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Multicountry surveillance study of acute febrile disease of unknown cause in sub-Saharan Africa

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ABSTRACT

Background Acute Febrile Disease of Unknown Cause (AFDUC) remains a major diagnostic and clinical challenge in sub-Saharan Africa (SSA), where malaria dominates while other aetiologies are under-recognised. The African Network for Improved Diagnostics, Epidemiology and Management of common Infectious Agents aimed to enhance understanding of the epidemiology of AFDUC in SSA through clinical and laboratory-based surveillance.

Methods A multicentre prospective sentinel surveillance case-control study was conducted across urban and rural sites in Côte d'Ivoire (CI), Burkina Faso (BF) and South Africa (SA). We enrolled 6100 AFDUC cases and 1455 healthy controls between 2018 and 2022 across all study sites. Standardised clinical, laboratory and follow-up data were collected. Diagnostics included biomolecular multiplex PCR, serology and blood culture. Associations between pathogens and AFDUC were assessed using adjusted odds ratios.

Results *Plasmodium falciparum* remained the leading pathogen in BF and CI, and EBV was the most frequent viral detection (up to 23% in SA). HBV prevalence matched WHO estimates, while DENV and CHIKV IgM seropositivity reached 20–22% in BF and CI, with marked regional and rural-urban differences. Blood cultures had low positivity (5.4%), with *Staphylococcus aureus* and *Salmonella* spp predominating. Mortality was highest in BF (22%), particularly in adults ≥45 years, and largely attributed to unresolved febrile illness. Across sites, HIV infection, comorbidities and neurological symptoms were linked to poor outcomes. Mortality patterns mirrored health system disparities, with lowest physician density and health spending in BF. Despite broad testing, no pathogen was detected in 65% of cases.

Conclusions Our results highlight the need for improved diagnostics of common and zoonotic pathogens in SSA and provide insights into febrile disease aetiologies. Strengthened laboratory surveillance, improved case management and targeted vector control are critical to reduce the burden of febrile illness.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Aetiologies of non-malarial Acute Febrile Diseases of Unknown Cause (AFDUC) in sub-Saharan Africa remain largely uncharacterised due to limited diagnostic capacity.

WHAT THIS STUDY ADDS

⇒ We provide the first harmonised, multicountry overview of AFDUC across Burkina Faso, Côte d'Ivoire and South Africa, showing that viral infections, particularly Epstein-Barr virus, hepatitis B virus and arboviruses, are major contributors, with important country-specific differences. We also identify HIV infection, neurological symptoms and comorbidities as potential predictors of poor outcomes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings highlight priority pathogens and risk factors that can guide diagnostic strategies, strengthen fever management and inform surveillance and policy in limited-resource settings.

INTRODUCTION

Acute febrile illness is a common reason for seeking healthcare and is a major cause of morbidity and mortality in sub-Saharan Africa (SSA).[1] While malaria remains the leading cause, its incidence has declined over the past two decades due to improved control measures.[2] However, causes of non-malarial fevers often remain unexplored, as they present with non-specific symptoms and require complex laboratory diagnostics often unavailable in resource-limited settings.[1 3 4]

Acute Febrile Disease of Unknown Cause (AFDUC) presents as a recent onset of fever with no obvious cause and can be due to a variety of infectious agents, including bacteria, viruses, parasites and fungi.

Evidence on AFDUC aetiology remains limited in many African countries,[5] with most studies focusing on single pathogens or small pathogen groups (eg, *Salmonella* spp[6] or arboviral infections[7]). Multicountry syndromic studies remain rare,[8–10] though some country-specific research has provided insights into pathogens circulating across the continent.[4 11]

Exposure risks for infectious diseases in SSA vary along an urban-rural gradient. In rural areas, close contact with livestock and wildlife facilitates bacterial zoonoses such as brucellosis and leptospirosis.[5 11] In contrast, urban and periurban settings, characterised by high population density and anthropogenic breeding sites, harbour distinct mosquito species and ecological conditions impacting arboviral transmission. This ecological variation generates different exposure risks for vector-borne diseases across the urban-rural spectrum.[12 13] Arboviruses, including dengue (DENV), West Nile (WNV), chikungunya (CHIKV) and Sindbis (SINV) viruses, represent important but often underdiagnosed AFDUC aetiologies.[4 14–16] Bloodstream infections are another major contributor, with *Salmonella* spp causing more than one-third of bacterial infections and showing high antimicrobial resistance.[6] Tuberculosis, HIV and related opportunistic infections continue to pose significant challenges in SSA,[17] and the incidence of co-infections among febrile patients further complicates the management of febrile illness.[3 4]

To address the need for comprehensive epidemiological data on AFDUC, the African Network for Improved Diagnostics, Epidemiology and Management of common Infectious Agents (ANDEMIA) conducted a syndromic sentinel surveillance study across eight healthcare facilities in urban and rural settings in three sub-Saharan countries. We combined multiplex molecular testing for a panel of known human, vector-borne and zoonotic pathogens[18] with classical bacteriology and arboviral IgM serology to establish a baseline of common AFDUC aetiologies in SSA. Because AFDUC symptoms may be short-lived, the case definition included acute fever or self-reported fever with arboviral-like signs (neurological manifestations, arthralgia, myalgia or rash). By improving our understanding of the aetiologies behind acute febrile illnesses, this research contributes to the evidence base on AFDUC in SSA, provides data to support immediate public health interventions and facilitates future investigation of emerging and zoonotic diseases in unsolved cases.

METHODS

Study design and participants

This study was designed as a prospective sentinel case-control surveillance as previously described.[19] We collected data from 2018 to 2022 in eight healthcare facilities in Côte d'Ivoire (CI), Burkina Faso (BF) and South Africa (SA) including urban (Bouake and Guiglo in CI, Bobo-Dioulasso in BF, Kalafong in SA) and rural sites (Brobo and Tai in CI, Dano/Dissin in BF and Mapulaneng/Matikwana in SA). We enrolled patients of all ages who met the definitions for cases or controls and provided informed consent. We aimed to enrol two-thirds of paediatric cases and one third of adult cases.[19]

The AFDUC case definition was adapted from the arboviral disease criteria of the Centres for Disease Control and Prevention (CDC)[20] and included the following: fever ≥38°C measured at enrolment and/or self-reported fever in the last 10 days; absence of evident cause of the febrile disease (eg, acute respiratory tract, gastrointestinal, renal, urinary tract or postoperative infections) and enrolment within 48 hours of admission. Signs of arboviral infections (eg, myalgia, arthralgia, rash or headache) or acute neurological disease (eg, meningitis, encephalitis, acute flaccid paralysis, recent onset of Guillain-Barré syndrome or other acute signs of central or peripheral neurologic dysfunction) were required for cases with only self-reported fever. New-borns directly hospitalised after delivery and patients admitted to the intensive care units were not included. In endemic areas, enrolment initially required a negative malaria test or persistent fever 48 hours after malaria treatment. To increase sample size, we later included untreated malaria-positive cases (22 in BF, 104 in CI) after sensitivity analyses showed no effect on odds ratios (ORs) for the main pathogens (detailed in the Data management and analysis section).

