









Case Study

Coliform bacteria as dominant pathogens of bovine mastitis in arid dairy systems: A case study

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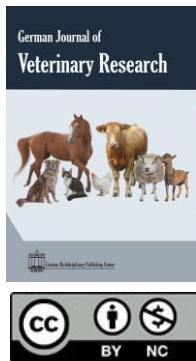
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Abstract

Bovine mastitis remains a significant barrier to dairy production globally, especially in arid regions where climate adaptation strategies may unintentionally facilitate pathogen proliferation. In the United Arab Emirates (UAE), intensive dairy operations utilize evaporative cooling, resulting in humid microenvironments that can favor environmental mastitis pathogens. This study aimed to characterize bacterial pathogens associated with clinical and subclinical mastitis on large-scale dairy farms in the UAE under arid conditions and to evaluate their zoonotic potential. A cross-sectional survey was conducted in two major dairy farms in Abu Dhabi from May to June 2023. Researchers collected 442 milk samples from cows diagnosed with clinical and subclinical mastitis. Bacterial isolation was performed on blood and MacConkey agar, and identification was performed using the VITEK 2® system. Data analysis included descriptive statistics and chi-square tests. In total, 113 bacterial isolates representing 30 species were identified. Gram-negative bacteria were predominant (63.7%), with *Escherichia coli* (38.9%) and *Klebsiella pneumoniae* (7.1%) as the most frequent species. Among Gram-positive isolates, *Enterococcus gallinarum* (5.4%) and *Staphylococcus aureus* (4.5%) were most common. Importantly, 60% of species and 77.9% of isolates exhibited documented zoonotic potential. Pathogen diversity did not differ significantly between clinical and subclinical cases of mastitis. Coliform bacteria were the dominant mastitis pathogens in UAE dairy farms, likely due to the humid conditions produced by evaporative cooling systems. The high prevalence of zoonotic species highlights the necessity for integrated One Health strategies in mastitis control. Future research should include antimicrobial resistance profiling and molecular diagnostics to enhance understanding of pathogen dynamics and associated public health risks.

Keywords: Arid climate, Bovine mastitis, Coliforms, Dairy cattle, Gram-negative bacteria, UAE, Zoonosis

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Introduction

Bovine mastitis presents a persistent challenge with substantial economic, animal welfare, and public health consequences. The condition leads to decreased milk yield and quality, increased veterinary expenses, premature culling, and extensive use of antimicrobials (Gonzalez et al., 1986; Gomes et al., 2016; Giraudo et al., 1997). The global economic impact is estimated at USD

35 billion annually, with milk production losses comprising approximately 70% of this total (Hogeveen et al., 2019). Clinical mastitis is characterized by visible udder inflammation and altered milk, whereas subclinical mastitis, which is often asymptomatic, is more prevalent and causes greater cumulative harm due to undetected chronic inflammation and elevated somatic cell counts (Bradley 2002; Halasa et al., 2007; Zhao and Lacasse, 2008). Both clinical and subclinical forms impair reproductive performance and raise public health concerns through the transmission of foodborne pathogens and the development of antimicrobial resistance (Deluyker et al., 1993; Taylor et al., 2025).

Mastitis is predominantly caused by bacteria and is generally classified as either contagious or environmental. Contagious mastitis is transmitted during milking and is associated with pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Zeng et al., 2023; Schreiner and Ruegg, 2002). In contrast, environmental mastitis is caused by opportunistic pathogens, particularly coliforms, including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., which proliferate in bedding, manure, water, and soil (Gonzalez et al., 1986; Bradley, 2002). The incidence of environmental mastitis is strongly influenced by climate and hygiene conditions and is often more severe, frequently resulting in systemic illness and mortality (Garcia, 2004). In the United Arab Emirates (UAE), the expansion of the dairy sector under the national food security agenda has led to the adoption of intensive farming systems that rely on evaporative cooling. Although these systems enhance cow comfort in extreme desert climates, they also create a persistently wet and humid environment that may promote coliform growth. Despite the sector's growth, epidemiological data on mastitis within UAE dairy systems remain scarce. A retrospective study reported a high incidence of clinical mastitis, reaching up to 49 cases per 1,000 cows per month (Zeinhom et al., 2016), although the causative organisms were not identified. The present study seeks to characterize mastitis pathogens in intensively managed dairy farms in arid ecosystems, thereby establishing a baseline for surveillance and informing evidence-based control strategies.

