Improving Aetiological Diagnosis of Travellers' Diarrhoea Using Multiplex PCR Gastrointestinal Panels: A Retrospective Cross-Sectional Study from a High-Travel Tropical Setting

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ABSTRACT

Background: *Travellers' diarrhoea is a leading cause of acute gastroenteritis (AGE) worldwide. In Indonesia, diagnosis still relies largely on conventional stool microscopy despite its limitations, while access to multiplex PCR gastrointestinal (GI) panels remains limited. This study provides an early comparison between multiplex PCR and routine stool microscopy for AGE in a high-travel setting in Indonesia.*

Methods: *A retrospective cross-sectional study of patients presenting with AGE was conducted at a private international hospital in Bali (January 2024-June 2025). All patients underwent stool microscopy and multiplex PCR GI panel testing. Descriptive, bivariate, and multivariable analyses assessed patient characteristics, pathogen distribution, and associations with microscopy findings.*

Results: *A total of 114 patients were included (mean age 36.1 ± 14.4 years; 55.3% male). Multiplex PCR detected bacterial pathogens in 88.6% of cases, viral pathogens in 41.2%, and parasitic pathogens in 3.5% of cases. The most common bacteria were enteropathogenic Escherichia coli (47.4%), enteroaggregative E. coli (28.1%), and Campylobacter spp. (26.3%), while norovirus (11.4%) predominated among viruses. Routine stool microscopy parameters showed no meaningful association with bacterial or viral detection. Importantly, erythrocytes were independently associated with lower odds of viral infection (AOR 0.09; 95% CI 0.01–0.71) and higher odds of parasitic infection (AOR 8.16; 95% CI 1.05–63.41).*

Conclusion: *Multiplex PCR substantially improves pathogen detection and clarifies the microbial profile of travellers' diarrhoea in Bali. Although stool microscopy has limited diagnostic value, the presence of erythrocytes may provide a useful interpretive clue when molecular diagnostics are unavailable.*

Keywords: *Acute Gastroenteritis (AGE), Multiplex PCR, Stool Microscopy, Travellers' Diarrhoea*

ABSTRAK

Latar Belakang: *Diare wisatawan merupakan salah satu penyebab utama gastroenteritis akut (GEA) di seluruh dunia. Di Indonesia, praktik diagnostik masih didominasi oleh pemeriksaan mikroskopis feses konvensional meskipun memiliki berbagai keterbatasan, sementara akses terhadap panel gastrointestinal (GI) berbasis multiplex PCR masih terbatas. Penelitian ini memberikan perbandingan awal antara multiplex PCR dan mikroskopi feses rutin pada kasus GEA di wilayah Indonesia dengan tingkat perjalanan internasional yang tinggi.*

Metode: *Studi potong lintang retrospektif dilakukan pada pasien yang datang dengan GEA di sebuah rumah sakit swasta internasional di Bali pada periode Januari 2024–Juni 2025. Seluruh pasien menjalani pemeriksaan mikroskopis feses dan panel GI multiplex PCR. Analisis deskriptif, bivariat, dan multivariat digunakan untuk*

menilai karakteristik pasien, distribusi patogen, serta hubungan antara temuan mikroskopi dan kategori patogen.

Hasil: *Sebanyak 114 pasien diikutsertakan (usia rata-rata 36,1 ± 14,4 tahun; 55,3% laki-laki). Multiplex PCR mendeteksi patogen bakteri pada 88,6% kasus, virus pada 41,2%, dan parasit pada 3,5%. Patogen bakteri yang paling sering ditemukan adalah* Enteropathogenic Escherichia coli *(47,4%),* Enteroaggregative E. coli *(28,1%), dan* Campylobacter *spp. (26,3%), sedangkan norovirus (11,4%) merupakan etiologi virus tersering. Parameter mikroskopi feses rutin tidak menunjukkan hubungan bermakna dengan deteksi bakteri maupun virus. Namun, keberadaan eritrosit berhubungan secara independen dengan kemungkinan infeksi virus (AOR 0,09; 95% CI 0,01–0,71) yang lebih rendah dan infeksi parasit yang lebih tinggi (AOR 8,16; 95% CI 1,05–63,41).*

