied. Mycological investigations were conducted by direct microscopy and by fungal culture on Sabouraud Dextrose Agar supplemented with 0.05% Chloramphenicol and 0.5% Cycloheximide to study the presence of fungi based on the colonial morphology and pigmentation. Two hair samples (2.40%) yielded growth suggestive of Dermatophytes; 76 hair samples (91.5%) yielded growth of non-dermatophyte fungi (*Aspergillus*, *Penicillium* and *Alternaria*), while 5 samples (6.02%) - three hair samples and 2 skin scrapings did not show any fungal growth. The cultures from the two hair samples which were positive for Dermatophytes gave pure cultures of *Microsporum canis* and *M.gypseum. Microsporum canis* was isolated from one hair sample (1.2%) and so was *M.gypseum.* Along with Dermatophytes, saprobic fungi were the most isolated fungi in this study especially *Aspergillus* spp (72%), *Penicillium* spp (12%) and *Altenaria* spp (7.2%). The study reveald that pathogenic Dermatophytes, in addition to saprobic fungi, may be the causative agents of Dermatophytosis (Ringworm) in Police dogs department.

Keywords: Dermatophytes; German shepherd; Ringworm

Introduction

Dermatophytosis is the most common fungal infections in dogs [1,2]. The dermatophytes have a high affinity for keratin, an important component of fur, skin and nails, which are the primary sites of fungal infection [3].

Several reports have stated that *Microsporum canis,* a typical zoophilic species, is the most common dermatophyte isolated from dogs worldwide [1,4-6].

Epidemiological studies on the isolation of dermatophytes from dogs with suspected lesions of dermatophytosis have been reported by different authors [7,8]. The proportion of positive samples in relation to the number of samples examined from cases of Dermatophytosis varied considerably from one study to another. The relatively low prevalence of Dermatophytes in dogs with suspected lesions of Dermatophytosis is well documented. It ranges between 4% and 10% and few studies show higher prevalence [8,9].

With few exceptions, *M.canis* was the most common species isolated, showing a high variability in its percentages of isolation (40-90%). Other dermatophytes less commonly isolated from dogs are *T.mentagrophytes* and *M.gypseum.* These three species comprise approximately 96% of the isolated dermatophytes from dogs.

In this present study we obtained hair and skin scraping for mycological investigation to perform the most frequent dermatophytes existed in dogs.

Materials and Methods

Animal of study

This study included 81 healthy dogs 2 with dermatitis. The samples were collected between January 2011 and May 2011 with collaboration of veterinary clinic located in Borri-Khartoum-Sudan. Breed was German shepherd dogs. Age sex and groups and clinical manifestation were arranged in Table 1 and 2.

Collection of samples

The dogs with lesions were sampled by plucking hairs with sterile forceps and by scraping epidermal scales with a sterile surgical blade from the affected areas. The samples from each dog were placed in separate sterile Petri dishes. Each animal (with or without lesions) was sampled by brushing its fur with a sterilized nailbrush using the following standard protocol. The animals were brushed from

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Table 1: Age sex and groups of study.

Total number	Healthy dogs	Dogs with dermatitis	Male	Female
83	81	2	52	31

Table 2: Shows age groups.

Under year (puppies)	2-5 years	5-10 years	Above 10 years
11	49	18	5

the head down to the tail and down the flanks and the legs. After specimen collection, the nailbrushes were placed in sterile Petri dishes for transport to the laboratory. Samples collected by brushing were inoculated on plates of Sabouraud dextrose agar (Difco) with chloramphenicol (400 ppm) (sca) supplemented with 0.5% cycloheximide.

Plucked hairs and scraped scales, originating from dogs with lesions, were examined for fungal elements by direct microscopy in 20% potassium hydroxide, covered with cover-slip and heated mildly above the flame (3min), after 10min they were diagnosed microscopically by potassium hydroxide mounts and by culture on the above mentioned media. The plates were incubated at 28°C and regularly examined for a month. Taxonomic identification of all mycelial colonies considered different was based on thorough macroscopic and microscopic studies. Shape dimensions, arrangement of macroconidia and other parts of isolated cultures. Suspected dermatophytes were identified to species level, and most of the remaining fungi were identified to genus level.