Materials and methods

Study setting

The study was conducted at two large-scale dairy farms in the Eastern region of the Emirate of Abu Dhabi, UAE. These farms, separated by two kilometers, maintain herds of approximately 6,000 and 4,000 lactating Holstein-Friesian cows, respectively. Both facilities employ advanced milking technologies, including automated milking parlors and individual cow monitoring systems based on somatic cell count (SCC). Cows are milked four times daily and receive a total mixed ration (TMR) formulated from globally sourced feed ingredients, which are routinely analyzed using near-infrared spectroscopy. To mitigate extreme heat during the summer months, the barns are equipped with evaporative cooling systems (Figure 1), which maintain internal temperatures at approximately 22°C even when external temperatures reach 50°C. Although effective in enhancing animal comfort, this system increases humidity and may facilitate the proliferation of environmental pathogens such as coliforms (Gonzalez et al., 1986; Klaas and Zadoks, 2018).

Study design

A cross-sectional study was conducted from May to June 2023 on two large dairy farms in the Eastern region of Abu Dhabi, UAE, with herds of approximately 6,000 and 4,000 Holstein-Friesian cows. One-fourth of the cows calving during the study period were purposively sampled. The study included 40 cows with clinical mastitis and 402 cows screened for subclinical mastitis. The absence of healthy controls is a noted limitation.

Case identification and sample collection

Clinical mastitis was identified by physical examination of the udder and observation of abnormal milk. Subclinical mastitis was detected using the California Mastitis Test (CMT) according to established protocols (Hogan and Larry Smith, 2003). In total, 442 milk samples were collected aseptically.

Bacteriological isolation and identification

Bacterial isolation followed National Mastitis Council guidelines (Schukken et al., 2012). From each selected quarter, approximately 10 mL of milk was aseptically collected into sterile, screw-capped bottles after discarding the initial streams. Teats were cleaned and disinfected with 70% ethanol (VWR Chemicals, France) before sampling. Samples were transported chilled to the bacteriology laboratory at the Department of

Veterinary Medicine, UAE University. Each milk sample (0.01 mL) was inoculated onto 5% sheep blood agar and MacConkey agar (HIMEDIA, India) to recover Gram-positive and Gram-negative organisms, respectively. Plates were incubated (memert incubator Germany) aerobically at 37 °C for 18 to 48 hours. Mixed cultures were differentiated, and pure colonies were sub-cultured onto nutrient agar slants. For identification, isolates were processed using the automated VITEK 2 Compact system (bioMérieux, France). Bacterial suspensions were adjusted to a 0.50 to 0.63 McFarland turbidity standard, and Gram-positive or Gram-negative

cards were used according to manufacturer instructions. Identification relied on colorimetric and turbidity changes interpreted by the integrated database. Species diversity was assessed using species richness and the Shannon diversity index.

Data analysis

Data were analyzed using SPSS version 29. Descriptive statistics and chi-square tests ($p < 0.05$) were applied. Confounders such as parity, lactation stage, and hygiene practices were not adjusted for, which is acknowledged as a limitation.

