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FROM STATIONERY TO STAIN: CAMLIN INK FOR *MALASSEZIA* DIAGNOSIS IN DOGS

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ABSTRACT

Malassezia species are opportunistic yeasts frequently implicated in canine dermatological conditions. While conventional staining techniques such as methylene blue, Gram stain, and Diff-Quik are routinely employed for identification, there is a growing need for cost-effective alternatives, particularly in resource-limited or field settings. This study assessed the efficacy of Camlin fountain ink (phthalocyanine blue) as a viable staining method for *Malassezia* detection. Impression smears were obtained from dogs presented with clinical signs of *Malassezia* dermatitis. Following confirmation of *Malassezia* positivity, smears were stained with Camlin ink. The results revealed that *Malassezia* yeasts cells effectively retained the stain, enhancing their visualization under oil immersion (100x) microscopy. These findings suggest that Camlin ink offers a practical, affordable, and accessible alternative for in-clinic and field-based diagnosis of *Malassezia* infections in dogs, potentially improving diagnostic efficiency in veterinary dermatology.

Keywords: Yeast, dogs, *Malassezia, phthalocyanine blue*

INTRODUCTION

Malassezia is a genus of lipophilic yeast that constitutes a normal component of the cutaneous microbiota in mammals. However, under specific predisposing conditions, it can transition into a pathogenic state, leading to *Malassezia* dermatitis, a common skin disorder in dogs (Bond *et al*., 2020). *Malassezia pachydermatis* has been identified as a primary etiological agent of dermatitis in both canine and feline populations, with clinical manifestations including alopecia, hyperpigmentation, pruritus, erythema, and a characteristic musty odour (Kano *et al.,* 2020).

The diagnosis of *Malassezia* dermatitis relies primarily on direct microscopic examination of skin scrapings, which are typically processed using staining techniques such as methylene blue, Gram stain, Diff-Quik, and periodic acid–Schiff (PAS) staining (Guillot & Bond, 2020). Recent studies have highlighted the utility of 20 per cent KOH combined with Blue-Black Parker ink, achieving a 90 per cent success rate in spore identification, thereby offering a valuable diagnostic tool for *Malassezia* folliculitis (Rahadiyanti *et al.,* 2020).

Among cytological sampling techniques, impression smears remain the most accessible method for collecting skin samples, as they enable rapid, non-invasive detection of yeast cells. Conventional staining methods include methylene blue staining, which provides a simple contrast; Gram staining, which differentiates *Malassezia* as Gram-positive oval to bottle-shaped yeast cells; and Diff-Quik staining, which rapidly highlights yeast structures in a blue to purple hue (Reddy & Sivajothi, 2016; Cafarchia *et al.,* 2011; Paterson, 2016). PAS staining, a histochemical method used in histopathological sections, selectively stains fungal cell walls magenta (Midgley *et al.,* 2012), while lactophenol cotton blue enhances fungal structure visualization (Gaitanis *et al.,* 2012). India ink staining, though primarily employed for encapsulated yeasts, is less commonly used for *Malassezia* (Jayanthy *et al.,* 2015).

Despite the effectiveness of these methods, their accessibility can limit their application in field settings, particularly in resource-limited environments. The need for a readily available, economical staining alternative is therefore imperative. This study aims to assess the diagnostic utility of Camlin ink as a cost-effective staining method for *Malassezia* in clinical samples, offering a potential alternative for routine field-based diagnosis.

MATERIALS AND METHODS

A total of 30 paired impression smears were collected from clinically affected dogs exhibiting dermatological signs consistent with *Malassezia* dermatitis, including alopecia, pruritus, hyperpigmentation, lichenification, and a characteristic musty odour. Impression smears were obtained by gently pressing clean glass slides against affected skin areas to facilitate yeast transfer. To confirm the presence of *Malassezia* yeast cells, one set of impression smears from each lesion was air-dried, methanol-fixed, and stained using methylene blue for 20 minutes. Microscopic examination was performed under oil immersion (100×) to identify *Malassezia* cells, characterised by round, budding, human-footprint-shaped yeast structures.