Healthy controls were enrolled as patients presenting to the sentinel sites (eg, for vaccination or dental treatment) with neither fever nor signs of infection in the previous 3 weeks and provided informed consent. Exclusion criteria were admission to the hospital for longer than 48 hours, pregnancy, use of antibiotics in the last 24 hours and being previously enrolled in the ANDEMIA study. We aimed to match the controls by frequency based on age group and location of healthcare facility.

Clinical, demographic, socioeconomic and behavioural data were collected using a standardised structured case investigation form, and follow-up calls were performed to record disease outcome post enrolment.[19]

Patient and public involvement statement

Patients or members of the public were not involved in the design, conduct, reporting or dissemination plans of this study. Bacteriology results were communicated to treating clinicians, contributing to patients care, and overall study findings were shared with participating hospital teams.

Sampling procedures

All samples were collected at enrolment. Blood samples were collected in EDTA tubes (0.5 mL for minors <15 years of age, 1–4 mL for patients ≥15 years of age). Clotted blood tubes (1–4 mL) were sampled from adult cases and controls in CI and BF and from all cases and controls in SA. Sera were separated by centrifugation. When requested for routine diagnostics by the treating physician, an aliquot of cerebrospinal fluid (CSF) was included. Samples were transported to the central laboratories for aliquoting, storage at –80°C and laboratory analyses.

For cases with fever ≥38°C, a total of 10 mL (adult) or 5 mL (children) of whole blood was collected in bacterial culture bottles and transferred to the bacteriology laboratory.

Laboratory testing

Bacteriology

Blood cultures (BCs) were manually processed in Tai and Guiglo in CI. In all other sites in BF and CI, BCs were automatically processed using BacT/ALERT 3D system (bioMérieux, Marcy L'Etoile, France). Commercially produced 10 mL Bi-state BC bottles (IClear Healthcare, Guangzhou, China) or BacT/ALERT FA bottles were used for cases ≥15 years of age and 5 mL Bi-state BC bottles or BacT/ALERT PF bottles were used for children <15 years of age. BCs were incubated up to 5 days according to manufacturer's instructions. A sterility test was performed for the negative BCs. A Gram stain was performed from positive BCs and relevant agar plates were then inoculated and incubated at 37±2°C in ambient or CO_2-enriched air (candle jar) for 24–48 hours. Isolate identification was based on morphological, biochemical and antigenic characteristics[21] In SA, bacteriology analyses were not routinely performed as part of the study; however, bacteriological results were collected when available from the National Health Laboratory Services diagnostic results database.

Molecular biology

Nucleic acids were extracted from EDTA blood, serum or CSF samples using the QIAamp Viral RNA mini kit (Qiagen, Hilden Germany) or the IndiSpin Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany) followed by reverse transcription using Expand (Roche, Mannheim, Germany), Superscript IV (Invitrogen, Massachusetts, USA) or Lunascript (New England Biolabs, Massachusetts, USA) following the manufacturer's instructions. A previously described multiplex PCR macroarray-based platform, referred to as fever chip, was used for the first line screening of 29 targets (online supplemental table 1).[18] Briefly, we performed two multiplex PCRs using biotinylated primers to generate biotin-labelled products. The PCR products were hybridised on a DNA macroarray containing DNA probes for each target (Chipron GmbH, Berlin, Germany). The reaction was detected using a streptavidin-peroxidase conjugate according to the manufacturer's instructions. The results were read using a scanner and software (Chipron GmbH, Berlin, Germany), identifying pixel density in specific areas, corresponding to the pathogen targets. Positive results were recorded for a colourimetric score >1500.

Since the fever chip results are not quantitative, confirmation of positive chip reactions by individual real-time PCRs, referred to here as 'confirmation PCR', was recommended. In SA, confirmation was performed using in-house Taqman real-time PCR (online supplemental table 1). For BF and CI, a subset of fever chip positive samples was sent to the Robert Koch Institute (RKI) (Berlin, Germany) for confirmation of selected pathogens with the following multiplex PCR commercial kits following the manufacturer's instructions: Neuro 9 (Siemens Healthcare, Berlin, Germany), targeting enterovirus (EV), Epstein-Barr virus (EBV), Herpes simplex viruses 1 and 2, human adenovirus (AdV), human cytomegalovirus and Varicella zoster virus and Tropical fever core PCR (Siemens Healthcare, Berlin, Germany) targeting CHIKV, DENV, *Leptospira* spp, *Rickettsia* spp, and WNV. Hepatitis B virus (HBV) positive samples from BF and CI were confirmed at RKI with an in-house conventional PCR.[22]

Serology

Serum specimens from BF and CI were tested by commercial ELISA for the presence of anti-DENV and anti-CHIKV IgM (Euroimmun, Lübeck, Germany) following the manufacturer's instructions. Screening of WNV and SINV was conducted in SA and has been previously reported.[15 16]

Data management and analysis

Data were entered into a customised electronic system (Voozanoo, Epiconcept, France) and checked for completeness and eligibility. Analyses included cases and controls with at least one molecular result; follow-up analyses were restricted to cases contacted within 21–365 days.

Molecular analyses excluded pathogens detected in fewer than 10 participants (online supplemental table 2). When possible, fever chip results were confirmed by PCR; for EBV and HBV, missing confirmations were imputed. Unless otherwise specified, final results refer to blood samples and are based on fever chip, confirmation PCR and imputation (online supplemental figure 1).

Multivariable logistic regression models estimated adjusted ORs (aOR) for key pathogens, AdV, EV, EBV, HBV and *Plasmodium falciparum*, adjusting for demographic and clinical covariates. Stratified, interaction and sensitivity analyses were conducted. Serology for DENV and CHIKV and bacteriological results were analysed similarly, with common contaminants excluded.

A detailed description of data processing, statistical modelling and additional analyses is available in the online supplemental annex 1, online supplemental figure 1 and online supplemental tables 2–9.

All statistical analyses were performed using R (V.4.2.2). Multiple imputation was done using the R library 'mice'[23].

RESULTS

Study population

Overall, 6100 AFDUC cases and 1455 controls were enrolled between January 2018 and December 2022. Among cases, 37% (n=2227) were from BF, 43% (n=2637)