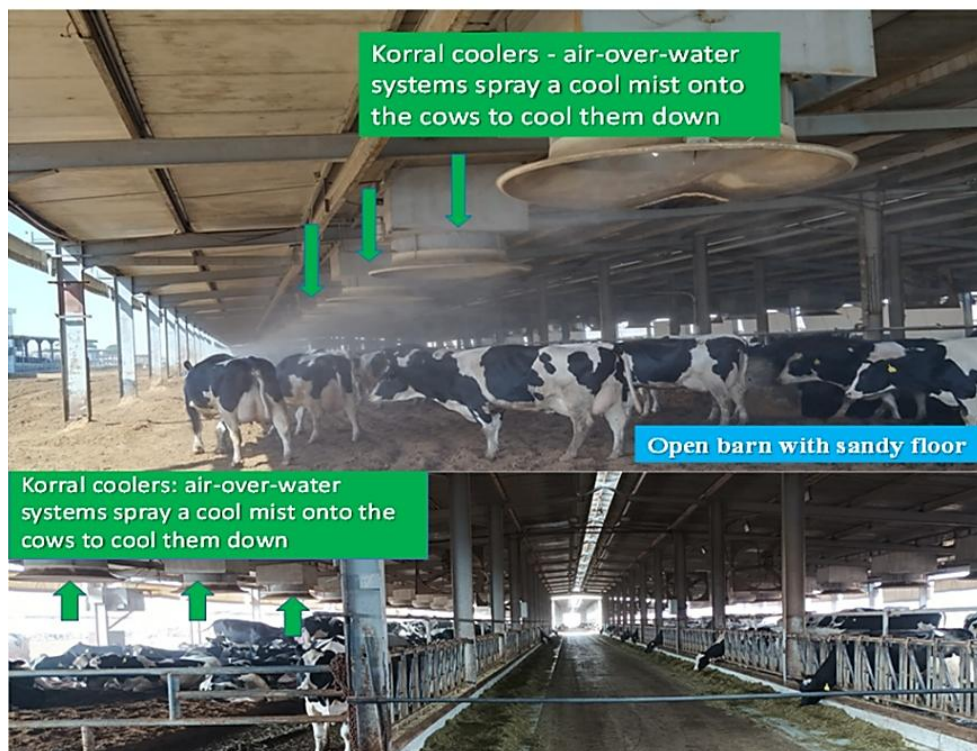


Figure 1: One of the barns of a dairy farm in the United Arab Emirates. The sides of the barns are open, the floor is sandy, and the barns are equipped with Korral coolers, which spray cool, moist air onto the cows and the floor, which is conducive for replication of coliforms.

Results

Overall bacterial profile

From 442 milk samples, 113 bacterial isolates were obtained (55 clinical, 58 subclinical) (Table 1). Gram-negative bacteria predominated (63.7%, $n = 72$) compared with Gram-positive bacteria (36.3%, $n = 41$), a statistically significant difference ($\chi^2 = 16.89$, $p < 0.0001$).

Subclinical mastitis

Among 402 cows screened, 66 (16.4%) were CMT-positive. From these, 58 isolates representing 22 species were recovered. Gram-negatives slightly

exceeded Gram-positives (51.8% vs. 48.2%). *E. coli* (36.2%) was the most frequent species, followed by *K. pneumoniae* (5.2%) and *Enterococcus gallinarum* (10.3%) (Table 2).

Clinical mastitis

From 40 cows with clinical mastitis, 55 isolates were identified, spanning 16 species (Table 3). Gram-negative organisms predominated (76.4%). *E. coli* (42%) was the most common, while *K. pneumoniae* and *S. marcescens* each accounted for 10.8%. Gram-positive isolates included *Streptococcus agalactiae* and *Streptococcus uberis* (6.5% each).

Table 1: Species of bacteria ($n = 58$) isolated from sub-clinical bovine mastitis at the study farm in the UAE.

Gram-positive	Frequency	Gram-negative	Frequency
<i>Enterococcus gallinarum</i>	6 (10.3%)		
<i>Staphylococcus aureus</i>	3 (5.2%)	<i>Escherichia coli</i>	21 (36.2%)
<i>Staphylococcus lentus</i>	3 (5.2%)	<i>Klebsiella pneumoniae</i>	3 (5.2%)
<i>Streptococcus thoraltensis</i>	3 (5.2%)	<i>Klebsiella oxytoca</i>	2 (3.4%)
<i>Staphylococcus hominis</i> spp. <i>hominis</i>	2 (3.4%)	<i>Serratia marcescens</i>	2 (3.4%)
<i>Staphylococcus pseudintermedius</i>	2 (3.4%)	<i>Rauotella ornithinolytica</i>	1 (1.7%)
<i>Aerococcus viridans</i>	1 (1.7%)	<i>Rauotella planticola</i>	1 (1.7%)
<i>Gardnerella vaginalis</i>	1 (1.7%)	<i>Pseudomonas fluorescens</i>	1 (1.7%)
<i>Kocuria kristinea</i>	1 (1.7%)		
<i>Staphylococcus chromogens</i>	1 (1.7%)		
<i>Staphylococcus gallinarum</i>	1 (1.7%)		
<i>Staphylococcus haemolyticus</i>	1 (1.7%)		
<i>Staphylococcus saprophyticus</i>	1 (1.7%)		
<i>Staphylococcus warneri</i>	1 (1.7%)		
<i>Staphylococcus xylosum</i>	1 (1.7%)		
	28 (48.3%)		30 (51.7 %)

Table 2: Bacterial species (n = 55) isolated from clinical mastitis cases in dairy cattle at the study farms in the UAE.

