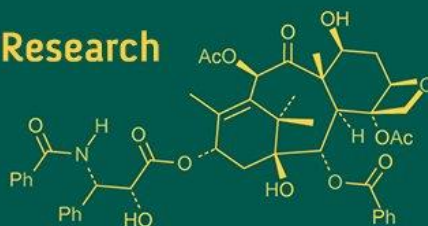


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 982-985
www.biochemjournal.com
 Received: 15-04-2026
 Accepted: 17-05-2026

Elaiyaraja Govindaraj
 Assistant Professor, Department
 of Veterinary Microbiology,
 Veterinary College and Research
 Institute, TANUVAS, Salem,
 Tamil Nadu, India

Shanmugasundaram Udhayavel
 Assistant Professor, Department
 of Veterinary Microbiology,
 Veterinary College and Research
 Institute, TANUVAS, Salem,
 Tamil Nadu, India

S Jaisree
 Associate Professor and Head,
 Department of Veterinary
 Microbiology, Veterinary College
 and Research Institute,
 TANUVAS, Salem, Tamil Nadu,
 India

E Venkatesakumar
 Professor and Head, Department
 of Veterinary Medicine,
 Veterinary College and Research
 Institute, TANUVAS, Salem

PA Enbavelan
 Assistant Professor, Department
 of Veterinary Medicine,
 Veterinary College and Research
 Institute, TANUVAS, Salem,
 Tamil Nadu, India

E Madesh
 Assistant Professor, Veterinary
 Clinical Complex, Veterinary
 College and Research Institute,
 TANUVAS, Salem, Tamil Nadu,
 India

E Kalaiselvan
 Assistant Professor, Department
 of Veterinary Surgery and
 Radiology, Veterinary College
 and Research Institute,
 TANUVAS, Salem, Tamil Nadu,
 India

Corresponding Author:
Elaiyaraja Govindaraj
 Assistant Professor, Department
 of Veterinary Microbiology,
 Veterinary College and Research
 Institute, TANUVAS, Salem,
 Tamil Nadu, India

Isolation and characterization of *Rhizopus stolonifer* associated with otitis in cattle

Elaiyaraja Govindaraj, Shanmugasundaram Udhayavel, S Jaisree, E Venkatesakumar, PA Enbavelan, E Madesh and E Kalaiselvan

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6l.8810>

Abstract

Otitis in cattle is commonly associated with bacterial pathogens; however, fungal agents may also contribute to chronic and recurrent infections. The present study describes the isolation and characterization of *Rhizopus stolonifer* from bovine otitis cases. Ear swab samples were collected aseptically from cattle exhibiting clinical signs of otitis, including ear discharge, pruritus, head shaking, and inflammation of the external ear canal. Samples were cultured on Sabouraud Dextrose Agar (SDA) and incubated at 28 °C. Fungal isolates were characterized based on cultural characteristics and microscopic examination using Lactophenol Cotton Blue staining. The isolates produced rapidly growing cottony white colonies that gradually turned grayish-black with age. Microscopic examination revealed broad aseptate hyphae, sporangiophores, sporangia, and rhizoids characteristic of *Rhizopus stolonifer*. The findings suggest that *Rhizopus stolonifer* may act as an opportunistic pathogen in bovine otitis, particularly under conditions favoring fungal proliferation. Early identification of fungal agents is essential for appropriate therapeutic intervention and prevention of chronic otitic infections in cattle.

Keywords: Fungal otitis, Mucorales, *Rhizopus stolonifer*, characterization, cattle

Introduction

Otitis is an inflammatory condition affecting the external, middle, or inner ear and is associated with reduced animal welfare, decreased productivity, and economic losses in livestock production systems. Bacterial pathogens such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas aeruginosa*, and *Proteus* spp. are frequently implicated in bovine otitis; however, fungal pathogens are increasingly recognized as an important etiological agents, particularly in chronic and recurrent otitis cases (Duarte *et al.*, 2004; Quinn *et al.*, 2025 Elaiyaraja *et al.*, 2026) [1, 7, 3].