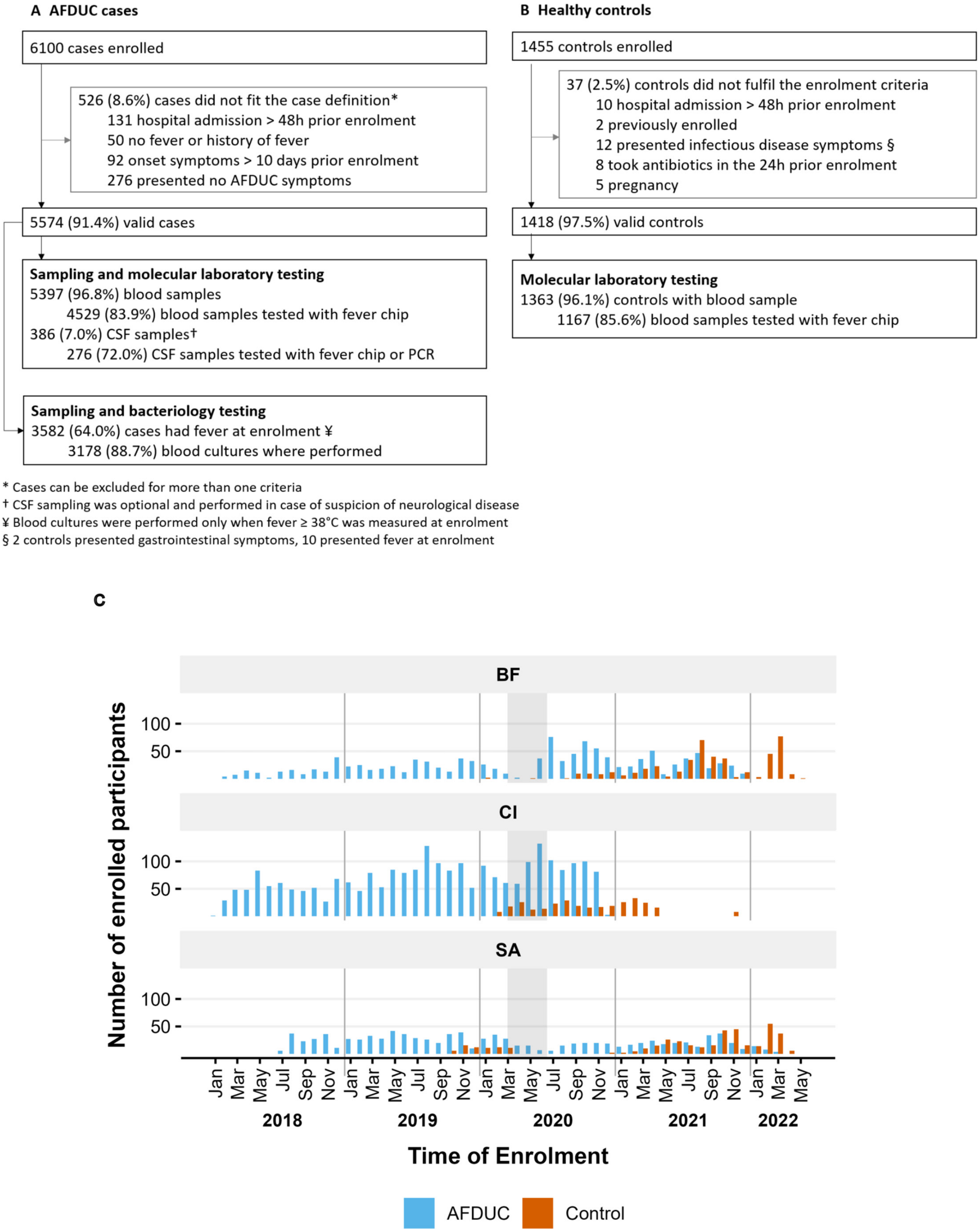

Figure 1 AFDUC cases and healthy controls enrolment, sampling and testing. (A) Number of cases and (B) controls, exclusion criteria, number of specimens collected and tested are indicated for all countries. Blood samples refer to EDTA blood specimens and sera. (C) Enrolment timeline showing AFDUC cases and controls who fulfilled the enrolment criteria and were tested with the fever chip for blood or CSF, greyed period corresponds to the first COVID-19 lockdowns. AFDUC, acute febrile disease of unknown cause; BF, Burkina Faso; CI, Côte d'Ivoire; CSF, cerebrospinal fluid; SA, South Africa.

from CI and 20% (n=1236) from SA. Among controls, 43% (n=631) were enrolled in BF, 23% (n=333) in CI and 34% (n=491) in SA. All analyses except bacteriology focused on 4649 cases and 1167 controls with at least one molecular result for blood or CSF (figure 1; online supplemental figure 2).

Half of the AFDUC cases were enrolled between 2018 and 2019. In BF, most cases were enrolled in 2020 and

2021. CI consistently enrolled cases from 2018 to 2020 and SA from 2018 to 2022. Control enrolment occurred mainly between 2020 to 2022 with a peak in 2021 (52%). CI enrolled the majority of its controls in 2020 whereas BF and SA did so in 2021. Due to delays in the implementation of control enrolment and the COVID-19 containment measures, a mismatch occurred between case and control enrolment periods (figure 1, table 1).

Overall, half of the cases were <18 years old with substantial differences between countries (47% BF, 44% CI and 76% SA). In CI, the age distribution was similar in cases and controls (median age; cases: 20, controls: 21). In BF, controls were generally younger than cases (median age; cases: 19, controls: 4) and the opposite trend was observed in SA (median age; cases: 2, controls: 18).

Across countries, 65% of cases were enrolled in urban healthcare facilities. As expected from frequency matching, a majority of controls (59%) were recruited at urban sites. The majority of cases in CI and SA resided in cities (65% and 67%, respectively) with similar proportions for enrolment of cases at urban healthcare facilities (62% and 68%). In BF, although 61% of cases resided in villages, 67% were enrolled in urban healthcare facilities, indicating that many village residents sought care in urban health centres.

Overall female (f) and male (m) participants' proportions were similar among cases (f/m=1) and controls (f/m=0.9). In BF and SA, there were slightly fewer female cases (f/m=0.8), whereas the opposite was observed in CI (f/m=1.2).

Hospitalisation or need for hospitalisation (referred to hereafter as hospitalisation) was reported in 51% of the cases. Marked country-specific differences were observed, with nearly all cases hospitalised in SA (98%) compared with 78% in BF and 20% in CI.

HIV status was available for 26% of cases with significant intercountry variations (BF 6%, CI 10%, SA 88%). Overall, 5% of the cases were positive, reaching 15% in SA where HIV status was significantly associated with age (p<0.001, online supplemental figure 3). Among controls, HIV status was known for 25%, of whom 7% were positive.

Vaccination records were available for 39% of cases and 34% of controls (detailed in online supplemental table 10). Overall, 20% of cases and controls received an HBV vaccine, with the highest proportions observed among children under 1 year of age (cases: 57%, controls: 55%; online supplemental figure 4).

Clinical presentation

Across countries, 94% of AFDUC cases reported fever in the last 10 days and 64% had fever measured at enrolment. The most frequent symptoms in cases were fatigue (71%), followed by headache (53%) and chills (42%) (figure 2; online supplemental table 11). Non-hospitalised cases reported significantly higher frequencies of milder symptoms such as headache, chills and myalgia. Neurological symptoms were predominantly observed in hospitalised cases with marked intercountries variations: 80% of hospitalised cases in SA, 24% in BF and 18% in CI (figure 2; online supplemental table 12). This difference could not be explained by the higher proportion of young cases in SA (online supplemental figure 5). The most common neurological symptoms were seizures and meningitis (online supplemental table 13, online supplemental figure 6). Among all hospitalised SA cases, 45% had seizures compared with 3% and 1% in CI and BF. Meningitis was reported for 25% of hospitalised cases in SA and for 17% and 9% in BF and CI, respectively.

Overall, 58% of cases responded to follow-up calls between 21 and 364 days after enrolment (median: BF 200, CI 30 and SA 31 days). The follow-up rates were highest in SA (73%, n=723), followed by CI (56%, n=1395) and BF (50%, n=577). Among those followed up, deaths were recorded in 22% of cases in BF (n=127), 4% in CI (n=58) and 2% in SA (n=15). Among deceased cases with reported date of death, the median time from enrolment to death was 34 days in BF (n=96), 4 days in CI (n=58) and 16 days in SA (n=13). Across all countries, most deaths occurred in participants aged ≥18 years (BF: 74%, CI: 84%, SA: 87%). In BF, the highest mortality (40%) was observed in the age group of 45+years.