Gram-positive	Frequency	Gram-negative	Frequency
<i>Streptococcus agalactiae</i>	3 (6.5%)	<i>Escherichia coli</i>	23 (42%)
<i>Streptococcus uberis</i>	3 (6.5%)	<i>Klebsiella pneumoniae</i>	5 (10.8%)
<i>Staphylococcus aureus</i>	2 (4.3%)	<i>Serratia marcescens</i>	5 (10.8%)
<i>Staphylococcus lentus</i>	2 (4.3%)	<i>Klebsiella oxytoca</i>	3 (6.5%)
<i>Staphylococcus sciuri</i>	1 (2.1%)	<i>Citrobacter freundii</i>	2 (4.3%)
<i>Streptococcus parasanguinis</i>	1 (2.1%)	<i>Serratia ficaria</i>	1 (2.1%)
<i>Staphylococcus pseudintermedius</i>	1 (2.1%)	<i>Sphigomonas paucimolis</i>	1(2.1%)
		<i>Rautella ornithinolytica</i>	1 (2.1%)
		<i>Rhizobium radiobacter</i>	1 (2.1%)
	13 (23.6%)		42 (76.4%)

Table 3: Bacteria species isolated from both clinical and subclinical mastitis in dairy cattle.

Gram-positive	Frequency	Gram-negative	Frequency
<i>Staphylococcus aureus</i>	5 (6.6%)	<i>Escherichia coli</i>	44 (57.9%)
<i>Staphylococcus lentus</i>	3 (3.9%)	<i>Klebsiella pneumoniae</i>	8 (10.5%)
<i>Staphylococcus pseudintermedius</i>	2 (2.6%)	<i>Serratia marcescens</i>	7 (9.2%)
		<i>Klebsiella oxytoca</i>	5 (6.6%)
		<i>Rautella ornithinolytica</i>	2 (2.6)
Sub-total	10 (13.2%)		66 (86.8)

Comparative findings

In total, 30 bacterial species were identified: 16 associated with clinical mastitis, 22 with subclinical mastitis, with 8 species common to both. Diversity, assessed by species richness and Shannon index, did not differ significantly between clinical and subclinical cases ($\chi^2 = 1.87$, $p = 0.17$). Of the 113 total isolates, 76 (67.3%) were shared between mastitis forms, predominantly Gram-negative (86.8%). *E. coli*

(57.9%), *K. pneumoniae* (10.5%), and *S. marcescens* (9.2%) were the most frequent, whereas *S. aureus* was the leading Gram-positive (6.6%).

Zoonotic potential

Overall, 18 of 30 species (60%) and 88 of 113 isolates (77.9%) had documented zoonotic potential (Table 4), including *E. coli*, *K. pneumoniae*, *S. marcescens*, and *E. gallinarum*. These findings highlight potential public health

risks associated with consuming raw milk controls without adequate heat treatment or hygiene

Table 4: Bacterial species with zoonotic potential isolated from bovine clinical and subclinical mastitis in the UAE.