Fungal otitis is often underdiagnosed because routine diagnostic investigations primarily focus on bacterial pathogens. Opportunistic fungi may colonize the external auditory canal upon bacterial infection or following excessive moisture accumulation, tissue injury, or immunity compromised animals (Dworecka-Kaszak *et al.*, 2020) [2]. The warm and humid microenvironment of the ear canal provides favorable conditions for fungal proliferation and persistence.

Rhizopus stolonifer, a member of the order Mucorales, is a ubiquitous environmental fungus commonly found in soil, decaying vegetation, animal bedding, and feed materials (Ribes *et al.*, 2000) [8]. Although generally regarded as a saprophyte, *Rhizopus* species have been increasingly reported as opportunistic pathogens capable of causing localized and systemic infections in both humans and animals (Spellberg *et al.*, 2005) [10]. The pathogenic potential of these fungi is primarily attributed to their rapid growth, thermotolerance, and ability to invade host tissues through broad aseptate hyphae.

Members of Mucorales also possess a unique capacity for angioinvasion, resulting in vascular thrombosis, tissue ischemia, and necrosis, which are considered hallmarks of mucormycosis (Ibrahim *et al.*, 2012) [4]. In animals, mucormycetes have been associated with respiratory infections, gastrointestinal disease, cutaneous lesions, mastitis, abortions, and systemic mycoses (Richardson, 2009) [9]. However, reports describing *Rhizopus*-associated otitis in cattle remain limited.

The emergence of fungal pathogens in bovine otitis highlights the necessity of comprehensive microbiological investigations that include both bacteriological and mycological examinations. Accurate identification of fungal pathogens is essential for appropriate therapeutic interventions and for understanding their epidemiological significance in livestock populations. Therefore, the present study was undertaken to isolate and characterize *Rhizopus stolonifer* associated with otitis in cattle.

2. Materials and Methods

2.1. Study Animals and Sample Collection

Cattle presented with clinical signs suggestive of otitis, including discharge from the ear, irritation, head shaking, foul odor, and inflammation of the external ear canal, were selected for the study. Following clinical examination, ear swab samples from the affected ears were collected aseptically using sterile cotton swabs. The swabs were immediately transported to the department of veterinary microbiology laboratory for microbiological examination.

2.2. Fungal Isolation

Samples were inoculated onto Sabouraud dextrose agar (SDA) as well on brain heart infusion agar. The inoculated plates were incubated aerobically at 28 ± 2 °C and monitored daily for fungal growth for up to seven days and at 37 °C for bacteriological growth. Emerging fungal colonies were subcultured onto fresh SDA plates to obtain pure cultures.

2.3. Cultural characteristics

The fungal isolates were characterized based on growth rate, pigmentation, surface and reverse colony appearance. The observations were recorded periodically during incubation.

2.4. Microscopic Characterization

A small portion of fungal growth was mounted in lactophenol cotton blue (LPCB) stain and examined under a light microscope for its microscopic features.

3. Results

3.1. Clinical Findings

Affected cattle exhibited varying degrees of otitic inflammation characterized by head shaking, ear scratching, purulent or serous ear discharge, foul-smelling exudate and pain upon manipulation of the ear.

3.2. Isolation of Fungal Organism

Pure fungal growth was obtained from affected ear samples following incubation on SDA.

3.3. Cultural characteristics

The fungal isolate produced rapidly spreading cottony colonies that initially appeared white and fluffy. As incubation progressed, colonies developed grayish-black pigmentation due to maturation of sporangia (Figure 1).