The hospitalisation rate was higher among deceased cases (BF: 98%, CI: 69%, SA: 100%). For the majority of deaths in BF, the declared cause corresponded to the disease reported at enrolment. The cause of death was generally unknown in CI and meningitis was the primary cause reported in SA. HIV, comorbidities and neurological symptoms were more frequently observed across countries in deceased cases compared with survivors or cases with unknown survival status. In SA, 60% (n=9) of deceased cases were HIV positive, compared with 13% of survivors and 18% of cases with unknown survival status.

Overall, integrating biomolecular, serological and bacteriological results, a pathogen was identified in 35% of cases.

Biomolecular results and risk factor analyses

Biomolecular results in AFDUC cases and controls

Overall, 25% of cases' blood samples tested positive for at least one pathogen with varying percentages by country (BF: 31%, CI: 25%, SA: 17%) (figure 3, online supplemental figure 7). In comparison, 14% of controls' blood samples tested positive for at least one pathogen (BF: 25%, CI: 7%, SA: 6%). *P. falciparum* (BF: 27%, CI: 21%), EBV (BF: 6%, CI: 2%) and HBV (BF: 4%, CI: 2%) were the most frequently observed pathogens in cases in West Africa. In SA, the most frequent pathogens were EBV (15%), EV (1%) and AdV (1%).

Codetection of two or more pathogens was observed in 2.3% of cases and 1.1% of controls, most often involving *P. falciparum* (1.8% of cases and 1% of controls).

After PCR confirmation of fever chip results, EBV and HBV detection rates declined sharply in BF and

Table 1 Main enrolment and sociodemographic characteristics of the ANDEMIA study participants overall and by country

	Burkina Faso		Côte d'Ivoire		South Africa		Total		
Characteristic	**AFDUC n=1164**	**Control n=447**	**AFDUC n=2494**	**Control n=309**	**AFDUC n=991**	**Control n=411**	**AFDUC n=4649**	**Control n=1167**	**P value**
Year of enrolment									
2018	145 (12.5%)	0 (0.0%)	567 (22.7%)	0 (0.0%)	140 (14.1%)	0 (0.0%)	852 (18.3%)	0 (0.0%)	**<0.001**
2019	284 (24.4%)	0 (0.0%)	946 (37.9%)	0 (0.0%)	352 (35.5%)	34 (8.3%)	1582 (34.0%)	34 (2.9%)	
2020	407 (35.0%)	42 (9.4%)	981 (39.3%)	201 (65.0%)	227 (22.9%)	36 (8.8%)	1615 (34.7%)	279 (23.9%)	
2021	328 (28.2%)	271 (60.6%)	0 (0.0%)	108 (35.0%)	246 (24.8%)	229 (55.7%)	574 (12.3%)	608 (52.1%)	
2022	0 (0.0%)	134 (30.0%)	0 (0.0%)	0 (0.0%)	26 (2.6%)	112 (27.3%)	26 (0.6%)	246 (21.1%)	
Sex									
Female	522 (44.8%)	218 (48.8%)	1355 (54.3%)	148 (47.9%)	437 (44.1%)	193 (47.0%)	2314 (49.8%)	559 (47.9%)	0.285
Male	640 (55.0%)	227 (50.8%)	1137 (45.6%)	161 (52.1%)	549 (55.4%)	216 (52.6%)	2326 (50.0%)	604 (51.8%)	
Missing	2 (0.2%)	2 (0.4%)	2 (0.1%)	0 (0.0%)	5 (0.5%)	2 (0.5%)	9 (0.2%)	4 (0.3%)	
Age group in years									
<1	89 (7.6%)	120 (26.8%)	132 (5.3%)	50 (16.2%)	202 (20.4%)	67 (16.3%)	423 (9.1%)	237 (20.3%)	**<0.001**
1–4	274 (23.5%)	112 (25.1%)	532 (21.3%)	42 (13.6%)	436 (44.0%)	69 (16.8%)	1242 (26.7%)	223 (19.1%)	
5–17	184 (15.8%)	59 (13.2%)	424 (17.0%)	41 (13.3%)	115 (11.6%)	66 (16.1%)	723 (15.6%)	166 (14.2%)	
18–44	377 (32.4%)	97 (21.7%)	1014 (40.7%)	92 (29.8%)	175 (17.7%)	173 (42.1%)	1566 (33.7%)	362 (31.0%)	
45+	240 (20.6%)	56 (12.5%)	391 (15.7%)	84 (27.2%)	61 (6.2%)	36 (8.8%)	692 (14.9%)	176 (15.1%)	
Missing	0 (0.0%)	3 (0.7%)	1 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)	3 (0.1%)	3 (0.3%)	
Age									
Median (IQR)	19.0 (3.0–40.0)	4.0 (0.8–31.0)	20.0 (4.0–36.0)	21.0 (2.0–47.0)	2.0 (1.0–13.0)	19.0 (1.0–32.0)	16.0 (2.0–35.0)	13.0 (1.0–35.0)	**<0.001**
Location of healthcare facility									
Urban	784 (67.4%)	275 (61.5%)	1536 (61.6%)	214 (69.3%)	677 (68.3%)	194 (47.2%)	2997 (64.5%)	683 (58.5%)	**<0.001**
Rural	380 (32.6%)	172 (38.5%)	958 (38.4%)	95 (30.7%)	314 (31.7%)	217 (52.8%)	1652 (35.5%)	484 (41.5%)	
Place of residence									
City	452 (38.8%)	187 (41.8%)	1622 (65.0%)	196 (63.4%)	668 (67.4%)	195 (47.4%)	2742 (59.0%)	578 (49.5%)	**<0.001**
Village	706 (60.7%)	256 (57.3%)	872 (35.0%)	113 (36.6%)	315 (31.8%)	212 (51.6%)	1893 (40.7%)	581 (49.8%)	
Missing	6 (0.5%)	4 (0.9%)	0 (0.0%)	0 (0.0%)	8 (0.8%)	4 (1.0%)	14 (0.3%)	8 (0.7%)	
Contact with animals									
Yes	785 (67.4%)	221 (49.4%)	1219 (48.9%)	125 (40.5%)	86 (8.7%)	45 (10.9%)	2090 (45.0%)	391 (33.5%)	**<0.001**
No	364 (31.3%)	223 (49.9%)	1251 (50.2%)	183 (59.2%)	903 (91.1%)	362 (88.1%)	2518 (54.2%)	768 (65.8%)	
Unknown	10 (0.9%)	0 (0.0%)	24 (1.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)	34 (0.7%)	1 (0.1%)	
Missing	5 (0.4%)	3 (0.7%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	4 (1.0%)	7 (0.2%)	7 (0.6%)	

