Original Research Article

DERMATOPHYTES FROM ANIMALS IN THE MALWA REGION OF PUNJAB: A PRELIMINARY STUDY

ABSTRACT:

In recent decades, there has been an increase in the incidence of dermatophytosis among humans, especially those who are in close contact with animals. It is caused by dermatophytes, a zoophilic fungi group that can infect animals and humans. These fungi invade the superficial keratinised tissue causingnails, hair, and skin infection. Inflammatory response to fungi on the outer layer of skin or other tissues results in clinical symptoms like itching, red rashes, alopecia, hair loss etc.Despite reports of dermatophytes from various regions, there is increasing evidence of geographical variation in fungal species, their spread to new areas, and growing resistance to antifungal treatments. The ability of these infections to transmit from animals to humans underscores the need for vigilance and proactive management. Addressing this issue can help protect animal and human health, making early detection and treatment vital in preventing outbreaks and ensuring overall well-being. Hence, we conducted a preliminary study on the incidence of dermatophytosis in animals of the Malwa region witha hot-humid climatic condition anda large animal population.Aseptically collected samples were examined for fungal infection by direct microscopy (10% KOH), fungal isolation & lactophenol cotton blue staining. Of the 51 clinically suspected dermatitis cases of various animal species, 27 (52%) were found positive by KOH preparations. Dermatophytes were isolated from 11(40%) samplesdemonstrating their significant presence. The results of the present study highlight the incidence of dermatophyte infections in animals within the Malwa region, offering valuable insights into the prevention and control of dermatophytosis in both animals and humans. This information is a critical reference for public health strategies and underscores the importance of effective monitoring and managing these infections.

Keywords: Dermatophytosis, Phenotypic, hot-humid climate, Microspore, Zoonotic.

1. INTRODUCTION:

Microbial infections seriously threaten public health, and the mortality ratefrom drug resistance is rising worldwide.Among various microbialinfections, mycotic diseases also growingand developing resistance to available antifungal agents.In recent decades, these infectionshave significantly increased, affecting approximately 20-25% of the global population (Kim et al., 2015). The increase in fungal infections can be attributed to various factors such as climate change, pandemics, drug resistance, population growth, and longer life spans (WHO, 2022). In contrast to the considerable attention directed towards bacterial and viral infections, fungal infections are relativelyneglected. Fungal infections encompass a wide range of diseases, from superficial mycoses like dermatophytosis and candidiasis to deep-tissue invasive infections caused by *Cryptococcus* and *Blastomyces* (Moskaluk and VandeWoude, 2022).Confirmatory diagnosis of these infections can take weeks, and treatment often requires longer therapy duration. Among these infections, superficial mycoses caused by dermatophytes are typically self-curing in healthy animals but may have zoonotic potential (Łagowski et al., 2019). Dermatophytes, which include the important genera *Trichophyton,Microsporum*, and *Epidermophyton*, are keratinophilic fungi that produce keratinase enzymes to digest the keratin present on the nails, skin, and hair. This enzymatic reaction can attract inflammatory cells, leading to an inflammatory response at the site of infection, resulting in severe itching and tissue damage. Timely diagnosis and treatment are crucial for a positive outcome as they can cause high morbidity and indirect psychological effects.

Lifestyle changes and increased interaction with pets, farms, and wild animals have impacted the epidemiology of zoonotic dermatophytosis (Ameen, 2010). Report on epidemiological studies on anthropophilic fungi shows the changing trends of various etiological agents of superficial mycoses (Ameen, 2010, Verma et al., 2021, Kumar et al.,2021). Similar studies of the prevalence and epidemiological trends of zoophilic fungi provide knowledge about the major genera circulating in animalsand further improve the understanding of risk factors associated with mycological infection (Lopes et al., 2024).Humans and animals in tropical and subtropical regions are more vulnerable to superficial mycosis, and increasing interactions between them may serve as a potential source of zoonotic infections. Identification of the etiologic agent and the pattern of skin infection serves as a reliable indicator of health status in both humans and animals, enabling timely intervention and effective management of the infection (Krishnan and Almheiri, 2024).Therefore, a preliminary study was envisaged to detect the incidence of

dermatophytes in animals of the Malwa region of Punjab. This region has a large number of animal populations and agriculture farming is one of the main occupations. The data on dermatophytes of animals inPunjab especially in the Malwa region is scarcely available. Hence, the studies on dermatophytosis are dire necessary to understand the depth of these neglected infections in animals and the outcome of the study will provide insight into the zoonotic potential of dermatophytes in animals of the Malwa region of Punjab.

2. MATERIALS AND METHODS:

2.1 Collection of clinical samples: The samples were collected from animals with clinical signs of superficial mycoses.Skin scrapings and hair sample(s) n=51of animalsshowing symptoms such as itching, alopecia, scaling areas, ring-like lesions, and red rashes with round skin lesions were aseptically collected and submitted for microbiological evaluation. After swabbing the sight with 70% ethanol, hairs were plucked from the periphery of round lesions. Skin scrapings were collected aseptically using a sterile scalpel blade from the borders of the round lesion and collected in a sterile container.