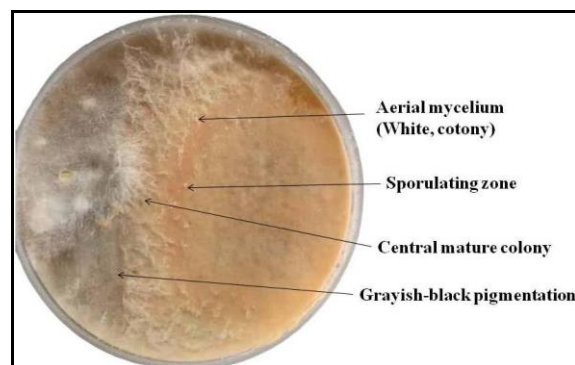


Fig 1: Cultural characteristics of *Rhizopus stolonifer* isolated from bovine otitis showing rapidly growing cottony aerial mycelium on Sabouraud Dextrose Agar

3.4. Microscopic Characteristics

The LPCB staining results revealed that the broad ribbon-like aseptate hyphae, long sporangiophores, spherical sporangia, abundant sporangiospores, well-developed rhizoids and these microscopic features were consistent with *Rhizopus stolonifer* (Figure 2).

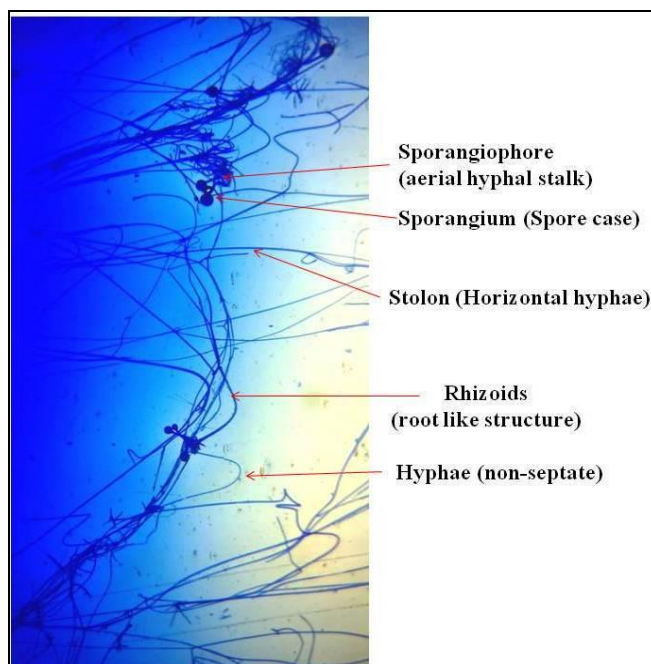


Fig 2: Microscopic morphology of *Rhizopus stolonifer* stained with Lactophenol Cotton Blue showing broad aseptate hyphae, sporangiophores, sporangia, and rhizoids.

3.5. Associated Bacterial Isolates

Gram staining of bacterial isolates recovered from otitic samples revealed predominantly Gram-positive cocci arranged in pairs and short chains.

4. Discussion

The present study reports the isolation and characterization of *Rhizopus stolonifer* from bovine otitis cases. The recovery of this fungus from clinical ear samples suggests that opportunistic fungal pathogens may contribute to the pathogenesis of otitis in cattle. Although bacterial agents remain the predominant causes of bovine otitis, fungal involvement should be considered, particularly in chronic, recurrent, or treatment-refractory cases.

The cultural characteristic observed in the present study was characterized by rapidly growing cottony white colonies that subsequently developed gray to black pigmentation upon maturation. These findings are consistent with previous descriptions of *Rhizopus* species reported by Larone (1994)^[6], Kidd *et al.*, (2022)^[5] who described *Rhizopus* colonies as fast-growing, fluffy, and initially white before becoming grayish-black due to the development of mature sporangia (Quinn *et al.*, 2025)^[7].

Microscopic examination revealed broad aseptate hyphae, sporangiophores, sporangia, and rhizoids, which are considered characteristic features of *Rhizopus stolonifer* (Walther *et al.*, 2019, Winn 2006)^[11, 12]. The presence of well-developed rhizoids directly beneath sporangiophores is an important distinguishing feature that differentiates *Rhizopus* species from other members of the Mucorales.

The isolation of *Rhizopus stolonifer* from otitic lesions suggests a potential role in chronic inflammatory processes of the ear. The moist microenvironment within the external auditory canal may facilitate fungal colonization and proliferation, particularly in the presence of tissue damage or concurrent bacterial infection. *Rhizopus* spores are commonly present in soil, feed, and organic debris and can readily contaminate damaged epithelial surfaces (Ribes *et al.*, 2000)^[8]. Conditions such as prolonged antimicrobial treatment, excessive moisture, trauma, or concurrent bacterial infections may facilitate fungal colonization and subsequent infection (Spellberg *et al.*, 2005)^[10].