Continued

Table 1 Continued

Characteristic	Burkina Faso AFDUC n=1164	Burkina Faso Control n=447	Côte d'Ivoire AFDUC n=2494	Côte d'Ivoire Control n=309	South Africa AFDUC n=991	South Africa Control n=411	Total AFDUC n=4649	Total Control n=1167	P value
Requires hospitalisation*									
Yes	907 (77.9%)	123 (27.5%)	490 (19.6%)	21 (6.8%)	966 (97.5%)	281 (68.4%)	2363 (50.8%)	425 (36.4%)	**<0.001**
No	256 (22.0%)	316 (70.7%)	2004 (80.4%)	288 (93.2%)	24 (2.4%)	120 (29.2%)	2284 (49.1%)	724 (62.0%)	
Missing	1 (0.1%)	8 (1.8%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	10 (2.4%)	2 (0.0%)	18 (1.5%)	
HIV status									
Positive	46 (4.0%)	30 (6.7%)	42 (1.7%)	1 (0.3%)	147 (14.8%)	48 (11.7%)	235 (5.1%)	79 (6.8%)	**0.022**
Negative	21 (1.8%)	7 (1.6%)	216 (8.7%)	4 (1.3%)	723 (73.0%)	203 (49.4%)	960 (20.6%)	214 (18.3%)	
Unknown	1097 (94.2%)	410 (91.7%)	2236 (89.7%)	304 (98.4%)	121 (12.2%)	160 (38.9%)	3454 (74.3%)	874 (74.9%)	
Reported ever being vaccinated									
Yes	271 (23.3%)	120 (26.8%)	353 (14.2%)	57 (18.4%)	425 (42.9%)	96 (23.4%)	1049 (22.6%)	273 (23.4%)	**<0.001**
No	52 (4.5%)	19 (4.3%)	670 (26.9%)	73 (23.6%)	25 (2.5%)	38 (9.2%)	747 (16.1%)	130 (11.1%)	
Missing	841 (72.3%)	308 (68.9%)	1471 (59.0%)	179 (57.9%)	541 (54.6%)	277 (67.4%)	2853 (61.4%)	764 (65.5%)	
Among those ever being vaccinated									
Documented receipt of Hepatitis B containing vaccine									
Yes	261 (96.3%)	96 (80.0%)	299 (84.7%)	47 (82.5%)	378 (88.9%)	86 (89.6%)	938 (89.4%)	229 (83.9%)	**<0.001**
No	10 (3.7%)	24 (20.0%)	38 (10.8%)	8 (14.0%)	3 (0.7%)	2 (2.1%)	51 (4.9%)	34 (12.5%)	
Missing	0 (0.0%)	0 (0.0%)	16 (4.5%)	2 (3.5%)	44 (10.4%)	8 (8.3%)	60 (5.7%)	10 (3.7%)	

Denominators are given by AFDUC cases and controls who fulfilled the case and control definitions, respectively, and were tested with the fever chip on blood or CSF. P values are calculated based on the 'total' column, assessing statistically significant differences in characteristics between the AFDUC and control group. The Mann-Whitney U test is used for continuous variables, and the χ^2 test (or Fisher's exact test when needed) is used for categorical variables. P values in bold indicate statistical significance. Additional sociodemographic and clinical characteristics can be found in online supplemental table 10.

*Does the patient's condition require hospitalisation or has the patient been hospitalised?

AFDUC, acute febrile disease of unknown cause; ANDEMIA, African Network for Improved Diagnostics, Epidemiology and Management of Common Infectious Agents; CSF, cerebrospinal fluid.

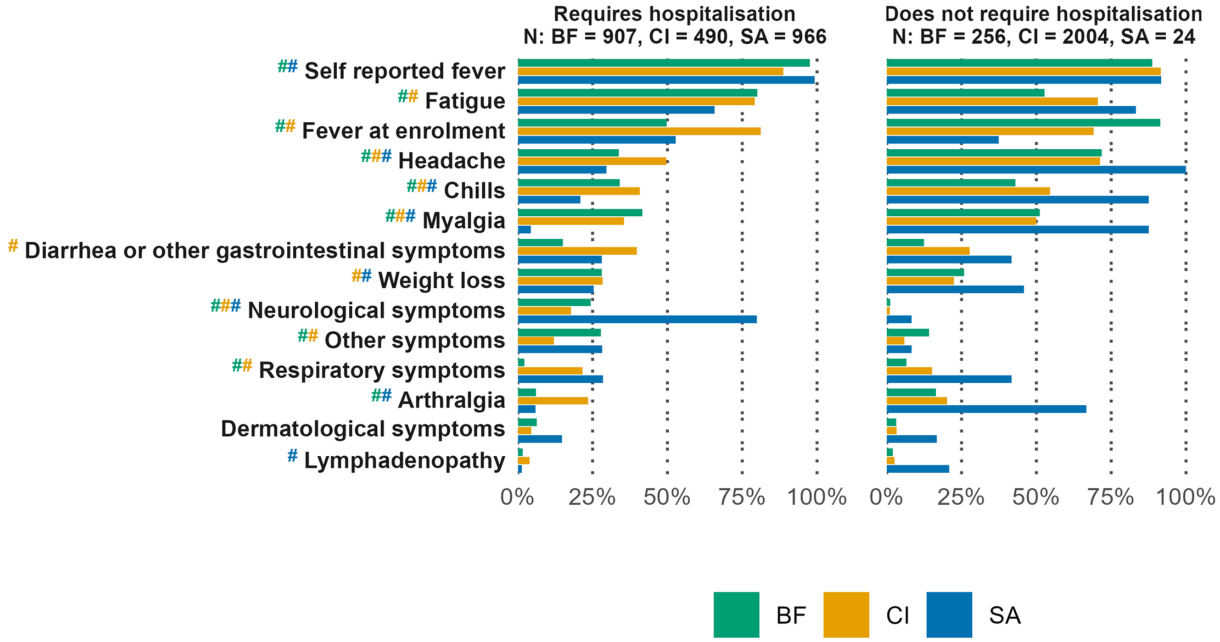

Figure 2 Symptom frequencies (%) in AFDUC cases by country and hospitalisation status. Symptoms are presented from highest to lowest overall frequency. The left panel shows percentages among cases who were hospitalised or required hospitalisation. The right panel shows percentages among cases who did not require hospitalisation. Two cases were excluded due to missing data for hospitalisation (BF n=1, SA n=1). Hashtags next to the symptoms' names indicate statistically significant (p<0.05) differences in the frequency of reported symptoms by hospitalisation status (χ^2 or Fisher's exact test). The colour of the hashtag corresponds to the country for which this difference was significant. There is more than 5% missing data for self-reported fever in SA among those who do not require hospitalisation (n=2). AFDUC, acute febrile disease of unknown cause; BF, Burkina Faso; CI, Côte d'Ivoire; SA, South Africa.

CI (online supplemental figure 8). Only 23% of EBV-positive cases in BF and 44% in CI were PCR-confirmed. For HBV, confirmation rates were 47% in BF and 49% in CI. SA confirmation rates were higher for EBV (84%) and HBV (100%). Confirmation PCR was performed on only 14% of *P. falciparum*-positive cases with a 97% confirmation rate. Confirmation rates for inconclusive tests are shown in online supplemental table 14.

CSF biomolecular analyses were performed in 32 cases in BF and 244 cases in SA. The pathogens identified in BF were EBV (13%), HBV (5%) and *P. falciparum* (3%). EBV was the most frequently detected pathogen in SA (3%), while all other pathogens had detection rates under 1% (online supplemental figure 8, online supplemental table 15). All EBV positive cases in BF (n=4) and the majority of EBV positive cases in SA (88%, n=7) had neurological symptoms. Details about the population with CSF tests can be found in online supplemental table 16.

