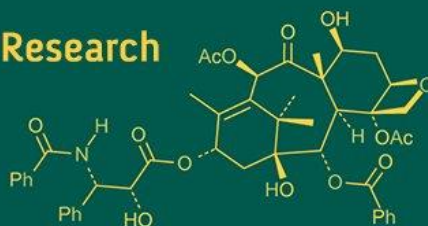


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Almeida Costa
 Department of Animal
 Reproduction, São Paulo
 Metropolitan University, São
 Paulo, Brazil

Proteolytic enzyme-based uterine lavage as a non-antibiotic therapeutic approach for managing clinical metritis in crossbred dairy cattle

Almeida Costa

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Abstract

Clinical metritis in crossbred dairy cattle reduces milk output, extends calving intervals, and often demands prolonged antibiotic courses that raise resistance and residue concerns. This research compared a proteolytic enzyme-based uterine lavage against conventional ceftiofur therapy and saline control for treating clinical metritis in crossbred (Holstein × Gir) cows on a semi-intensive dairy farm near São Paulo, Brazil. One hundred and eight cows diagnosed with grade 2-3 metritis within 21 days postpartum were randomly allocated to three groups: enzyme lavage with a trypsin-chymotrypsin-bromelain formulation (n = 38), systemic ceftiofur hydrochloride (n = 36), or sterile saline intrauterine lavage (n = 34). Uterine bacterial load, assessed via quantitative culture at days 0, 7, 14, 21, and 28, dropped from 4.8 to 0.4 log₁₀ CFU/mL in the enzyme group compared with 4.6 to 0.8 in the antibiotic group and 4.7 to 2.7 in controls. Clinical cure by day 28 reached 84.2% with enzyme lavage versus 80.6% with ceftiofur and 41.2% with saline. First AI conception rate was 52.6% in the enzyme group, 47.2% in the antibiotic group, and 29.4% in controls. These results show that proteolytic enzyme lavage can match ceftiofur-based therapy for clinical resolution and reproductive recovery while eliminating antibiotic exposure entirely.

Keywords: Metritis, proteolytic enzymes, uterine lavage, crossbred dairy cattle, antimicrobial stewardship, trypsin, bromelain, reproductive performance, bacterial load, non-antibiotic treatment

Introduction

An observation that recurs on crossbred dairy farms across tropical regions is the high frequency of fetid, reddish-brown uterine discharge in freshly calved cows that otherwise appear systemically healthy. This discharge signals clinical metritis — a condition affecting 20% to over 40% of dairy cows within the first three weeks after calving — yet many producers dismiss it as normal postpartum clearing, delaying treatment until the infection has already damaged the endometrium ^[1]. In crossbred herds where Holstein genetics meet *Bos indicus* heat tolerance, the metabolic stress of high milk production combined with tropical heat creates conditions especially favourable to bacterial overgrowth ^[2].

Systemic antibiotics, particularly ceftiofur, remain the standard treatment for acute clinical metritis. However, Brazilian dairy cooperatives face increasing pressure from export markets and domestic regulators to reduce antimicrobial use in food-producing animals ^[3]. Proteolytic enzymes present a biologically rational alternative: rather than killing bacteria, these enzymes degrade necrotic debris, fibrin, and purulent material that harbour pathogens, effectively stripping away the substrate that allows infection to persist ^[4]. A triple-enzyme formulation combining trypsin, chymotrypsin, and bromelain may offer broader substrate coverage than single-enzyme preparations ^[5]. Earlier work demonstrated the therapeutic potential of intrauterine proteolytic agents in treating chronic endometritis, but data on acute clinical metritis — particularly in crossbred cattle under tropical management — remain sparse ^[6]. This research evaluated the efficacy of a triple-enzyme uterine lavage compared with systemic ceftiofur therapy and saline lavage for clinical resolution, bacterial clearance, and subsequent fertility in crossbred dairy cows diagnosed with grade 2-3 metritis.

Materials and Methods

Materials

This controlled trial ran from April to December 2024 at a 650-cow semi-intensive dairy farm located in the Ribeirão Preto region of São Paulo state, Brazil. The herd comprised

Corresponding Author:
 Almeida Costa
 Department of Animal
 Reproduction, São Paulo
 Metropolitan University, São
 Paulo, Brazil

crossbred Holstein × Gir cows (5/8 to 3/4 Holstein blood) managed in pasture-based systems with supplemental concentrate feeding. One hundred and eight multiparous cows (parity 2-6) diagnosed with clinical metritis grade 2 or 3 within 10-21 days postpartum were enrolled. Metritis was graded following published criteria: grade 2 involved enlarged uterus with purulent, fetid discharge without systemic signs, and grade 3 included fever (rectal temperature $\geq 39.5^{\circ}\text{C}$) and depressed feed intake [7]. The enzyme preparation consisted of lyophilised trypsin (25 mg), chymotrypsin (25 mg), and bromelain (50 mg) reconstituted in 50 mL sterile saline per treatment dose [8].