Kesimpulan: *Multiplex PCR secara signifikan meningkatkan deteksi patogen dan memperjelas profil mikroba diare wisatawan di Bali. Meskipun mikroskopi feses memiliki nilai diagnostik terbatas, temuan eritrosit dapat menjadi petunjuk interpretatif yang bermanfaat ketika pemeriksaan molekuler tidak tersedia.*

Kata kunci: *Gastroenteritis Akut (GEA), Multiplex PCR, Mikroskopi Feses, Diare Wisatawan*

INTRODUCTION

Travellers' diarrhoea (TD) remains the most common travel-related illness globally, affecting up to 40% of international travellers and typically presenting within the first week of travel.[1] Although often self-limiting, TD can substantially disrupt itineraries and may lead to longer-term sequelae such as post-infectious irritable bowel syndrome, reactive arthritis, and Guillain–Barré syndrome.[1,2] The aetiology of TD is diverse: rotavirus A is a major cause in young children, while adults and travellers are more frequently affected by bacterial pathogens such as diarrhoeagenic *Escherichia coli* and *Campylobacter spp*.[3] Accurately identifying these pathogens is therefore essential for appropriate management and antimicrobial stewardship.

In many low- and middle-income countries, including Indonesia, conventional stool microscopy and culture remain the primary diagnostic modalities for acute diarrhoea. Despite their accessibility, these methods have well-recognised limitations: microscopy has modest sensitivity and high observer variability, while culture requires viable organisms, specialised laboratory capacity, and prolonged turnaround times.[3] In practice, these constraints often lead to empirical antibiotic prescribing. Evidence from Indonesian hospitals suggests that fewer than half of diarrhoeal cases receive rational antibiotic therapy, reflecting an ongoing challenge in mitigating antimicrobial resistance (AMR).[4] This underscores the need for diagnostic approaches that provide faster and more reliable aetiological insights.

Syndromic multiplex PCR gastrointestinal (GI) panels represent an important technological advance, allowing simultaneous, high-sensitivity detection of multiple viral, bacterial, and parasitic enteropathogens within a short timeframe.[5-7] Although increasingly adopted in high-income settings, their use in Indonesia remains uncommon due to cost, limited availability, and infrastructural constraints. As a result, real-world data on the performance and clinical utility of multiplex GI panels in the Indonesian context, particularly among travellers with diarrhoea, are scarce.

Bali occupies a unique epidemiological position as one of Southeast Asia's busiest international tourist destinations. The island functions as a natural sentinel site for monitoring patterns of gastrointestinal pathogens among travellers from diverse geographical backgrounds. Despite this, few studies have characterised the microbiological landscape of TD in Bali using advanced molecular diagnostics, and even fewer have compared multiplex PCR with conventional stool microscopy in this population. This study aimed to evaluate the diagnostic utility of a multiplex PCR-based GI pathogen panel compared with routine stool microscopy in travellers presenting with acute diarrhoea at a high-travel tropical destination. By integrating molecular diagnostics with conventional methods, this research provides novel real-world evidence from a setting where PCR-based diarrhoeal testing is rarely available. The findings may offer valuable insights for clinicians, policymakers, and public health stakeholders seeking to strengthen diagnostic capacity, guide rational antibiotic use, and enhance surveillance of enteric pathogens in resource-limited environments.

METHODS

Study Design and Setting

This retrospective analytical cross-sectional study was conducted at a private international hospital located in a high-tourism region of southern Bali, Indonesia.

The setting is characterised by a predominantly traveller population, many of whom present with acute gastrointestinal symptoms. Multiplex PCR testing for gastrointestinal (GI) pathogen is not routinely available in most Indonesian healthcare facilities; however, it is selectively used at this centre for clinical decision-making. The study covered all eligible patient encounters between January 2024 and June 2025. This study has obtained research ethics from the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Warmadewa University (Reference No. 74/Unwar/FKIK/KEPK/VI/2025; Protocol No. 639.02.06.25).

Participants and Eligibility Criteria

The target population consisted of travellers presenting with acute diarrhoea. Eligible participants were those aged >13 years with symptom onset ≤7 days, who underwent both routine stool microscopy and multiplex PCR-based GI panel testing with at least one detected pathogen (bacterial, viral, or parasitic). Exclusion criteria included patients requiring intensive care during admission, surgical patients presenting with diarrhoea, individuals with complex chronic medical disorders (e.g., cystic fibrosis, congenital gastrointestinal anomalies), and patients with incomplete or missing medical record data. A consecutive sampling approach ensured inclusion of all eligible cases during the study period.