Among deceased cases with available blood results, at least one pathogen was detected in 18% of cases in BF (n=22), 33% in CI (n=19) and 42% in SA (n=5). *P. falciparum* was most frequently detected in BF (7%, n=7) and CI (21%, n=12) but was significantly less frequent in deceased compared with surviving cases in BF (aOR 0.3). HBV was detected in 6% of deceased cases in BF (n=7) and 9% in CI (n=5) and EBV in 6% in BF (n=6) and 9% in CI (n=5). EBV was the only pathogen detected in 42% of deceased cases in SA (n=5), but further analyses were not possible due to the sample size. Among cases with follow-up and CSF results (BF n=1, SA n=4), one deceased SA case tested EBV-positive in CSF. Serology was performed for 54% of followed-up cases in BF (n=309) and for 45% in CI (n=634). Detection rates of CHIKV or DENV in serology in BF and CI did not differ significantly between deceased and surviving cases.

The effect of infection on AFDUC

P. falciparum was a strong risk factor for AFDUC in BF (aOR 2.1) and CI (aOR 6.4) (figure 3). The association remained significant across both villages (aOR: BF 2.3, CI 5.9) and cities (aOR: BF 6.7, CI 8) (online supplemental table 4, online supplemental figure 9) with no evidence of interaction by residence. Age-stratified analyses indicated consistent association across age groups with sufficient data in CI; in BF, however, this association was limited to children aged 1–4 years (online supplemental table 3).

EBV, the second most frequent pathogen, was significantly associated with case-control status only in SA (aOR 2.6). An interaction analysis suggested that the effect was driven by cases from villages (aOR 5.8), whereas no association was observed in cities (aOR 0.9) (online supplemental table 4). Age-specific analysis further indicated that the association was limited to individuals aged 18–44 years (online supplemental table 3, online supplemental figure 9).

HBV, mainly observed in BF and CI, showed no significant association with AFDUC (table 2).

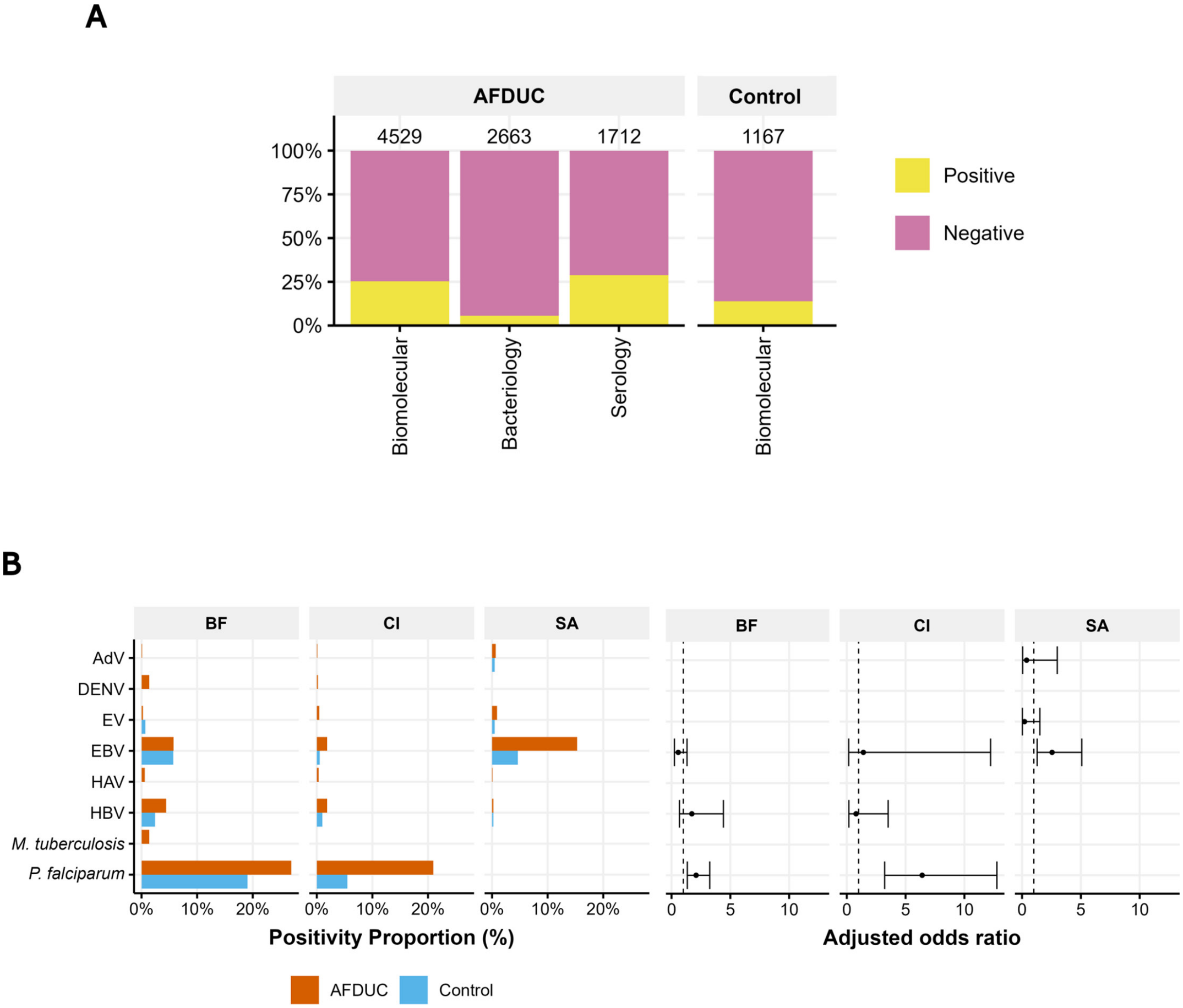

Figure 3 Overview of all blood test results, final result detection rates (%) in blood and adjusted ORs in AFDUC cases and controls by country. (A) Detection rates for pathogens in biomolecular analyses on blood, bacteriological or serological tests. Denominators are shown on top of the bars. Results for each type of test correspond to positives divided by all tested. (B) Detection rates were computed using the final result and taking the ratio of number of positives over number of tested. Only pathogens with at least ten detections on the fever chip for cases or controls are shown. The denominators for AFDUC cases differ by country: CI: n=2494; SA: n=889; for BF due to inconclusive test results, the number of blood samples tested varies by pathogen: DENV, EV, HAV, *Mycobacterium tuberculosis* n=1146; HBV n=1106; EBV n=1008; *Plasmodium falciparum* n=980; AdV n=952. The denominators for controls by country are: CI: n=309; SA: n=411; BF: DENV, EV, HAV, HBV, *M. tuberculosis* n=447; AdV, EBV, *P. falciparum* n=446. ORs are shown with 95% CI and were adjusted for age group, hospital location, residence, HIV status, year of enrolment, month of enrolment, exposure to domestic or wild animal. BF and CI models include the pathogens EBV, HBV and *P. falciparum* and SA model includes the pathogens EBV, EV and AdV. AFDUC, acute febrile disease of unknown cause; AdV, adenovirus; BF, Burkina Faso; CI, Côte d'Ivoire; DENV, dengue virus; EBV, Epstein-Barr virus; EV, enterovirus; HAV, Hepatitis A virus; HBV, Hepatitis B virus; SA, South Africa.