2.2 Direct examination of the clinical specimen by 10% KOH: A few drops of 10% KOH were placed on a grease-free glass slide and samples were placed on it. Carefully put a coverslip and heated the glass slide for a few seconds. The processed samples were kept at room temperature for 5-10 minutes and observed under the law power objective of the microscope for preliminary identification of fungal elements.

2.3 Isolation of organism: The sample found positive for fungal elements (spores/hyphae) was inoculated on Sabouraud's dextrose agar (SDA) supplemented with chloramphenicol and cycloheximide (Himedia) and Dermatophyte test medium (DTM) containingDermato supplement (Himedia). All the inoculated plates were incubated at 27°C for 21-28 days and observed daily for fungal growth.

2.4 Identification of isolated fungal organism

Phenotypic identification

Macromorphological examination: The isolated fungal colonies were observed for their growth rate, consistency, surface/or reverse colour and colour change in the DTM.

*Microscopic examination:*The obtained colonies were examined under a microscope using lactophenol cotton blue (HiMedia) staining.

3. RESULTS AND DISCUSSION:

The present study was conducted to isolate and identify the dermatophytes from the suspected cases of mycoses in animals. Alopecia with erythematous round lesions were the most observed clinical sign along with other symptoms like itching, weight loss, anorexia etc. It was found that most of the clinical cases were presented from March to September. This agrees with previous studiessuggesting that hot, humid and rainy seasons favour the growth of dermatophytes in humans and animals (Segal and Elad, 2021).

Animals of all ageswere susceptible to infection and most were between 3 to 5years of age.

3.1 Direct examination of the clinical specimen by 10% KOH: Out of 51samples, 27(52%) were found positive by microscopic examination by 10% KOH preparations. Direct microscopic examinationshowed characteristic fungal microspores and elements (Fig 1.a) which are suggestive of Dermatophytes. Among the different types of samples, hair was found frequently affected. Multiple outgrowths of fungal elements and microspores indicate fungal involvement in the skin infection. Conventional methods for identifying dermatophytes involve detecting fungal elements through direct microscopy of clinical specimens and isolating them on specific culture media.Identifying fungal elements by direct microscopic method may not be conclusive for dermatophytosis as there may be other nonspecific/contaminant fungal agents. Fungal species may be determined by isolating fungi on a selective culture medium (Lopes*et al.,* 2024).

3.2 Isolation of organism: Out of 27KOH-positive samples, dermatophytes were isolated from 11 samples on DTM (40.74%).The growth of fungal colonies was observed after 8-9 days and radially expanded on extended incubation. Fungal growth was observed in both DTM and SDS medium. The characteristic yellow-to-red colour change in the medium was observed when a fluffy white-coloured colony formed on DTM (Fig.1.b and c).

The presence of antibacterial and antifungal agents in the DTM such as chloramphenicol, gentamicin, and cycloheximide prevent/inhibit the growth of contaminating bacteria and saprophytic fungi (Byrne, 2014). The pH of the medium will change to alkaline by the metabolic activity of dermatophytes, giving a characteristic red colour(Greenacre, 2017). The time of colour change is also important as contaminating fungi can change the colour after a longincubation period (Hnilica and Patterson, 2017). Hence, the observationtime at which colour change is initiated is significant in identifying dermatophytes. Further microscopic characterization ofthe isolated colony was done by staining with lactophenol cotton blue which showed septate hyphae, microspores, segmented arthroconidia, and macroconidia (Fig.1.d). Fewmacroconidia were observed in isolated cultures.This could be because dermatophytes grown on DTM may not have adequate conidial production for identification. The different culture media like rice agar, oatmeal agar and potato dextrose agar were reported to be used for macroconidia production. Though, MAT agar was found to be best for conidia production (Achterman et. al., 2011). Even though the gold standard method for the identificationis isolation and characterisation, KOH preparation of clinical specimens can be considered for the preliminary identification of dermatophytes in resourceless laboratories. It is a simple and cost-effective method that clinicians can use to begin treatment before the culture reports are available, which often take several days to weeks. Taha et al., 2016 suggest

that advanced molecular methods like PCR, RFLP etc. are rapid but too expensive for the regular diagnosis.

Several factors that can impact the incidence of dermatophytosis include climate change, natural disasters, chemotherapy, socioeconomic conditions, migration and transportation. Incidents of zoophilic dermatophytes among humansare related to the increased prevalence of dermatophytosis in animals. Moreover, increased interaction of humans with wild animals can cause the emergence and re-emergence of zoonotic fungal infection (Segal and Elad 2021; IJkelenstam-Koek et al.,2024). Gupta et. al., 2021 report the high prevalence and increasing trend of dermatophytosis among humans. They also suggest that the hot-humid climatic conditions and occupation of the people of the Malwa region are contributing factorsto the growing incidence of dermatophytosis. This region of Punjab has a significant population of animals of various species, which play a crucial role in the local economy. The residents of this area primarily rely on livestockfarming as a key source of income.The present study identified a significant prevalence of mycotic infectionsamong animals in this region, particularly fromMarch to August, characterised byhot and humid climatic conditions.The bovine, canine, feline and rabbit species included in the present study were infected with dermatophytes. Hence, there may be a correlation between the increased dermatophytosis in humanswho are in close contact with these animals.

