Indian Ink for the Diagnosis of *Malassezia Pachydermatis* in Dogs

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Abstract
The present study was designed for an economical, accurate and rapid method of diagnosing canine malasseziosis. The table side diagnosis would favour faster diagnosis and early treatment and management. 50 paired tape preparations were collected from 50 dogs and stained using Giemsa-Leishmann stain and Indian ink. Indian ink proved to be an effective faster and economical method of diagnosing *Malassezia pachydermatis*. Indian ink compared to Giemsa-Leishmann stain saved 29 minutes and reduced cost by around 300 times. Indian ink is a good table side diagnosis technique based on easily availability, lower cost and ease of performing test.

Keywords: *Malassezia pachydermatis*, Indian ink; tape preparation.

Introduction
*Malassezia pachydermatis* is a slightly elongated oval or bottle shaped yeast measuring 1-2 X 2-4 μm. It is a unipolar budding yeast and reproduces via bud fission in which the bud detaches from mother cell by septum. Though it is a commensal, environmental and other factors can result in primary or secondary pathological manifestation leading to classical clinical presentation. Genus *Malassezia* is taxonomically located in the family Cryptococcaceae and class Basidiomycetes. *Malassezia* spp are globose to ellipsoidal lipophilic yeast (Matousek and Cambell, 2002) with thick wall and do not produce pseudomycelium.

Predisposing factors
It includes excessive sebum production, accumulation of moisture, damage of epidermis and concurrent dermatoses. It could be secondary to other primary diseases like allergic dermatoses, parasitic dermatoses, endocrinopathies, keratinisation disorders, immunologic dysfunction and skin neoplasia. The lipids and cells of *stratum corneum* protect the skin against invasion by microorganism. When epidermal layer is disrupted secondary bacterial and yeast infection occurs. Environmental factors like high temperature and humidity can be a cause of proliferation of *Malassezia pachydermatis* (Bond and Lyod, 1996).

Clinical signs
Severe itching, self mutilation and typical musty odour will be emanated from affected dogs. Skin lesions are predominant in ventral part of body especially in intertriginous areas. The distribution of lesions are commonly seen in ventral neck, axilla, inguinal, medial thigh, interdigital, perianal, perigenital, hock and ear pinna. *Malassezia* is normal resident of skin, but it is generally isolated in low numbers from skin and in high numbers from mucosa of healthy animals. This has led to speculation that *Malassezia pachydermatis* is shed on to skin from mucosal carriage sites (Bond et al., 2000). In some cases, it is also noticed in periocular and perioral areas. Excessive wax or cerumen in ear canal has been reported as a predisposing factor of *Malassezia* (Masuda et al., 2000). Human cerumen is reportedly mycotic while canine -cerumen often supports yeast growth (Gobal, 1988). Rusty appearance of nails is also observed in most cases. Skin will be erythematous, scaly, excoriated with hairless patches and dry or greasy coat. Often, papules, pustules or epidermal collaret are seen because of secondary bacterial infection. In chronic cases, hyperpigmentation and lichenification is also noticed. Yeast adherence to *stratum corneum* may be an important factor in skin colonisation and infection.
Indian Ink for demonstration of *Malassezia pachydermatis* in tape preparations

Cytologic preparations are the most common technique used to detect Malassezia yeasts. There are several ways to collect cytologic samples like superficial skin scraping, cotton swab, acetate tape impression of skin and direct impression with glass slide. All these methods are effective for moist lesions and acetate tape impressions or skin scraping are effective for drier areas. With exception of tape preparations the slides are heat fixed and stained using Diff-quik, Giemsa, Gram’s or Methylene blue. The tape preparations are not heat fixed. The tape acts as a coverslip through which the sample is viewed. Microscopic examination using 1000X magnification reveals round to oval yeasts. Diagnosis is based on presence of yeast in affected areas of skin with classical clinical signs.

The stain commonly recommended for table side diagnosis of Malassezia *spp* and used in most part of the globe is Diff-Quik which is not available for regular use in India. Regular staining procedures with other standard stains are time consuming (up to 30 minutes) and cannot be used for table side diagnosis in heavily case loaded small animal practice. Hence, an alternative for rapid identification of Malassezia *spp.* organisms was attempted. Review of literatures revealed that *Cryptococcus neoformans* infection in humans was diagnosed using Indian ink from spinal fluid samples collected from affected humans. The capsule of *C. neoformans* are seen as halo around organism distributed in black background in liquid medium. Basic Indian ink is composed of fine soot known as lamp black, combined with water to form a liquid. Malassezia also belong to family Cryptococcaceae. So, the above technique was modified for dry skin samples collected from suspected dogs for identification of Malassezia infection in dogs.

A total of 100 tape preparations from 50 affected dogs were subjected to Giemsa- Leishmann staining method and their pairs with Indian Ink method as described below and found successful.

The sample is collected by pressing the cellophane tape over the lesion and stuck over a drop of Indian Ink in degreased glass slide. Immediately the excess ink is squeezed out by pressing with cotton. Any excess air pockets should be squeezed out. The tape should have a homogenous light black stain. A drop of oil is placed over the tape and examined under 100X
Fig. 7: *Malassezia* yeast cells with a clear halo on black background in a light microscope (100 X)

Fig. 8: Comparative time employed

Fig. 9: Comparative cost

Fig. 10: Comparative efficacy and accuracy

Table 1: Comparative evaluation of Giemsa-Leishman Stain and Indian ink

<table>
<thead>
<tr>
<th>Stain</th>
<th>Time taken (mins.)</th>
<th>Cost (INR.)</th>
<th>Accuracy (50 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giemsa-Leishmann</td>
<td>30</td>
<td>600/-</td>
<td>50</td>
</tr>
<tr>
<td>Indian ink</td>
<td>01</td>
<td>15/-</td>
<td>50</td>
</tr>
</tbody>
</table>

Results and Discussion

1-2 organism per microscopic field are usually considered normal, if the sample is taken from normal harbouring areas like ear pinna and perianal areas. A good number of organisms in combination with classical clinical signs are found if sample is collected from affected areas of skin lesions and is considered pathological.

The Indian ink can be procured from any stationary shops without any restrictions and available round the year. Diff-Quik, if procured should be cleared by importing authorities and should be ordered well in advance. The Giemsa-Leishmann stain can be purchased only from dealers of scientific chemicals.

Staining of tape preparations takes one minute to stain with Indian ink. For instant diagnosis of Malassezia dermatitis Diff-Quik is recommended. Unlike in Giemsa-Leishmann staining it takes 30 minutes. Hence, Indian ink staining to diagnose Malassezia pachydermatis infection is equally rapid.

The Indian ink is cheaper. The cost of 50 ml of Indian ink is INR 15/- which could be used for around 200 clinical cases. Unlike the Diff - Quik, if imported costs approximately INR 6000/-. The conventional Giemsa-Leishmann costs
Indian ink for Malassezia pachydermatis

approximately INR 600/-. So, Indian ink is economical and effective (Table 1).

The tape preparations were examined by conventional staining technique and results were compared with Indian ink staining. The results of both the technique were similar. Hence this method of staining is reliable.

Conclusion
Indian ink could be used to diagnose Malassezia economically, rapidly and recommended as a reliable alternative for Diff-Quik. Rapid table side diagnosis of canine Malassezia dermatitis can be done using Indian ink.

References