For the evaluation of Camlin ink as a staining alternative, impression smears were first air-dried and fixed with methanol for one minute. The prepared smears were then flooded with Camlin ink and incubated at room temperature for varying time intervals of 10, 15 and 20 minutes. After incubation, excess stain was gently rinsed off with distilled water, and the slides were blotted dry. Microscopic examination was conducted under 100× oil immersion to assess the visibility and morphological integrity of *Malassezia* yeast cells.

RESULTS AND DISCUSSION

All the stained smears that were positive with methylene blue stain were found to be positive for Camlin ink stain as well indicating *Malassezia* yeast cells being effectively stained with Camlin ink, which is in correspondence with the findings of Jayanthy *et al.* (2015).

The cells remained unstained in the absence of methanol fixation, which indicated the use of methanol fixation enhanced stain uptake, improving clarity and specificity in identification.

The staining quality was comparable to conventional methods, highlighting the potential of Camlin ink as an economical alternative for field use. Unlike traditional staining methods, Camlin ink is cost-effective and widely available, making it particularly useful for veterinarians and field practitioners working in resource-limited environments.

The effectiveness of Camlin ink in staining *Malassezia* yeast cells can be attributed to the ink's chemical composition and the structural characteristics of the

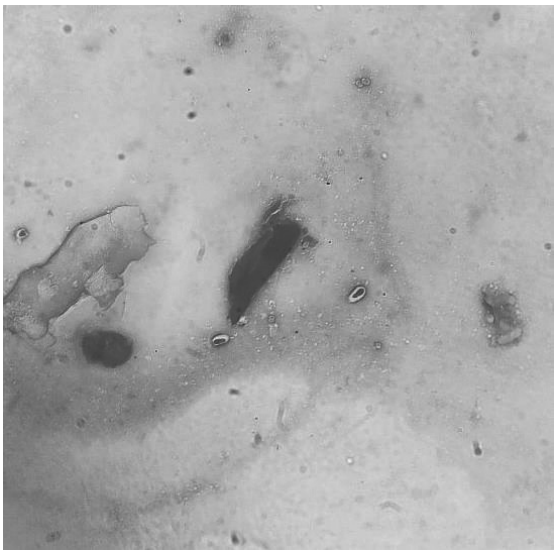

Figure 1. Malassezia pachydermatis stained with Camlin Ink in 10 minutes, 100x Oil immersion

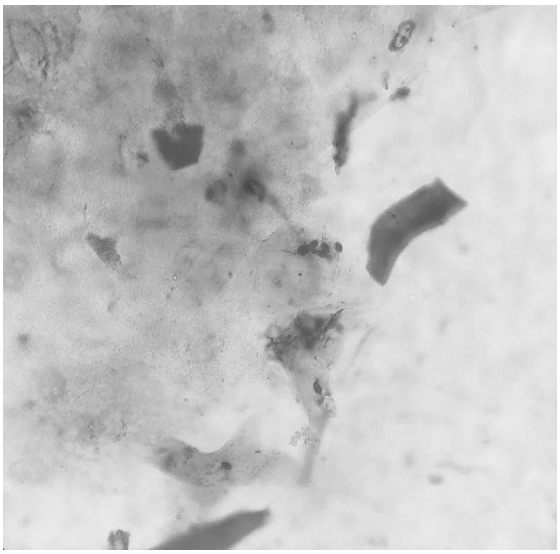

Figure 2. Malassezia pachydermatis stained with Camlin Ink in 15 minutes, 100x Oil immersion