Sample Size Justification

The minimum required sample size was estimated using a standard formula for proportions, assuming a regional gastroenteritis prevalence of 26%,[8] a 10% margin of error, and a 95% confidence level. This yielded a minimum of 74 participants. Accounting for potential missing data, a 10% buffer was added, resulting in a target of 81 patients. Ultimately, 114 complete cases were available and included. Given the exploratory nature of the study, no post-hoc power calculation was performed.

Variables and Operational Definitions

The primary independent variable was multiplex PCR-based pathogen detection. The dependent variables were stool microscopy findings, including macroscopic parameters (colour, consistency, mucus, visible blood) and microscopic elements (erythrocytes, leukocytes, ova, cysts, trophozoites, yeast, and other microorganisms). Potential confounders included age, sex, comorbidities, degree of dehydration, symptom duration, region of origin, length of hospital stay, and recent antibiotic use.

Diagnostic Procedures

Conventional stool microscopy

Stool samples were examined by accredited laboratory staff using standard light microscopy techniques. Observations were recorded using a semi-quantitative method in accordance with institutional laboratory protocols. Parameters assessed included stool appearance, presence of mucus or blood, inflammatory cells, protozoa, helminth ova, and yeast.

Multiplex PCR gastrointestinal panel

A commercially available syndromic multiplex PCR GI panel capable of detecting 24 bacterial, viral, and parasitic targets within approximately 70 minutes was used.[7] Tests were performed in accordance with manufacturer instructions, and results were categorised into bacterial, viral, and parasitic groups for analysis.

Data Collection

Data were extracted retrospectively from electronic medical records using a structured data collection tool, with no direct contact with patients at any stage. All potentially identifying information was removed and replaced with coded study identifiers before analysis. The anonymised dataset was stored in encrypted files accessible only to the research team.

Statistical Analysis

Descriptive statistics summarised baseline demographic and clinical characteristics. Numerical variables were presented as means ± standard deviations or medians with interquartile ranges, depending on distribution. Categorical variables were reported as frequencies and percentages.

Associations between stool microscopy parameters and pathogen categories detected via the PCR panel (bacterial, viral, parasitic) were examined using chi-square or Fisher's exact tests as appropriate. Variables with $p < 0.25$ in bivariate analysis were entered into multivariable logistic regression models to identify independent predictors. Statistical significance was set at $p < 0.05$. Analyses were performed using IBM SPSS Statistics version 26.0.

RESULTS

Patient Characteristics

A total of 114 travellers met the eligibility criteria and were included in the analysis. The mean age was 36.12 ± 14.43 years, and slightly more patients were male (55.3%) than female (44.7%). Symptom onset prior to presentation averaged 3.54 ± 6.59 days, with 60.5% seeking medical care within the first three days.

The largest demographic group consisted of European nationals (51.8%), followed by Australians (19.3%), Asians (13.2%), North Americans (10.5%), and South Americans (4.4%). Nausea was the most frequently reported symptom (77.2%), followed by vomiting (50.0%) and epigastric tenderness (46.5%). Comorbidities were present in 42.1% of patients, and dehydration was noted in 28.1%. Most patients received combination antibiotic therapy (76.3%). The mean length of hospital stay was 1.34 ± 1.91 days **(Table 1).**

Table 1. Patient Demographic and Clinical Characteristic

Variable	n	%
Age (year) (mean±SD)	36.12 ± 14.43	
Gender		
Female	51	44.7
Male	63	55.3
Onset (day) (mean±SD)	3.54 ± 6.59	
>7 days	7	
3-7 days	38	
<3 days	69	
Races		
European	59	51.8
North American	12	10.5
South American	5	4.4
Australian	22	19.3
Asian	15	13.2
African	1	0.9
Nausea		
Yes	88	77.2
None	26	22.8
Vomiting		
Yes	57	50.0
None	57	50.0
Epigastric Tenderness		
Yes	53	46.5
None	61	53.5
Comorbid		
Yes	48	42.1
None	66	57.9
Dehydration		
Yes	32	28.1
None	82	71.9
Antibiotic use		
Single therapy	27	23.7
Combination therapy	87	76.3
Length of Stay (day) (mean±SD)	1.34 ± 1.91	

