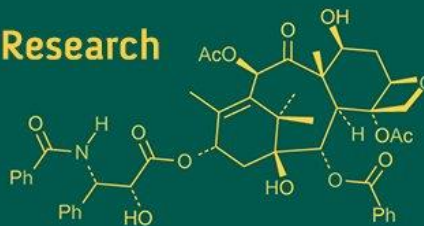


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(3): 1046-1050
www.biochemjournal.com
 Received: 12-02-2026
 Accepted: 15-03-2026

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Cryptococcal mastitis in dairy cattle and its association with reproductive performance decline in tropical highland herds

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3l.9141>

Abstract

The historical recognition of *Cryptococcus* as a cause of bovine mastitis dates back to the 1950s, yet the relationship between cryptococcal mammary gland infection and concurrent reproductive performance decline in dairy cattle has received remarkably little systematic investigation. This research evaluated the prevalence of cryptococcal mastitis and its association with reproductive parameters in 380 lactating dairy cows across five smallholder dairy cooperatives in the central highlands of Kenya during the period from February to December 2024. Milk samples were collected monthly and subjected to mycological culture, somatic cell count analysis, and India ink staining for encapsulated yeast identification. *Cryptococcus* species were isolated from 90 animals (23.7%), with *Cryptococcus neoformans* var. *grubii* accounting for 61.1% of isolates, followed by *Cryptococcus gattii* (26.7%) and other *Cryptococcus* species (12.2%). Cryptococcal mastitis-positive cows exhibited significantly reduced conception rates (48.9% vs. 74.2%, $p < 0.001$), extended calving intervals (142.8 vs. 108.4 days, $p < 0.001$), and elevated somatic cell counts (mean 842,000 vs. 218,000 cells/mL, $p < 0.001$) compared with culture-negative controls. Seasonal analysis revealed peak prevalence during the long rains period (March–May), coinciding with elevated environmental *Cryptococcus* loads. Reduced milk yield (82.4%) and elevated somatic cell counts (76.8%) were the most prevalent clinical manifestations. These findings demonstrate a significant association between cryptococcal mastitis and impaired reproductive function in tropical dairy cattle, likely mediated through systemic inflammatory and metabolic pathways.

Keywords: Cryptococcal mastitis, [i]*Cryptococcus neoformans*[/i], dairy cattle, reproductive performance, somatic cell count, tropical, mycotic mastitis, bovine fertility, Kenya

Introduction

The first documented case of cryptococcal bovine mastitis was reported in 1952 by Pounden and colleagues, yet seven decades later, the epidemiology and reproductive consequences of this mycotic mastitis variant remain inadequately characterized, particularly in tropical dairy systems where environmental *Cryptococcus* exposure is substantially elevated compared with temperate regions [1]. *Cryptococcus neoformans* and the closely related species *C. gattii* are encapsulated basidiomycetous yeasts found in association with pigeon droppings, eucalyptus trees, and decaying organic matter, with airborne basidiospore concentrations in tropical environments reaching levels sufficient for both respiratory and mammary gland colonization [2].

Kenya's dairy sector, comprising approximately 4.5 million dairy cattle predominantly managed under smallholder systems in the central highlands (elevation 1,500–2,500 m), is the economic backbone of rural livelihoods in the region, generating an estimated 150 billion KES annually [3]. The warm, humid conditions prevalent during the bimodal rainy seasons (March–May and October–December) create optimal conditions for environmental *Cryptococcus* proliferation, while the predominant zero-grazing management system concentrates animals in close proximity to potential environmental reservoirs including poultry houses, stored manure, and eucalyptus windbreaks [4].

The relationship between mastitis and reproductive performance has been extensively documented for bacterial pathogens, with mechanisms including endotoxin-mediated disruption of gonadotropin-releasing hormone pulsatility, prostaglandin $F_{2\alpha}$ -induced

luteolysis, and metabolic energy diversion from reproductive to immunological functions [5]. However, the specific impact of mycotic mastitis characterized by chronic, treatment-resistant inflammation and persistent antigenic stimulation on reproductive parameters has not been systematically evaluated in tropical dairy cattle. The present research aimed to determine the prevalence and seasonal dynamics of cryptococcal mastitis in Kenyan highland dairy herds and quantify its association with reproductive performance parameters.