Bacterial species	Number (percent)	Gram stain	Subclinical mastitis	Clinical mastitis
Gram-negative				
<i>Escherichia coli</i> (if EHEC) *	44 (50.0)	Gram-negative	Yes	Yes
<i>Klebsiella pneumoniae</i>	8 (9.1)	Gram-negative	Yes	Yes
<i>Serratia marcescens</i>	7 (8.0)	Gram-negative	Yes	Yes
<i>Klebsiella oxytoca</i>	5 (5.7)	Gram-negative	Yes	Yes
<i>Citrobacter freundii</i>	2 (2.3)	Gram-negative	No	Yes
Subtotal	66 (75.0)			
Gram-positive				
<i>Enterococcus gallinarum</i>	6 (6.8)	Gram-positive	Yes	No
<i>Streptococcus thoraltensis</i>	3 (3.4)	Gram-positive	Yes	No
<i>Staphylococcus hominis</i>	2 (2.3)	Gram-positive	Yes	No
<i>Staphylococcus pseudintermedius</i>	2 (2.3)	Gram-positive	Yes	Yes
<i>Aerococcus viridanis</i>	1 (1.1)	Gram-positive	Yes	No
<i>Gardnerella vaginalis</i>	1 (1.1)	Gram-positive	Yes	No
<i>Staphylococcus haemolyticus</i>	1 (1.1)	Gram-positive	Yes	No
<i>Staphylococcus sciuri</i>	1 (1.1)	Gram-positive	No	Yes
<i>Streptococcus parasanguinis</i>	1 (1.1)	Gram-positive	No	Yes
<i>Staphylococcus xylosus</i>	1 (1.1)	Gram-positive	Yes	No
<i>Staphylococcus gallinarum</i>	1 (1.1)	Gram-positive	Yes	No
<i>Staphylococcus warneri</i>	1 (1.1)	Gram-positive	Yes	No
<i>Staphylococcus saprophyticus</i>	1 (1.1)	Gram-positive	Yes	No
Subtotal	22 (25.0)			
Total	88/113 (77.9%)			

*EHEC: Enterohemorrhagic *Escherichia coli*

Discussion

This study offers detailed insights into the bacterial causes of bovine mastitis in UAE dairy farms, where dairy production is central to national food security. Coliforms, especially *E. coli*, *K. pneumoniae*, and *S. marcescens*, were predominant, differing from temperate regions where contagious pathogens like *S. aureus* and *S. agalactiae* are more common (Zeng et al., 2023; Shum et al., 2009; Zadoks and Fitzpatrick, 2009). This difference likely results from climate and management practices, particularly the use of evaporative cooling systems. While essential for reducing heat stress, these systems create humid conditions that promote coliform growth (Gonzalez et al., 1986; Bradley, 2002; Zeinhom et al., 2016). These findings are consistent with studies from other hot climates linking environmental mastitis to poor hygiene and high moisture (Klaas and Zadoks, 2018).

The consistently high recovery of *E. coli* across

both clinical and subclinical cases underscores its environmental persistence and adaptability to diverse intramammary infection dynamics. Previous studies have documented that cows in intensive systems can develop partial immune adaptation, leading to subclinical infections persisting alongside acute clinical cases (Hogan and Larry Smith, 2003). This dual presence highlights the challenge of controlling *E. coli*, which can survive in bedding, manure, water troughs, and milking equipment. The high prevalence observed in the UAE adds to global evidence that *E. coli* is a dominant environmental mastitis pathogen in intensive dairy production systems.

K. pneumoniae and *S. marcescens* were also significant pathogens. *Klebsiella* species are associated with bedding materials, feed, and water (Massé et al., 2020; Taniguchi et al., 2021). A recent meta-analysis identified *Klebsiella* as a leading Gram-negative mastitis agent globally

(Song et al., 2023), supporting its importance in both clinical and subclinical cases in the UAE. *S. marcescens*, though less frequent, has been linked to outbreaks from contaminated teat dips and poorly maintained milking systems (Schukken et al., 2012). Its detection here, especially in clinical cases, aligns with reports from Europe and North America (Katholm et al., 2012), indicating a broader distribution. However, the lack of environmental sampling in this study limits confirmation of sources and should be addressed in future research.

Gram-positive organisms, though less frequent, add important context. *S. aureus* and *S. agalactiae* were isolated as Gram-positive organisms, though less common, and provide important context. *S. aureus* and *S. agalactiae* were found at low levels, likely due to improved milking hygiene and teat disinfection. This supports literature on the effectiveness of control programs for contagious mastitis pathogens (Taylor et al., 2025; Schreiner and Ruegg, 2002). The detection of *S. uberis* is notable because of its dual epidemiological role. While traditionally considered environmental, recent evidence suggests it may also be transmitted contagiously (Cattell, 1996). Its presence in clinical cases highlights the need for molecular studies to distinguish transmission routes, predominantly from subclinical mastitis. Increasingly, CoNS are recognized as emerging bovine mastitis pathogens (Dahms et al., 2014; Pyörälä and Taponen, 2009). While once dismissed as minor contaminants, many species now feature prominently in intramammary infections and human nosocomial disease, often exhibiting antimicrobial resistance (Marshall et al., 1998; Huber et al., 2011). Their occurrence in dairy herds, therefore, warrants close surveillance. Likewise, *Enterococcus gallinarum*, detected in several cases, is clinically important. It has been associated with nosocomial infections and carries intrinsic resistance to vancomycin (Hayes et al., 2004; Osterås et al., 2006). Its presence in milk poses food safety risks when raw milk is consumed, especially given the rising global concern about the dissemination of antimicrobial resistance.