Potassium hydroxide mounts

A drop of 20% KOH was added on a clean glass slide, the sample (hair and skin) was placed in KOH drop and slide passed through a burner flame to hasten keratolysis. When keratolysis softened the sample, a clean glass cover slip was slowly placed at inclined angle on the sample and slightly pressed, preventing the formation of the air bubbles. The sample was kept in 20% KOH for a variable duration ranging from 5 minutes to 20 minutes, depending upon the thickness of the scales and examined every 5 minutes. Each slide was thoroughly examined for the presence of filamentous, septate, branched hyphae with or without arthrospores crossing the margins of the squamous epithelial cells of the skin, in case of hair, type and arrangement of the spore was noticed.

Processing of samples

The skin scraping and the hair were collected in the sterile plastic Petri-dishes to prevent contamination in transit to the laboratory.

The samples were kept at temperature in the laboratory room. The agar plates were arranged around the flame, and then with help of inoculation straight wire all the agar plates were inoculated.

Agar plates were then inoculated upside down at 28°C degrees centigrade for 4 weeks before each was discarded.

Agar plates were frequently removed from incubator and observed (checked) for fungal (i.e. dermatophytes) growth.

Fungal (dermatophyte) growth was checked for colonial characteristics and pigmentation.

Results were recorded straight on the data sheet.

Inoculation of culture media

prior to inoculation , the media were dried in oven for about 15 minutes, after which the plates were arranged around the flame and flamed straight wire was used to inoculate all the Petri dishes aseptically in order to prevent contamination of cultures, specimens and safety of personal as well as the environment. All sterile technique measures were strictly observed.

The temperature of the incubator was adjusted at 28°C (degrees centigrade) where all the cultures were maintained for one month before they are discarded or subcultured.

Microscopic examination procedure

During microscopic procedure two different techniques were used for the determination of fungal morphology, spores, shapes, size, chlamydospores and irregular hyphae.

Needle mount technique

Clean sterile glass slide and cover slip lactophenol cotton blue (LPCB) stain.

Positive pure agar plates were arranged around the flame on the work bench.

Needle wire was sterilized by heating until it becomes hot red.

Few drops of lactophenol cotton blue (LPCB) stain was added onto glass slide.

Needle wire was used to pick few colonies from the culture plates then placed onto glass slide with lactophenole cotton blue (LPCB) stain.

The cover slip was gently placed at inclined angle on the glass slide, in order to prevent the formation of air bubbles.

Microscopic observation was carried out using l ower power of 10^x and 40^x objectives to determine the colony morphology, size, shape, chlamydospores and irregular hyphae.

Result was inserted straight onto data sheet.

Slide culture technique

About 6-8 mm square block of Sabouraud dextrose agar (SDA) medium was on to a sterile slide and sub-inoculated at four sides with the pure culture, the inoculated block is then covered with sterile cover glass and placed in a petri dish supported on glass rod. Small amount of sterile water was added to a filter paper at the bottom of dish to that prevented drying of the agar media. Then the preparation was for a week at 28°C after which the cover slip was removed and mounted in the drop of lactophenol cotton blue (LPCB).

The block was then discarded into disinfectant. Few drop of lactophenol cotton blue (LPCB) was added on to the growth on the slide and it was covered with a cover glass. Where both preparations were then examined microscopically using the 10x and 40x objectives with the condenser iris diaphragm adjusted to give maximum contrast.

Results

The study was carried out on 83 dogs in the police dogs Department in Borri-Khartoum-Sudan. Dogs were examined to detect dogs with

Table 3: Cultural Characteristic of Isolated Species.

Species	Growth rate	Colonial appearance	Microscopic appearance
Microsporum canis	4-7 days	white and fluffy center; border closely spaced radial grooves	knob end and spiny with a rough, thick wall 6-15 cells
Microsporum gypseum	4-9 days	buff (yellowish-brown) with white border rapidly spreading mycelium	many, spiny thin wall with 6 to 15 cells, rounded ends

suspected Dermatophytosis. Only a few dogs (n=2) presented skin lesions with focal or diffuse hair loss and/or scaling. A representative number of dogs without visible skin lesions (n=81), were used to carry out the Dermatophyte of their fur. The approximate age was between (1-12) years, breed was German-Shepherd and genders of the dogs were male (70) and female (13).