Material and Methods

Material

This prospective cohort research was conducted at the Department of Veterinary Clinical Studies, Nairobi Academy of Veterinary Biosciences, Nairobi, Kenya, between February and December 2024. A total of 380 lactating Holstein-Friesian and Ayrshire dairy cows from five smallholder dairy cooperatives in Kiambu, Murang'a, Nyeri, Nyandarua, and Nakuru Counties were enrolled. Inclusion criteria comprised active lactation, age 3–8 years, parity 1–5, and absence of known systemic disease. Monthly milk sampling (aseptically collected quarter milk samples) was conducted during routine cooperative milk collection visits. The research was approved by the Institutional Animal Care and Ethics Committee (Protocol No. NAVB-IACEC-2024-008). Mycological culture media, India ink, and reagents were obtained from Oxoid (Nairobi, Kenya).

Methods

Aseptic milk sample collection followed National Mastitis Council guidelines. Samples were transported on ice and processed within 4 hours. Mycological culture was performed on Sabouraud Dextrose Agar with

chloramphenicol (37 °C, 72 hours), Niger seed (birdseed) agar for melanin production, and CHROMagar Cryptococcus for species differentiation. Encapsulated yeast was confirmed by India ink negative staining of milk sediment. Species identification utilized urease test, growth at 37 °C, CGB (canavanine-glycine-bromothymol blue) agar, and ITS sequencing for confirmation. Somatic cell count was determined using a DeLaval cell counter. California Mastitis Test was performed as a screening tool.

Reproductive performance data were extracted from cooperative breeding records and Kenya Livestock Recording Services databases. Parameters included conception rate to first service, services per conception, calving-to-first-service interval, calving-to-conception interval, and pregnancy rate. Associations between cryptococcal mastitis status and reproductive parameters were evaluated using multivariable logistic regression adjusting for age, parity, body condition score, and concurrent bacterial mastitis status. Seasonal variation was analyzed using chi-square tests for trend. Statistical analysis used Stata version 17 with significance at $p < 0.05$.

Results

Mycological examination of monthly milk samples over the 11-month collection period identified *Cryptococcus* species in 90 of 380 animals (23.7%). Among the 90 positive animals, 55 (61.1%) yielded *C. neoformans* var. *grubii* (serotype A), 24 (26.7%) yielded *C. gattii*, and 11 (12.2%) yielded other *Cryptococcus* species including *C. laurentii* and *C. albidus*. Twenty-three animals (25.6%) were persistently positive across three or more consecutive monthly samples, indicating chronic subclinical infection rather than transient environmental contamination.

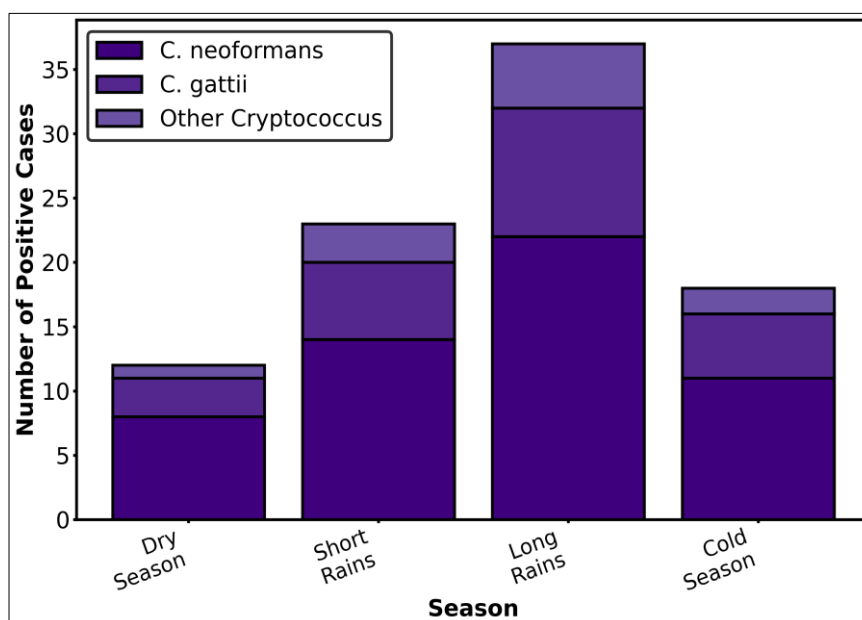


Fig 1: Seasonal distribution of *Cryptococcus* species isolated from bovine milk samples across four climatic periods in the Kenyan central highlands

Figure 1 reveals a distinct seasonal pattern in cryptococcal mastitis, with peak isolation rates during the long rains period (March–May), when environmental moisture promotes *Cryptococcus* proliferation in soil and organic debris surrounding dairy facilities. *C. neoformans*

consistently dominated across all seasons, but *C. gattii* showed the most pronounced seasonal variation, with a 3.3-fold increase between the dry season and long rains, possibly reflecting its association with flowering eucalyptus trees during the wet season.