4. CONCLUSION:

The preliminary study on the occurrence of dermatophytes in animals of the Malwa region suggests that various species of dermatophytes are circulating among them. Most of the infections were reported during the hot-humid, rainy season. The identification of dermatophytes by direct microscopic examination using 10% KOH showed fungal elements which were further confirmed by isolation in a specific culture medium. Most of the animals were between 3 to 5 years of age. Increased occurrence of dermatophytes in animals may be the reason for rising cases of human dermatophytosis. Our finding shows the importance of region-wise study of dermatophytes and clinical practitioners can take precautionary measures to contain the zoonotic fungal infections. It is also important to educate people to curtail the spread of infections. Current findings indicate theneed for a systematic study involving both humans and animals, along with various factors contributing to dermatophytosis, to determine the actual magnitude of this health issue in the area.

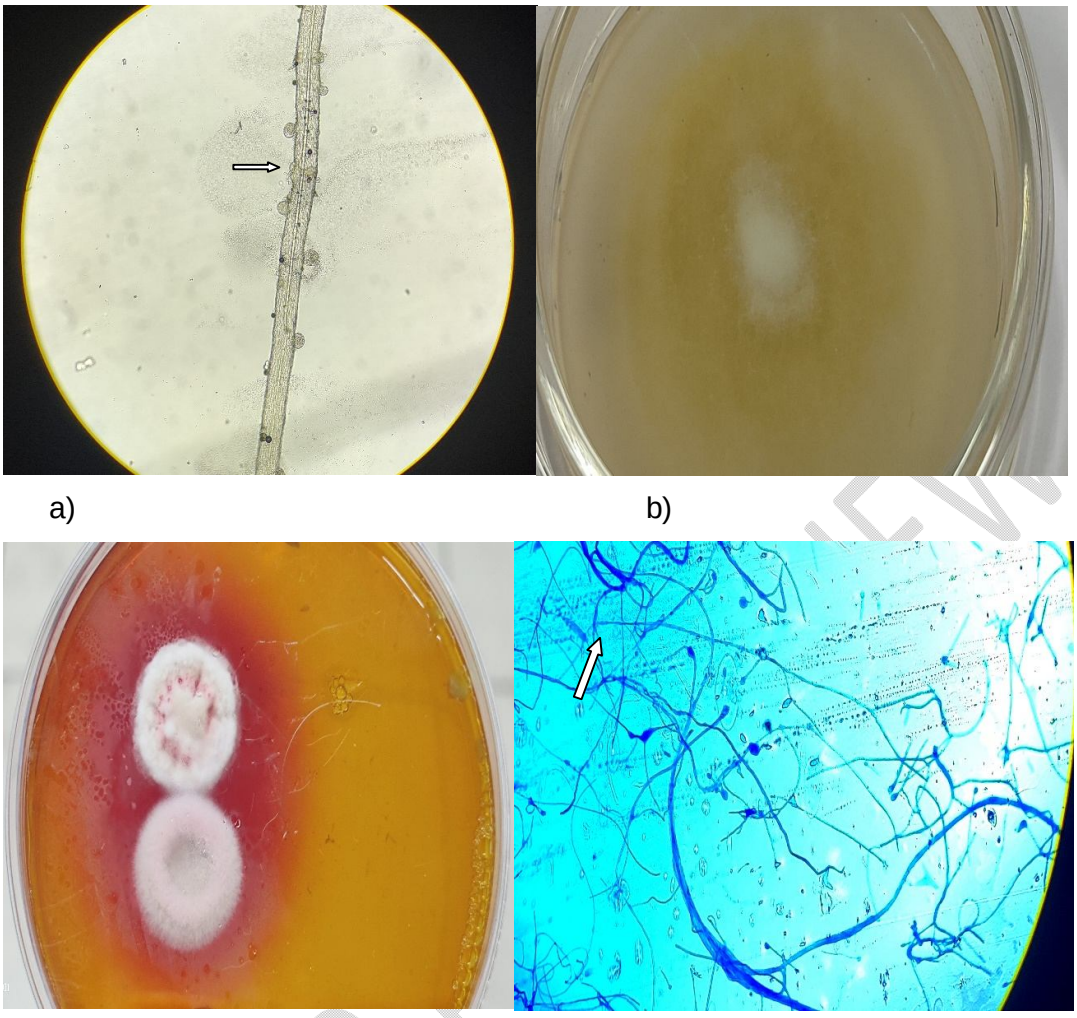

Figure 1.: Microscopical and Macromorphological identification of Dermatophytes a) 10% KOH preparation of hair sample showing microspores and hyphae; b) White fluffy growth on SDA; c) Growth on DTM showing white fluffy colony with a colour change from yellow to red; d) Lactophenol cotton blue staining showing fungal hyphae micro and macrospores (Arrowhead).

Data Availability Statement: The data supporting the findings of this study can be obtained from the corresponding author upon a reasonable request.

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