The effect of covariates on infection rates

EBV detection in cases and controls did not differ between villages and cities in SA or BF. In CI, analysis was limited to cases (due to low control positivity) and showed similar results. In contrast, *P. falciparum* was more frequent in village residents: in BF for both cases (aOR 2.6) and controls (aOR 3.4) and in CI for cases only (aOR 1.4). In SA, HIV-positive cases had higher odds of EBV detection (aOR 2.6); analysis was not possible in controls (online supplemental table 17).

No seasonal trends were observed for EBV, HBV, *P. falciparum*, AdV or EV (online supplemental figures 10 and 11). In CI, *P. falciparum* positivity remained stable, while BF patterns were inconsistent, likely reflecting enrolment variation. EBV detection in SA peaked in 2019–early 2020, with significant association with AFDUC only in 2020. DENV cases clustered in June–August 2021 in BF (14/16) and in May–June 2019 in CI (4/5). Full sensitivity results are available in online supplemental tables 3–9.

We analysed the association between *P. falciparum* fever chip results, prior antimalarial treatment and Malaria rapid diagnostic test (RDT) status at enrolment. Among AFDUC cases in BF and CI, we observed a 77% agreement

Table 2 Detection rates (%) in blood in AFDUC cases and controls, adjusted ORs (aOR) with 95% CI and p values stratified by country

	Burkina Faso				Côte d'Ivoire				South Africa			
Pathogen	**AFDUC**	**Control**	**aOR (95% CI)**	**P value**	**AFDUC**	**Control**	**aOR (95% CI)**	**P value**	**AFDUC**	**Control**	**aOR (95% CI)**	**P value**
AdV	1/952 (0.1%)	0/446 (0.0%)	–	–	2/2494 (0.1%)	0/309 (0.0%)	–	–	6/889 (0.7%)	2/411 (0.5%)	0.4 (0 to 3)	0.36
DENV	16/1146 (1.4%)	0/447 (0.0%)	–	–	5/2494 (0.2%)	0/309 (0.0%)	–	–	0/889 (0.0%)	0/411 (0.0%)	–	–
EV	3/1146 (0.3%)	3/447 (0.7%)	–	–	11/2494 (0.4%)	0/309 (0.0%)	–	–	8/889 (0.9%)	2/411 (0.5%)	0.2 (0 to 1.5)	0.12
EBV	58.2/1008 (5.8%)	25.4/446 (5.7%)	0.6 (0.2 to 1.3)	0.19	45.9/2494 (1.8%)	1.6/309 (0.5%)	1.4 (0.2 to 12.2)	0.75	136/889 (15.3%)	19/411 (4.6%)	2.6 (1.3 to 5.1)	**<0.05**
HAV	7/1146 (0.6%)	0/447 (0.0%)	–	–	8/2494 (0.3%)	0/309 (0.0%)	–	–	1/889 (0.1%)	0/411 (0.0%)	–	–
HBV	49.1/1106 (4.4%)	10.9/447 (2.4%)	1.7 (0.7 to 4.4)	0.25	46/2494 (1.8%)	3.1/309 (1.0%)	0.8 (0.2 to 3.5)	0.75	2/889 (0.2%)	1/411 (0.2%)	–	–
Mycobacterium tuberculosis	16/1146 (1.4%)	0/447 (0.0%)	–	–	0/2494 (0.0%)	0/309 (0.0%)	–	–	0/889 (0.0%)	0/411 (0.0%)	–	–
Plasmodium falciparum	264/980 (26.9%)	85/446 (19.1%)	2.1 (1.3 to 3.3)	**<0.05**	523/2494 (21.0%)	17/309 (5.5%)	6.4 (3.2 to 12.8)	**<0.001**	0/889 (0.0%)	0/411 (0.0%)	–	–

Detection rates were calculated using the final result and taking the ratio of number of positives over number of tested. Only pathogens with at least ten detections on the fever chip for cases or controls are shown. In BF, the number of samples tested varies by pathogen due to inconclusive test results. Multivariable logistic regression was performed with AFDUC/control status as the outcome and pathogen detection as the exposure. Models were adjusted for age group, hospital location, residence (excluded in SA due to high collinearity with hospital location), HIV status, year of enrolment, month of enrolment, exposure to domestic or wild animal. In BF and CI models were adjusted for detection of other pathogens (EBV, HBV, *P. falciparum*) when sufficient data were available. Models were adjusted whenever possible in SA for the pathogens EBV, EV and AdV. P values in bold indicate statistical significance.

AdV, adenovirus; AFDUC, acute febrile disease of unknown cause; BF, Burkina Faso; CI, Côte d'Ivoire; DENV, dengue virus; EBV, Epstein-Barr virus; EV, enterovirus; HAV, hepatitis A virus; HBV, hepatitis B virus; SA, South Africa.

between fever chip and RDT; however, Cohen's test indicated poor concordance (κ=0.02). In BF, prior antimalarial treatment (aOR 1.6) and RDT positivity (aOR 4.1) were both associated with higher odds of *P. falciparum* detection on the fever chip. Full descriptive results are presented in online supplemental table 18.

Serology

IgM serology was performed in 79% of adult cases from BF and CI, mostly from 2019 to 2020 in CI (85%) and less so in BF (45%) (online supplemental table 19).

DENV IgM was detected in 20% of the tested cases in BF (n=593) and 22% in CI (n=1119) (online supplemental table 20). Co-detection with CHIKV IgM occurred in 11% of BF cases and 15% of CI cases. In BF, 6% of DENV IgM-positive cases also tested positive for DENV by fever chip (online supplemental table 21). Multivariable analysis showed significantly higher DENV IgM detection in villages compared with cities in BF (aOR 2.2), while no village-city differences were seen in CI. However, in CI, DENV IgM rates were significantly higher (aOR 1.5) in the southwest sentinel sites (Guiglo and Tai) compared with the centre (Bouaké and Brobo).

CHIKV IgM was detected in 20% of BF cases and 21% of CI cases (online supplemental table 20). The apparent village-city difference in BF disappeared after adjusting for month and year of enrolment. In CI, no village-city effect was seen, but CHIKV IgM detection was significantly higher in the southwest compared with the centre (aOR 1.7).

No clear seasonal patterns for either virus were observed in BF or CI (online supplemental figure 12).

Bacteriology

At enrolment, 64% of cases (n=3582) had fever (≥38°C) and were eligible for BC. A total of 3178 cultures were collected, mainly from BF (n=1085) and CI (n=1800). Of these, 84% were negative (n=2682), 10% grew contaminants (n=325) and 5% yielded clinically relevant bacteria (n=171). Contamination was significantly more frequent in rural than urban sites in BF and CI (p<0.0001). The leading pathogens were *Staphylococcus aureus* and *Salmonella* spp (online supplemental figure 13).