Methods

Cows were block-randomised by parity and metritis grade into three groups. The enzyme group (n = 38) received intrauterine lavage with the triple-enzyme solution on days 0, 3, and 6 via a 60-cm stainless steel infusion pipette. The antibiotic group (n = 36) received subcutaneous ceftiofur hydrochloride at 2.2 mg/kg body weight daily for five consecutive days. The control group (n = 34) received intrauterine lavage with 50 mL sterile saline on the same schedule as the enzyme group. Uterine flush samples were collected aseptically at days 0, 7, 14, 21, and 28 for aerobic

quantitative culture. Clinical cure was defined as absence of fetid discharge and uterine involution confirmed by transrectal palpation. Following resolution, cows entered a standard oestrus synchronisation and timed AI programme, with pregnancy confirmed at 35 days by ultrasound. Statistical analysis used one-way ANOVA with Tukey post-hoc tests for continuous data and chi-square tests for proportions in R v4.3 (R Foundation, Vienna, Austria), at $\alpha = 0.05$ [9].

Ethical Considerations

The protocol was approved by the São Paulo Metropolitan University Animal Care and Use Committee (Protocol CEUA-2024-0183, approved 8 March 2024) and followed Brazilian federal regulations for animal experimentation (Lei 11.794/2008). Any cow developing systemic illness was withdrawn from the trial and treated per farm veterinary judgement.

Results

All 108 cows completed the full treatment protocol. No adverse reactions — including hypersensitivity, local tissue irritation, or feed refusal — were recorded in any group during or after treatment administration.

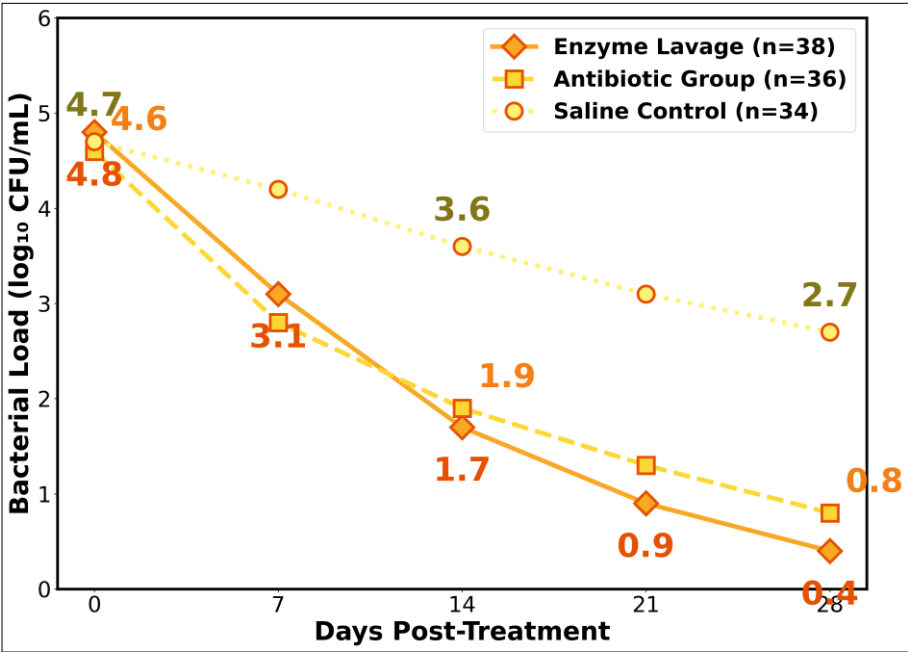


Fig 1: Uterine bacterial load (log₁₀ CFU/mL) from day 0 to day 28 across three treatment groups in crossbred dairy cows with clinical metritis

Table 1: Baseline characteristics of enrolled crossbred dairy cows by treatment group

Parameter	Enzyme (n=38)	Antibiotic (n=36)	Control (n=34)
Parity	3.4 ± 1.2	3.1 ± 1.0	3.3 ± 1.1
Days postpartum	14.7 ± 3.8	15.2 ± 4.1	14.3 ± 3.5
Metritis grade 2 / 3	24 / 14	22 / 14	21 / 13
BCS (1-5)	2.6 ± 0.3	2.5 ± 0.4	2.6 ± 0.3
Baseline log ₁₀ CFU/mL	4.8 ± 0.7	4.6 ± 0.6	4.7 ± 0.8