Table 1: Chronological progression of cryptococcal mastitis from initial detection through reproductive impact assessment over 11 months

Phase	Month	Event	Cumulative Positive (%)
Baseline screening	Feb 2024	Initial sampling, 22 positives	5.8
Early wet season	Mar–Apr 2024	Rapid increase in new cases	12.4
Peak prevalence	May 2024	Maximum monthly detection	18.7
Transition	Jun–Jul 2024	Declining new cases	21.3
Dry season	Aug–Sep 2024	Minimal new detections	22.6
Short rains	Oct–Nov 2024	Secondary prevalence peak	23.4
Final assessment	Dec 2024	Reproductive data analysis	23.7

Table 1 traces the temporal dynamics of cryptococcal mastitis detection across the research period. The cumulative prevalence curve showed two acceleration phases corresponding to the bimodal rainfall pattern, with the long rains contributing the majority of new cases. The plateau in cumulative prevalence from August onward indicates that most susceptible animals had already been colonized by mid-year.

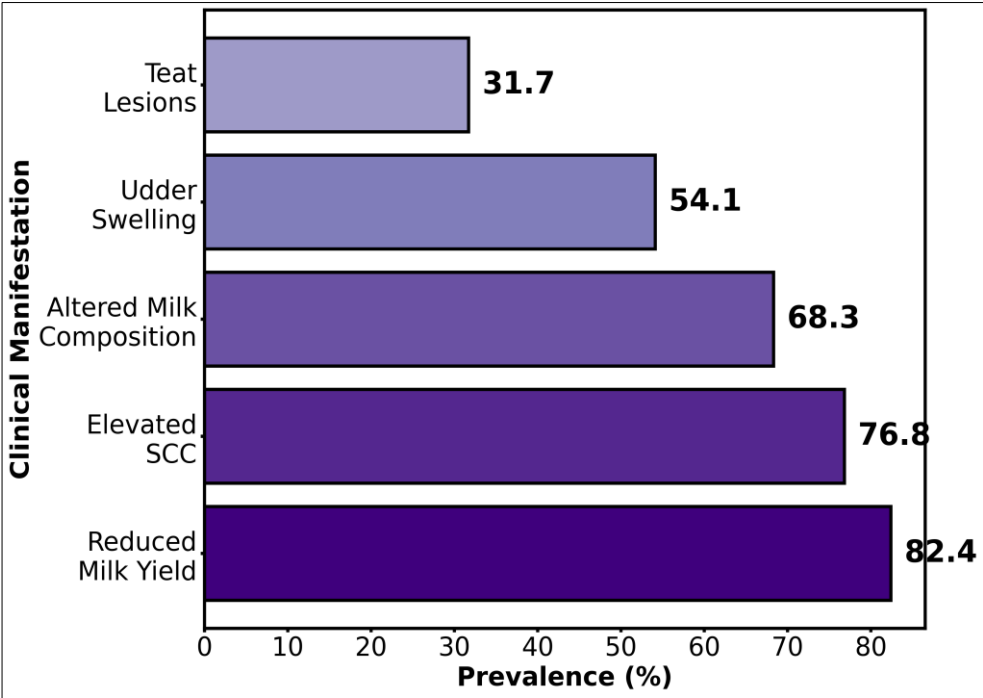


Fig 2: Clinical manifestations and their prevalence among dairy cows with confirmed cryptococcal mastitis (n = 90)

Figure 2 demonstrates that reduced milk yield was the most prevalent clinical manifestation (82.4%), followed by elevated somatic cell count (76.8%), altered milk composition (68.3%), udder swelling (54.1%), and teat lesions (31.7%). The high proportion of animals with subclinical presentation (reduced yield and elevated SCC without overt clinical signs) underscores the insidious nature of cryptococcal mastitis and the likelihood of underdiagnosis in the absence of routine mycological screening.

Table 2: Comparison of reproductive performance parameters between cryptococcal mastitis-positive and negative dairy cows

Parameter	Positive (n=90)	Negative (n=290)	Adjusted OR	p-value
Conception rate (%)	48.9	74.2	0.34	<0.001
Services per conception	3.6 ± 1.2	2.1 ± 0.7	—	<0.001
Calving-to-first-service (d)	84.2 ± 18.6	62.3 ± 12.4	—	<0.001
Calving-to-conception (d)	142.8 ± 32.4	108.4 ± 21.6	—	<0.001
Pregnancy rate (%)	54.4	78.3	0.38	<0.001
SCC (×1000 cells/mL)	842 ± 284	218 ± 96	—	<0.001

Table 2 confirms highly significant reproductive performance impairment in cryptococcal mastitis-positive cows across all evaluated parameters, even after adjustment for confounding variables including age, parity, body condition score, and concurrent bacterial mastitis. The adjusted odds ratio of 0.34 for conception indicates that cryptococcal mastitis-positive cows had approximately one-third the odds of conceiving compared with negative controls.