The pathogenesis of *Rhizopus* infections is largely attributed to the ability of the fungus to germinate rapidly and produce invasive hyphae capable of penetrating host tissues leading to local inflammation and tissue destruction. Mucorales fungi exhibit marked angioinvasive properties that promote vascular thrombosis, tissue infarction, and necrosis (Ibrahim *et al.*, 2012)^[4]. Although invasive mucormycosis is uncommon in cattle, localized infections can occur when host defenses are compromised.

The concurrent isolation of Gram-positive cocci from affected ears suggests the possibility of polymicrobial infections. Mixed bacterial-fungal infections have been reported in otitis and may contribute to disease chronicity and reduced therapeutic efficacy (Dworecka-Kaszak *et al.*, 2020)^[2]. The inflammatory environment created by bacterial infection may facilitate fungal establishment, while fungal colonization may further aggravate tissue damage and inflammation.

The findings of the present study emphasize the importance of incorporating mycological investigations into routine diagnostic protocols for bovine otitis. Failure to recognize

fungal involvement may result in prolonged disease duration and inappropriate antimicrobial usage.

5. Conclusion

The present study confirms the isolation and characterization of *Rhizopus stolonifer* from bovine otitis cases based on cultural characteristics and microscopic examination. The findings highlight the potential role of opportunistic fungal pathogens in bovine ear infections and emphasize the importance of including mycological investigations in chronic or recurrent otitis cases. Early diagnosis and appropriate therapeutic interventions may improve clinical outcomes and reduce economic losses associated with otitic diseases in cattle.

6. Declaration Conflict of Interest

On behalf of all author declare that no conflict of interest with the publication

7. Ethics Statement

The animals used in this study were in accordance with the ethics of the TANUVAS institutional animal ethics committee (IAEC).

8. Acknowledgement

I gratefully acknowledge and thankful to the TANUVAS University for funding and support during entire study period.

References

1. Duarte ER, Hamdan JS. Otitis in cattle: an aetiological review. J Vet Med B Infect Dis Vet Public Health. 2004;51(1):1-7.
2. Dworecka-Kaszak B, Biegańska MJ, Dąbrowska I. Occurrence of various pathogenic and opportunistic fungi in skin diseases of domestic animals: a retrospective study. BMC Vet Res. 2020;16(1):248.
3. Govindaraj E, Udhayavel S, Venkatesakumar E, Enbavelan PA, Kalaiselvan E, Jaisree S, *et al.* Antimicrobial susceptibility and multidrug resistance patterns of bacterial isolates associated with otitis in cattle. Int J Adv Biochem Res. 2026;10(6):814-818. doi:10.33545/26174693.2026.v10.i6j.8759.
4. Ibrahim AS, Spellberg B, Walsh TJ, Kontoyiannis DP. Pathogenesis of mucormycosis. Clin Infect Dis. 2012;54(Suppl 1):S16-S22.
5. Kidd S, Halliday C, Ellis D. Descriptions of Medical Fungi. Wallingford: CABI; 2022.
6. Larone DH. Medically important fungi. Rev Inst Med Trop Sao Paulo. 1994;36(4):362.
7. Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ. Veterinary Microbiology and Microbial Disease. 3rd ed. Hoboken (NJ): Wiley-Blackwell; 2025.
8. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev. 2000;13(2):236-301.
9. Richardson M. The ecology of the Zygomycetes and its impact on environmental exposure. Clin Microbiol Infect. 2009;15(Suppl 5):2-9.
10. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. Clin Microbiol Rev. 2005;18(3):556-569.

11. Walther G, Wagner L, Kurzai O. Updates on the taxonomy of Mucorales with an emphasis on clinically important taxa. *J Fungi*. 2019;5(4):106.
12. Winn WC. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2006.