Stool Microscopy Findings

Brown stool was the predominant colour (86.8%), with smaller proportions exhibiting greenish-brown (7.9%) or yellow (2.6%) stools. Most specimens were mushy (56.1%), followed by liquid (29.8%) and semi-liquid (14.0%). Mucus was present in 52.6% of samples, and visible blood was reported in 8.8%. Elevated erythrocytes were observed in 12.3% of specimens, and leukocytosis was noted in 20.2%. Amoebae were detected in 20.2% and yeast in 47.4%, while no helminth ova were identified **(Table 3).**

Pathogen Detection Via Multiplex PCR GI Panel

Bacterial pathogens were identified in 88.6% of patients, while viral and parasitic infections were detected in 41.2% and 3.5% of cases, respectively **(Table 2).** The most frequently detected bacterial species were enteropathogenic *Escherichia coli* (EPEC, 47.4%), enteroaggregative *E. coli* (EAEC, 28.1%), *Campylobacter spp.* (26.3%), *Plesiomonas shigelloides* (17.5%), and enterotoxigenic *E. coli* (ETEC, 15.8%). Shiga-like toxin-producing *E. coli* (STEC) was identified in 12.3% of cases. Viral detections were dominated by norovirus (11.4%) and rotavirus (11.4%), whereas parasitic infections consisted of *Cyclospora cayetanensis* (1.8%), *Giardia lamblia* (0.9%), and *Cryptosporidium spp.* (0.9%).

Table 2. Bacteria, Virus, and Parasite Detection Rate via Gastrointestinal Panel

Detection Rate	n	%
Bacteria		
Positive	101	88.6
Negative	13	11.4
Virus		
Positive	47	41.2
Negative	67	58.8
Parasite		
Positive	4	3.5
Negative	110	96.5

Pathogen Distribution by Geographical Origin

Distinct regional patterns of enteric pathogens were observed among travellers. Among travellers from Europe, enteropathogenic *Escherichia coli* (EPEC; 47.5%), enteroaggregative *E. coli* (EAEC; 33.9%), and *Campylobacter* spp. (28.8%) were the most frequently identified bacterial pathogens, with norovirus as the predominant viral agent (25.4%). Travellers from North America showed a similar distribution, with EPEC (41.7%), EAEC (33.3%), and *Campylobacter* spp. (25.0%) being commonly detected, while

norovirus accounted for the majority of viral infections (41.7%). In travellers from South America, EPEC (40%) and *Cyclospora cayetanensis* (40%) were the leading pathogens. Among Australian travellers, EPEC (45.5%), EAEC (27.3%), and *Campylobacter* spp. (27.3%) predominated, with norovirus detected in 31.8% of cases. Travellers from Asia demonstrated a distinct profile, characterised by higher proportions of EPEC (60%), *Campylobacter* spp. (26.7%), and *Plesiomonas shigelloides* (26.7%), while norovirus (33.3%) and rotavirus (20%) constituted the majority of viral infections.

Associations Between Stool Microscopy Parameters and PCR-Based Pathogen Detection

There were no significant associations observed between bacterial detection and any stool microscopy characteristics, including colour, consistency, mucus, visible blood, erythrocytes, or leukocytes ($p > 0.05$). Two stool microscopy findings showed significant associations with viral detection, i.e., elevated erythrocytes were less common among viral cases ($p = 0.006$), and elevated leukocytes were also less frequent in viral infections ($p = 0.034$). No stool parameter demonstrated a statistically significant association with parasitic detection ($p > 0.05$) **(Table 3).**

Table 3. Association Between Stool Examination Result and Gastrointestinal Panel Examination (Bacteria, Virus, and Parasite)