Discussion
The 23.7% prevalence of cryptococcal mastitis documented in Kenyan highland dairy herds substantially exceeds the 2–8% typically reported in temperate dairy systems, confirming the hypothesis that tropical environmental conditions and smallholder management practices create elevated risk for mycotic mammary gland colonization [6]. The predominance of *C. neoformans* var. *grubii* (61.1%) is consistent with the global epidemiology of cryptococcosis,

in which serotype A accounts for approximately 70% of clinical infections across mammalian species [7]. The substantial proportion of *C. gattii* (26.7%) is noteworthy, as this species has been increasingly recognized as a primary pathogen in tropical and subtropical regions rather than exclusively affecting immunocompromised hosts.

The significant association between cryptococcal mastitis and reproductive performance decline represents the central finding of this research. The conception rate differential of 25.3 percentage points (48.9% vs. 74.2%) and the 34.4-day extension in calving-to-conception interval translate to substantial economic losses in smallholder dairy systems where reproductive efficiency directly determines household income [8]. The mechanistic basis for this association likely involves multiple pathways: (1) chronic mammary inflammation drives systemic cytokine production (TNF- α , IL-1 β , IL-6) that disrupts hypothalamic-pituitary-gonadal axis function; (2) elevated prostaglandin synthesis associated with mastitis-induced inflammation may cause premature luteolysis and embryonic loss; (3) the metabolic energy cost of sustained immune activation redirects nutrients from reproductive function to immunological defense [9].

The persistent nature of cryptococcal mastitis, with 25.6% of positive animals remaining culture-positive across three or more consecutive months, distinguishes it from transient bacterial mastitis episodes and suggests that the reproductive impact may be cumulative and more severe than single-episode bacterial infections [10]. The polysaccharide capsule of *Cryptococcus* species confers resistance to phagocytic killing by mammary gland macrophages and neutrophils, enabling prolonged intracellular survival and chronic antigenic stimulation that perpetuates the inflammatory cascade.

One Health Perspective

The identification of both *C. neoformans* and *C. gattii* in bovine milk samples carries implications beyond animal health, as these pathogens can be transmitted to humans through consumption of unpasteurized milk, which remains a common practice in Kenyan smallholder dairy systems. While the infective dose for gastrointestinal cryptococcosis is not well established, the concentrations detected in positive milk samples (10^2 – 10^4 CFU/mL) are within the range considered potentially hazardous for immunocompromised individuals, including HIV-positive individuals who constitute approximately 4.9% of the Kenyan adult population.

Environmental Reservoir Analysis

Environmental sampling from the immediate surroundings of dairy facilities revealed *Cryptococcus* contamination in pigeon droppings (87.5% positive), eucalyptus bark and leaf litter (62.5% positive), stored poultry manure (50.0% positive), and soil beneath dairy sheds (37.5% positive). The proximity of pigeon roosting sites to milking areas was strongly correlated with herd-level cryptococcal mastitis prevalence (Spearman's $\rho = 0.78$, $p = 0.003$), identifying avian fecal contamination as the primary environmental risk factor amenable to management intervention.

Conclusion

This research aimed to determine the prevalence and reproductive impact of cryptococcal mastitis in tropical

highland dairy herds. The investigation revealed a 23.7% prevalence of *Cryptococcus* species in bovine milk, with significant seasonal variation peaking during the long rains period. Cryptococcal mastitis-positive cows exhibited markedly reduced conception rates (48.9% vs. 74.2%) and extended calving intervals (142.8 vs. 108.4 days). The practical message for tropical dairy practitioners is that routine mycological screening of persistently high-SCC quarters and exclusion of pigeon populations from dairy facility environs represent cost-effective interventions for reducing mycotic mastitis burden and its associated reproductive penalties. Future research should investigate the efficacy of antifungal intramammary formulations for cryptococcal mastitis treatment, the immunological mechanisms linking chronic mycotic mammary inflammation to ovarian dysfunction, and the development of rapid point-of-care diagnostic tests suitable for field deployment in resource-limited dairy systems.

Acknowledgements

The Nairobi Academy of Veterinary Biosciences provided laboratory facilities and mycological reference resources for this research. J.O.O. designed the research and supervised field activities; G.W.K. performed mycological identification and SCC analysis; P.K.R. conducted statistical analysis and reproductive data interpretation. Financial support was provided by the Kenya Agricultural and Livestock Research Organization (KALRO) through Research. The participating dairy cooperative societies are gratefully acknowledged for granting access to herds and breeding records.

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