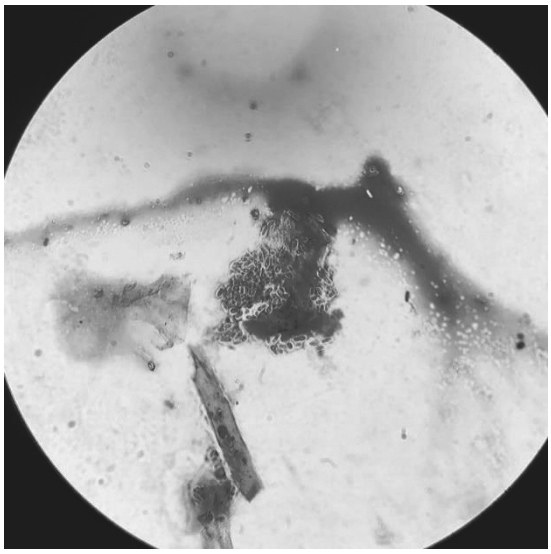

Figure 3. Malassezia pachydermatis stained with Camlin Ink in 20 minutes, 100x Oil immersion

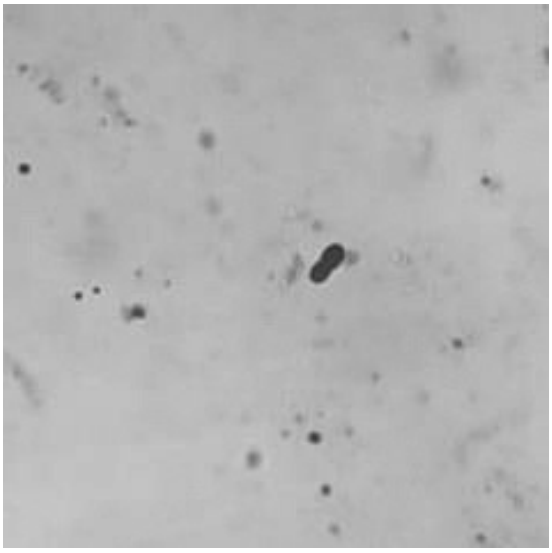

Figure 4. Malassezia pachydermatis (circled) stained with Methylene Blue for 20 minutes, 100x Oil immersion

yeast cell wall. The cell wall of *Malassezia* is rich in polysaccharides, proteins and lipids, which can interact with various staining agents. Studies have demonstrated that certain inks, such as Parker ink, can effectively stain fungal elements, including hyphae and spores, facilitating their identification under a microscope (Xiong and Dai, 2004). While the exact mechanism of Camlin ink's staining of *Malassezia* cells requires further investigation, it is plausible that the ink's components penetrate the yeast's cell wall or bind to specific cellular constituents, resulting in effective staining. This interaction increases the contrast between yeast cells and the surrounding background, facilitating clearer microscopic visualization.

Camlin ink staining offers advantages in terms of cost-effectiveness, accessibility, and ease of use. Comparison with traditional methods showed that Camlin ink provided similar visibility to methylene blue and Gram stain but was more accessible. Unlike PAS staining, which requires laboratory processing, Camlin ink staining can be performed easily in field conditions. Moreover, the staining time was comparable to that of methylene blue making it a practical option.

Despite its affordability and ease of use, Camlin ink staining presents certain limitations in the detection of *Malassezia* spp. Camlin ink smears aren't permanent mounts. They tend to fade, blur, or dry unevenly, making archival or delayed review nearly impossible. Variability in staining intensity with different incubation times may also affect the consistency of results.

CONCLUSION

The Camlin ink staining method is easy to perform, requires bare minimum equipment and expertise, and yields results reliable and equal to those obtained with more sophisticated staining techniques. It is particularly useful in field clinics, rural veterinary practices or emergency situations where access to laboratory facilities may be limited. By providing a cost-effective and efficient means of diagnosing yeast infections, this technique enables timely and targeted treatment, improving clinical outcomes and animal welfare.

However, further research is required to determine its long-term stability, specificity, and sensitivity compared to conventional stains. Additionally, future studies should explore its efficacy in different species and environmental conditions. Comparative studies with other staining techniques, including molecular diagnostics, can help establish its broader applicability in veterinary diagnostics and research.

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