Variable of Stool Examination	Bacteria			Virus			Parasite		
	Positive (+) n (%)	Negative (-) n (%)	p-value	Positive (+) n (%)	Negative (-) n (%)	p-value	Positive (+) n (%)	Negative (-) n (%)	p-value
Colour									
Brown	86 (85.1%)	13 (100%)	0.817	43 (91.5%)	56 (83.6%)	0.487	4 (100%)	95 (86.4%)	0.987
Green	1 (1%)	0 (0%)		0 (0%)	1 (1.5%)		0 (0%)	1 (0.9%)	
Greenish Brown	9 (8.9%)	0 (0%)		2 (4.3%)	7 (10.4%)		0 (0%)	9 (8.2%)	
Greenish Yellow	1 (1%)	0 (0%)		0 (0%)	1 (1.5%)		0 (0%)	1 (0.9%)	
Reddish Brown	1 (1%)	0 (0%)		0 (0%)	1 (1.5%)		0 (0%)	1 (0.9%)	
Yellow	3 (3%)	0 (0%)		2 (4.3%)	1 (1.5%)		0 (0%)	3 (2.7%)	
Consistency									
Liquid	32 (31.7%)	2 (15.4%)	0.276	14 (29.8%)	20 (29.9%)	0.336	2 (50%)	32 (29.1%)	0.556
Semi Liquid	15 (14.9%)	1 (7.7%)		4 (8.5%)	12 (17.9%)		0 (0%)	16 (14.5%)	
Mushy	54 (53.5%)	10 (76.9%)		29 (61.7%)	35 (52.2%)		2 (50%)	62 (56.4%)	
Mucus									
Positive	53 (52.5%)	7 (53.8%)	0,926	21 (44.7%)	39 (58.2%)	0.154	3 (75%)	57 (51.8%)	0.620
Negative	48 (47.5%)	6 (46.2%)		26 (55.3%)	28 (41.8%)		1 (25%)	53 (48.2%)	
Blood									
Positive	9 (8.9%)	1 (7.7%)	1.000	2 (4.3%)	8 (11.9%)	0.193	1 (25%)	9 (8.2%)	0.311
Negative	92 (91.1%)	12 (92.3%)		45 (95.7%)	59 (88.1%)		3 (75%)	101 (91.8%)	
Erythrocyte									
High	12 (11.9%)	2 (15.4%)	0.661	1 (2.1%)	13 (19.4%)	0.006*	2 (50%)	12 (10,9%)	0.073
Normal	89 (88.1%)	11 (84.6%)		46 (97.9%)	54 (80.6%)		2 (50%)	98 (89.1%)	
Leukocyte									
High	21 (20.8%)	2 (15.4%)	1.000	5 (10.6%)	18 (26.9%)	0.034*	2 (50.0%)	21 (19.1%)	0.181
Normal	80 (79.2%)	11 (84.6%)		42 (89.4%)	49 (73.1%)		2 (50.0%)	89 (80.9%)	
Amoeba									
Positive							2 (50.0%)	21 (19.1%)	0.181
Negative							2 (50.0%)	89 (80.9%)	
Yeast									
Positive							2 (50.0%)	52 (47.3%)	1.000
Negative							2 (50.0%)	58 (52.7%)	

*p-value significant (p<0.05)

Table 4. Multivariable Logistic Regression Analysis of Stool Erythrocytes and Pathogen Detection

Outcome Category	Variable	B	SE	Adjusted Odds Ratio (AOR)	95% Confidence Interval	*p*-value
Viral pathogens	Elevated erythrocytes	−2.405	1.057	0.09	0.01–0.71	0.023
	Constant	4.970	2.085	—	—	—
Parasitic pathogens	Elevated erythrocytes	2.100	1.046	8.16	1.05–63.41	0.045
	Constant	−0.308	1.686	—	—	—

In multivariable logistic regression models, elevated erythrocytes were the only stool microscopy parameter independently associated with pathogen detection outcomes, being linked to a significantly lower likelihood of viral pathogen detection (AOR 0.09; 95% CI 0.01–0.71; p = 0.023) and a significantly higher likelihood of parasitic pathogen detection (AOR 8.16; 95% CI 1.05–63.41; p = 0.045), while no other stool microscopy parameters retained statistical significance **(Table 4).**

DISCUSSION

This study offers one of the first detailed descriptions of the diagnostic and epidemiological landscape of travellers' diarrhoea (TD) in Bali using both conventional stool microscopy and multiplex PCR-based gastrointestinal (GI) pathogen panels. Across a diverse traveller population, enteropathogenic *Escherichia coli* (EPEC) and enteroaggregative *E. coli* (EAEC) were the predominant bacterial pathogens, accompanied by a considerable burden of norovirus infections. These patterns broadly mirror global TD literature, where diarrhoeagenic *E. coli* and norovirus remain leading aetiologies in both sporadic and travel-associated diarrhoea.[1-4]