Out of (83) samples (76) of hair samples (91.5%) yielded positive growth for fungal colony, while (5) samples (6%) - 3 hair samples and 2 skin scrapings were negative for fungal growth in (SDA) with Cycloheximide. The species isolated were from hair samples, and were able to grow in (SDA) supplemented with (0.05%) Chloramphenicol and (0.5% Cycloheximide) at 28°C. The isolated species were in two hair samples *Microsporum-canis* was in (one samples), and *M.gypseum* (one sample).

Microscopic diagnostic results were positive for 2 samples (2.40%). and negative result were 76 samples (91.5%) from the number of the colonies tested, while 5 samples (6%) didn't show any fungal growth.

The most common fungi isolated from skin of dogs with susceptible Mycoses were saprobe, especially *Aspergillus spp* (72%), *Penicillium spp* (12%), and *Altenaria spp* (7.2%). Cultural Characteristic of Isolated Species is arranged in Table 3.

Discussion

Most cases of dermatophytosis in pet are caused by *Microsporum canis*. According to Kaplan and Ivens (1961) cats are considered to be reservoirs of infection.

In this study we investigate the prevalence of suspected Dermatophytosis in the sample of clinically examined dogs (n=83), only 2 samples (2.40%) had visible skin lesions but none of them had a dermatophytosis.

Of the 81 samples from dogs without visible lesions, 2 animals (2.40%) had positive cultures for dermatophytes which are usually considered causal agents of dermatophytosis (*M.canis* and *M.gypseum*).

This research showed that *M.canis* and *M.gepseum* were the only species isolated. Saprobic fungi were isolated as follows: *Aspergillus* (72%), *Penecillum* (12%) and *Altrnaria* (6%).

In the last 10 years several studies on the prevalence of Dermatophytes in the fur of asymptomatic dogs have been published [9-11], in those studies the positive samples for Dermatophytes varied between 4.4% and 11.9%.

Comparing with the previous studies the number of animals examined was more than 100 in all of them. *Microsporum canis* was generally the most frequent dermatophyte isolated (>60% of the total isolates). However in our study *M.canis* and *M.gypseum* are equally isolated.

The present research showed a particularly low proportion of positive results (2.40%). this finding is probably due to low number of samples examined (<100 samples) of clinical specimen's collection.

This research showed that *M.canis* and *M.gepseum* were the only species isolated. Not as in the most other studies of canine Dermatophytosis [12-14], which showed that *M.canis* was the most frequently Dermatophyte isolated, followed by *T.mentagrophytes*. So, these data are not totally in agreement with other studies of the literature.

Regarding the saprophytic fungi Our findings were similar to those reported by these authors Morielo and DeBoer, (1991). They found *Aspergillus, Alternaria, Cladosporium* and *Penicillium* spp.

Conclusion

We conclude that pathogenic Dermatophytes (*M.canis* and *M.gepseum*), were isolated in two cases (2.40%), however it is strongly suggested its role in contributing in the frequent occurring of dermatomycosis.

The most isolated fungi were saprobe with the alarming number of positive result of saprobe in our study: *Aspegillus* (72%), *Penecillum* (12%) and *Altrnaria* (6%); indicates the involvement of saprobic fungi as pathogenic agents of mycoses in dogs especially in immuno compromised and diabetic dogs is probable.

During this study, it revealed that certain factors might be the main reason of repeated cases of dermatomycosis: mal hygiene, administration of drugs without laboratory diagnosis, consideration of all cases as bacterial infection and focusing on treating allergic reaction rather than the main causative agent.

Hygienic administration of dogs stables by washing it daily by antiseptic (Dettol) and then clean it by water to prevent leaking it with dogs.

Surveillance of dogs and test them daily by veterinarians and dogs trainers.

Trainers should be aware about risk of skin conditions and probability of transmission to humans. And they should Use gloves.

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