The zoonotic potential of mastitis pathogens identified here is considerable. Sixty percent of species and nearly 78% of isolates were of recognized or probable zoonotic significance (Hayes et al., 2004; Murinda et al., 2019; Navon-Venezia et al., 2017). These include *E. coli*, *K.*

pneumoniae, *S. marcescens*, and *E. gallinarum*. While this study did not assess virulence factors such as Shiga toxin genes, previous reports have documented STEC in milk from mastitic cows in the Middle East and Africa (Murinda et al., 2019). Hence, zoonotic claims remain literature-based and speculative, yet they highlight the risk of consuming raw milk. The lack of antimicrobial resistance testing represents a critical gap, as resistance patterns greatly influence both veterinary treatment outcomes and public health risk.

Overall, these results demonstrate that mastitis in arid climates, such as those in the UAE, is shaped by unique environmental and management pressures. Evaporative cooling, while mitigating heat stress, fosters coliform persistence; improved milking hygiene reduces traditional contagious pathogens but shifts the profile toward environmental agents. This interplay emphasizes the importance of tailoring mastitis control programs to local contexts. Beyond routine hygiene, emphasis should be placed on bedding management, water system maintenance, and regular culture of milking equipment to reduce Gram-negative prevalence. Future research should also incorporate antimicrobial susceptibility testing, molecular typing, and environmental surveillance to provide a more complete One Health perspective on mastitis in desert dairy systems.

While this study provides valuable baseline data, several methodological limitations should be noted. Bacterial identification was performed using the VITEK 2® Compact system, which, although widely accepted, may lack the precision of more advanced approaches such as MALDI-TOF mass spectrometry or 16S rRNA sequencing. Future work integrating these confirmatory tools would enhance taxonomic resolution and strengthen confidence in species-level identification. Similarly, antimicrobial susceptibility testing was not undertaken, which restricts direct assessment of resistance patterns and zoonotic transmission risks. Including AMR profiling in follow-up studies would provide critical insight into therapeutic options for mastitis and inform One Health risk assessments. In addition, the purposive sampling design, the absence of healthy controls, and reliance on descriptive and unadjusted analyses limit causal inference. Despite these constraints, the study establishes an important epidemiological benchmark for mastitis in arid,

intensively managed dairy systems and lays the foundation for future, more comprehensive molecular and resistance-focused investigations.

Conclusion

This study provides important baseline epidemiological data on mastitis pathogens in arid, intensively managed dairy systems in the UAE. The predominance of coliform bacteria, particularly *E. coli* and *K. pneumoniae*, reflects how climate adaptation strategies such as evaporative cooling shape pathogen ecology. These findings carry direct implications for mastitis control, underscoring the need to complement conventional hygiene measures with enhanced bedding, water, and equipment management practices. At the same time, limitations in bacterial identification and the absence of antimicrobial resistance testing constrain the depth of inference. Future research should integrate confirmatory identification methods, AMR profiling, and molecular typing to clarify zoonotic risks and resistance dynamics. Despite these constraints, the study establishes a valuable benchmark for understanding mastitis in desert dairy systems and lays the groundwork for targeted One Health surveillance and intervention strategies that will be critical for safeguarding animal health, milk safety, and public health in the region.

Article Information

Animal ethics and consent to participate declarations. The authors confirm that the journal's ethical policies, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the milk samples were collected by the farm's personnel as part of their routine bacterial check-up of milk by the laboratory of the farm.

Data availability. Data used for the preparation of this manuscript have can be provided by the corresponding upon reasonable request

Conflict of interest. The authors declare no conflict of interest.

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Authors' contribution. AZ, MMOA, HAAA, FAAA, MNKA collected samples and conducted laboratory investigations. AZ and GA supervised students and drafted the manuscript. TH collected samples and edited the manuscript. TM, OKD, TS and MT edited the manuscript.

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