Conventional stool microscopy remains widely used in many healthcare settings, including those across Indonesia, yet its diagnostic performance is constrained by modest sensitivity and operator variability.[5] In contrast, multiplex PCR GI panels offer rapid, syndromic detection of a broad range of pathogens, enabling earlier and more precise therapeutic decisions.[6] In this cohort, stool colour, consistency, mucus, and visible blood did not meaningfully differentiate bacterial or parasitic aetiologies. While elevated levels of erythrocytes and leukocytes were associated with viral detection in unadjusted analyses, only erythrocytes remained significant in multivariable modelling, suggesting that the presence of erythrocyte reduces the likelihood of a viral diagnosis.

Taken together, these findings reinforce a central theme in contemporary infectious diseases diagnostics: routine microscopy provides limited discrimination for bacterial or viral infections, and molecular testing adds substantial diagnostic clarity. The potential for multiplex PCR to detect colonisation, particularly with EAEC and EPEC, must be acknowledged. In this context, PCR-positive results should be interpreted with clinical correlation, as detection does not necessarily indicate causation, especially for organisms that are known to have potential for colonisation. Since quantitative indicators of pathogen load (such as cycle threshold values) are not included in routine reporting, distinguishing between active infection and colonisation depends on clinical assessment and supporting findings. This phenomenon is well recognised in high-sensitivity platforms and reflects the tension between analytical sensitivity and clinical specificity.[6,7]

Consistent with previous research, the use of syndromic PCR testing may support antimicrobial stewardship by reducing unnecessary empirical antibiotics, facilitating organism-directed therapy, and enabling timely infection control measures.[6-8] This is particularly relevant in Indonesia, where various studies have documented suboptimal antibiotic rationality in the management of diarrhoeal disease.[5]

This study contributes novel molecular epidemiology data from Bali, a globally significant travel destination and natural sentinel point for monitoring enteric pathogens introduced by international visitors. The predominance of EPEC and EAEC across all geographical groups aligns with trends reported in South-East Asia and other tropical regions.[1-3,9] The detection of *Plesiomonas shigelloides* among Asian travellers is notable and may reflect region-specific exposures, such as freshwater contamination or food-handling practices, consistent with prior reports linking this organism to environmental transmission.[10] Despite Bali's high travel volume, published molecular data describing TD in the region remain extremely scarce. The present findings therefore, provide a timely baseline from which to inform local surveillance efforts, travel medicine guidance, and future public health strategies.

Multivariable analyses revealed a coherent and biologically plausible pattern: increased erythrocytes on microscopy were independently associated with a lower likelihood of viral detection and a higher likelihood of parasitic detection; however, this finding should be interpreted cautiously. Viral gastroenteritis typically produces secretory, non-inflammatory diarrhoea with preserved epithelial integrity, explaining the inverse association.[11,12] In contrast, mucosal breach and microscopic bleeding are well-described in invasive protozoal infections, most notably *Entamoeba histolytica*, and in some contexts, *Cyclospora* and *Cryptosporidium*, providing a pathophysiological rationale for the positive parasitic association.[13-16]

The wide confidence interval for the parasitic model reflects imprecision due to small absolute numbers and should be considered as hypothesis-generating rather than definitive. Potential residual confounding, including timing of stool sample collection and prior antimicrobial exposure, can also not be excluded.[17] Inter-observer variability in microscopy, inherent to routine practice, may have further attenuated true associations.[18-19] Nevertheless, the directionality of these findings aligns with the established understanding of enteric pathogen biology.

This study underscores the limitations of relying solely on stool microscopy in the evaluation of TD or acute gastroenteritis (AGE), especially in resource-limited environments. While microscopy retains value for detecting parasites or gross abnormalities, its predictive capacity for bacterial or viral aetiologies is limited. Clinicians should therefore exercise caution when using microscopy alone to guide therapy, particularly antibiotic decisions.