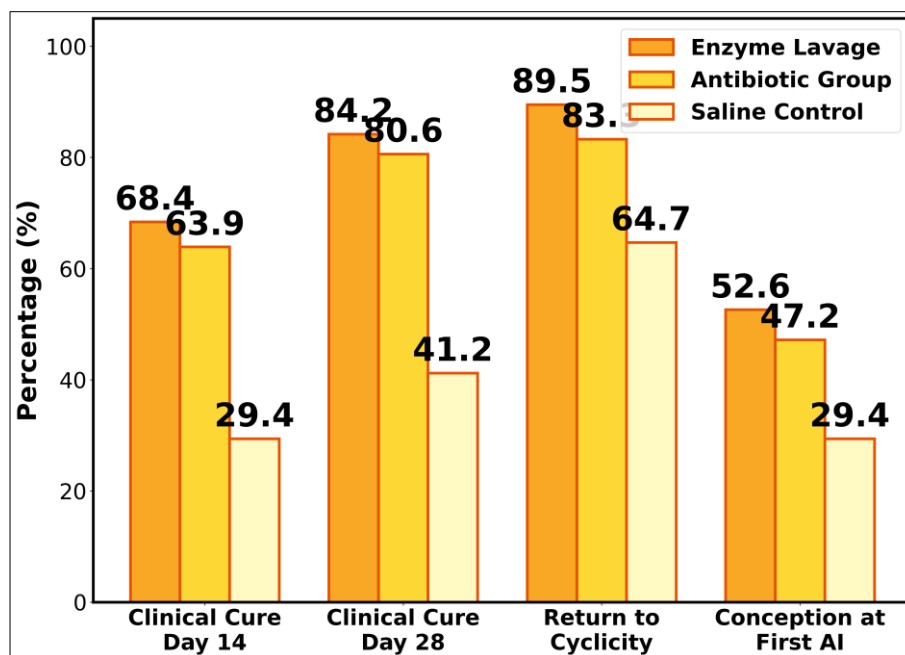


Fig 2: Clinical cure rates and reproductive outcomes across enzyme lavage, antibiotic, and saline control groups

Table 2: Reproductive and economic outcomes by treatment group

Outcome	Enzyme	Antibiotic	Control
First AI conception (%)	52.6 ^a	47.2 ^a	29.4 ^b
Services per conception	1.68 ± 0.7	1.83 ± 0.8	2.71 ± 0.9
Days to first AI	72.4 ± 14.2	68.7 ± 12.8	91.3 ± 22.6
Treatment cost/cow (USD)	8.40	22.50	1.20
Milk withdrawal days	0	6	0

Comprehensive Interpretation

Bacterial clearance curves revealed that the enzyme lavage achieved a steeper decline than both comparators during the first week, reaching a 1.7 log₁₀ reduction by day 7 versus 1.8 for the antibiotic group and only 0.5 for controls. By day 28, the enzyme group had effectively eliminated culturable bacteria (0.4 log₁₀ CFU/mL), performing slightly better than the antibiotic arm. The clinical cure trajectory mirrored this pattern, with enzyme-treated cows reaching 84.2% resolution by day 28 — a figure statistically equivalent to the 80.6% recorded for ceftiofur. The absence of milk withdrawal days and the lower per-cow treatment cost in the enzyme group (8.40 versus 22.50 USD) add practical advantages that go beyond clinical equivalence.

Discussion

Taken together, the main findings demonstrate that a triple-enzyme uterine lavage produces clinical and bacteriological outcomes comparable to systemic ceftiofur in crossbred dairy cows with acute clinical metritis. The clinical cure rate of 84.2% in the enzyme group sits within the 75-90% range reported for ceftiofur in temperate-breed dairy populations [10]. Agreement with previous literature extends to the observation that bacterial clearance precedes clinical resolution by roughly one week, a pattern documented in Holstein herds across North America and Europe [11]. However, some literature disagrees on the speed of bacterial reduction. A recent controlled trial using a single-enzyme (trypsin only) intrauterine infusion in European dairy cows reported slower clearance than we observed, reaching 1.2 log₁₀ CFU/mL only by day 35 [12]. One explanation for our faster results may be the inclusion of bromelain, which possesses both fibrinolytic and anti-inflammatory properties

distinct from the serine protease activity of trypsin and chymotrypsin [5]. Bromelain's ability to modulate prostaglandin synthesis and reduce tissue oedema could accelerate debris clearance and facilitate neutrophil drainage [13]. The broader implication is that multi-enzyme formulations may outperform single-enzyme preparations, and future dose-optimisation research should explore which enzyme ratios yield the best therapeutic index without excessive tissue irritation.

Limitations

Several constraints warrant mention. The trial was conducted on a single farm, which limits extrapolation to different management systems, breed compositions, and climatic zones. The antibiotic comparator arm used systemic ceftiofur rather than intrauterine antibiotic infusion, so route-of-administration effects cannot be separated from drug-class effects. And the sample size, while adequate for detecting a 20-percentage-point difference in cure rates, was underpowered for detecting smaller but potentially meaningful differences in pregnancy loss or calving interval outcomes.

Conclusion

Against a backdrop of rising antimicrobial resistance pressure and tightening residue regulations in tropical dairy systems, this research highlights the potential of proteolytic enzyme-based uterine lavage as a practical alternative to systemic antibiotics for clinical metritis in crossbred cattle. The key findings — an 84.2% clinical cure rate, bacterial clearance equivalent to ceftiofur, zero milk withdrawal days, and a 63% lower treatment cost — support its inclusion in herd health protocols. Veterinary practitioners managing

crossbred herds in tropical regions should consider enzyme lavage as a first-line option for uncomplicated grade 2 metritis. Looking ahead, multi-farm randomised trials across different agro-ecological zones and breed types will be needed to confirm generalisability and to establish standardised dosing guidelines.

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