For facilities without access to multiplex PCR, the models suggest a pragmatic clinical heuristic. The presence of erythrocytes makes a viral aetiology less likely and may prompt earlier consideration of parasitic coverage, where compatible with clinical and epidemiological context. However, the absence of erythrocytes is insufficient to infer viral disease and must be integrated with clinical assessment and local trends.

The integration of multiplex PCR-based GI panels into diagnostic pathways offers substantial potential for improving pathogen-directed management, reducing inappropriate antimicrobial use, and supporting stewardship initiatives. In a tourism-intensive region such as Bali, rapid molecular diagnostics also offer public health benefits by aiding outbreak detection and containment.

From a policy perspective, expanding access to point-of-care molecular diagnostics in high-travel settings could improve both individual patient care and broader surveillance capacity. This requires thoughtful considerations around cost, affordability, and equitable access, particularly to avoid restricting advanced diagnostics to private or higher-income groups. At a minimum, implementation would require access to basic molecular laboratory infrastructure, including PCR-capable instruments, reliable electricity supply, cold-chain storage for reagents, and quality control systems. In addition, trained laboratory personnel are required for sample handling, assay operation, and result interpretation. Cost considerations include not only the initial investment in equipment but also recurring expenses for consumables, maintenance, and quality assurance, which may limit widespread adoption. Initial laboratory setup may exceed USD 15,000, while per-test costs for molecular assays can exceed USD 100 depending on platform and reagents.[20] In Indonesia, cartridge-based platforms such as the GeneXpert System have been widely deployed for tuberculosis and COVID-19 diagnostics, and have been reported to cost approximately IDR 70,000–280,000 per test.[21] Complementary approaches, such as tiered diagnostic algorithms, targeted use of PCR, and integration of laboratory data into regional surveillance systems, may offer realistic pathways for implementation.

Strengths of this study include its relatively large sample of international travellers, use of a validated multiplex PCR platform, and the comparative assessment of molecular and microscopic diagnostics in a real-world clinical environment. The dataset provides valuable baseline information for a region with limited published molecular epidemiology of TD. Limitations include its retrospective, single-centre design, which constrains generalisability; reliance on medical records, which may introduce inaccuracies in symptom reporting; and the under-representation of local residents, limiting conclusions about the broader Balinese population. Seasonal variation was not assessed, and the selective availability of multiplex testing may reflect healthcare access patterns rather than population-wide disease burden. Multiplex PCR testing was performed selectively based on clinician judgement and availability, which may have biased the selection towards patients with more severe or persistent symptoms. As all included patients underwent both microscopy and PCR, comparison with those not tested by PCR was not possible, potentially

limiting generalisability. Larger, prospective, multi-centre studies are needed to validate these findings, examine cost-effectiveness, and assess the clinical impact of multiplex PCR-guided management.

CONCLUSION

This study demonstrates that multiplex PCR gastrointestinal panels substantially enhance pathogen detection and delineate the microbial profile of travellers' diarrhoea in a high-travel tropical setting, thereby improving aetiological diagnosis compared with routine stool microscopy. While conventional microscopy shows limited overall diagnostic utility, the presence of erythrocytes remains a clinically relevant interpretive marker, being associated with lower odds of viral and higher odds of parasitic infection, and may aid clinical decision-making when molecular diagnostics are unavailable. Together, these findings support a practical diagnostic strategy, where stool microscopy is used as an initial screening tool. Following this, multiplex PCR should be selectively utilized for patients with more severe, persistent, or unclear clinical presentations. This strategy aims to optimise resource use in settings with limited resources.

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Warmadewa University (Ref. No. 74/Unwar/FKIK/KEPK/VI/2025; Protocol No. 639.02.06.25). As this was a retrospective review of anonymised medical records, the requirement for individual informed consent was waived by the Ethics Committee. All study procedures adhered to the Declaration of Helsinki and relevant institutional guidelines. Institutional permission was granted by the participating private international hospital, which, in accordance with its internal policy, requested that its name not be disclosed in this manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

BB conceptualised the study, extracted and curated the dataset, performed the statistical analyses, and was the primary author responsible for drafting and revising the manuscript. LL contributed to the early conceptual framing of the study, provided critical revisions to the manuscript, and supported the integration of clinical context. NMAK provided senior clinical supervision, contributed to the interpretation of the findings, and guided the academic direction of the study through sustained mentorship. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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