DISCUSSION

The ANDEMIA study included a substantial, gender-balanced cohort of 6100 AFDUC cases and 1455 controls across CI, BF and SA, allowing for cross-context analyses through clinical surveillance in urban and rural settings. Using biomolecular, bacteriological and serological methods, we confirmed malaria's role but also highlighted the contribution of viral and bacterial pathogens to acute febrile illness. The detection of EBV and HBV as the most common viral pathogens and the high arboviral IgM seropositivity suggests that viral infections may be underappreciated contributors to AFDUC in SSA. Nevertheless, no aetiological agent was identified in two-thirds of cases, reflecting diagnostic gaps and the possible role of uncharacterised pathogens.

Consistent with previous studies, the common symptoms among AFDUC cases included fever, fatigue and headache.[24 25] Neurological symptoms were most common among hospitalised patients, particularly in SA, and were associated with poor outcomes, highlighting the need for increased awareness and monitoring of neurological complications in severe AFDUC.[15 26] In line with this finding, frequently detected viruses in our study such as EBV and arboviruses are known causes of acute neurological illness.[27 28] Notably, neurological manifestations were frequent among WNV (72%) and SINV (54%) positive cases previously reported in SA within the ANDEMIA study.[15 16] Outcome follow-up revealed strikingly high mortality in BF (22%), where deaths were often attributed to the same acute febrile episode that prompted enrolment. In contrast, mortality was lower in CI and SA, and the leading causes of death were respectively unknown or attributed primarily to meningitis. Across sites, HIV-positivity, comorbidities and neurological symptoms were more common among deceased individuals, indicating these as potential risk factors.[29 30] These findings emphasise the importance of close monitoring of severe AFDUC and better management of patients with comorbidities.

Among viruses tested by PCR, EBV was the most frequently detected in blood samples overall, consistent with its known endemicity in SSA[31] and was also present in CSF, pointing to possible neurological involvement. Its detection varied by year and country, with a decline during COVID-19 restrictions in SA, suggesting effects of reduced social contact.[32 33] A significant association between EBV and AFDUC was found in SA patients aged 18–44 years, supporting evidence that primary infection is more symptomatic in adults.[34] HBV detection rates matched the WHO estimates,[35] but were lower in SA, possibly reflecting earlier vaccine introduction (1995) compared with CI (2001) and BF (2006).[36 37] While PCR can also detect persistent infections,[38 39] we believe these pathogens still contribute to febrile illness, via viral reactivation or coinfection with *Plasmodium* spp exacerbating clinical presentation.[40 41] Further investigation into the disease associations and ensuring sustained HBV vaccine coverage are recommended.

Arboviral serology revealed high DENV and CHIKV IgM positivity in BF and CI (20–22%), with marked urban-rural and regional differences. In rural BF, higher seropositivity may relate to housing quality and water storage, while in CI, the western humid forested regions showed higher detection than central areas, consistent with ecological variation in vector activity. These findings are in line with prior reports linking vegetation as well as urbanisation to vector-borne disease spread.[12 42 43] Importantly, the discrepancy between high IgM rates and low PCR detection aligns with the short viremic phase of flaviviruses and alphaviruses, emphasising the need for serological surveillance. Prior ANDEMIA data also confirmed WNV and SINV as overlooked but important causes of neurological febrile illness in SA. Between 2019 and 2021, WNV IgMs were detected in 9.1% of patients while SINV IgM positivity reached 19.3%, both were more common in rural Mpumalanga.[15 16] Our findings align with previous studies indicating a substantial arboviral burden in SSA,[4 12] emphasising the need for improved surveillance and public health interventions.

Despite malaria screening at enrolment, *P. falciparum* remained the most common pathogen in BF and CI, with detection strongly associated with positive RDTs and prior antimalarial treatment in BF. Among pretreated individuals, malaria detection may reflect incomplete clearance or persistent low-level parasitaemia despite drug use.[44] Moreover, a proportion of RDT-negative cases were fever chip positive, reflecting both PCR's higher sensitivity and possible RDT limitations due to test handling or parasite mutations.[45–47] These findings reinforce the need for improved malaria diagnostic quality assurance and recognition of persistent parasitaemia after treatment.

BC positivity was low (5.4%) with higher contamination rates in rural sites, reflecting infrastructural challenges.[48] Yet, *S. aureus* and *Salmonella* spp emerged as predominant bloodstream pathogens, in line with previous studies in SSA.[6 49] Earlier ANDEMIA data analyses also revealed widespread use of WHO Watch antibiotics, which are prioritised for stewardship due to their higher resistance potential.[50] Our results highlight the urgency of improved diagnostic capacity and antimicrobial stewardship to ensure appropriate treatment of febrile illness.

Differences in case profiles and outcomes across sites are likely to reflect health system disparities. BF, with the lowest physician density and health spending,[51 52] showed the highest mortality. These findings underscore that weak health systems magnify the impact of undiagnosed febrile illness, while robust diagnostic and management capacity, as in SA, can mitigate outcomes despite disease severity.

This large, multicountry study provides valuable cross-national insights into infectious disease aetiologies, but several limitations must be noted. First, as with all case-control studies, recall bias, residual confounding and selection bias in controls cannot be excluded. Matching by age and hospital location was attempted but not fully achieved, and population differences (eg, older cases in BF and CI than in SA) limit generalisability. Although urban sites contributed a slightly higher proportion of cases, rural participants were well represented (41%), allowing setting-specific analyses. Clinical severity also varied: most SA cases were hospitalised with neurological symptoms, while milder cases predominated in BF and CI, likely reflecting disparities in healthcare access and care-seeking behaviour.

Second, pathogen distribution may have been shaped by demographic variation, healthcare access and diagnostic capacity. Because enrolment differed pre-COVID-19 and post-COVID-19, we cannot determine whether the pandemic influenced AFDUC pathogen detection rates in our settings. aORs for rare exposures should be interpreted with caution, and time lags between case and control enrolment may have biased frequencies, although sensitivity analyses suggested minimal impact.

Third, diagnostic constraints must be acknowledged. The fever chip, while sensitive, is not a gold standard and may have produced false positives, particularly in BF where confirmation rates were low. Confirmation capacity also varied, with fewer tests in CI. Bacteriological testing was hampered by sample quality and higher contamination in rural sites, likely due to equipment and training limitations. Limited sampling and absence of anaerobic culture may have reduced sensitivity compared with studies using gold-standard bacteriological methods or molecular approaches.[4 53] We also lacked detailed immune status measures (eg, lymphocyte counts), which may influence susceptibility to infection.

The ANDEMIA study demonstrates the complexity of diagnosing AFDUC in SSA, where parasites, viruses and bacteria co-circulate and malaria is not the sole contributor. Beyond *P. falciparum*, we found that EBV, HBV and arboviruses are important but often overlooked causes of febrile illness. Yet, despite broad testing, most cases remained without an identified aetiology, underlining the need for improved diagnostics and pathogen discovery. Strengthening laboratory capacity, including quality management, serology for pathogens with short viremia, and next-generation sequencing, will be essential to close this diagnostic gap.

From a public health perspective, our results emphasise the importance of surveillance systems that capture non-malarial febrile illnesses, particularly arboviruses, which are likely to expand with climate change and urbanisation. Differences across regions and rural-urban sites also highlight the need for tailored vector control and case management strategies. Finally, the high mortality in BF suggests that weak health systems amplify the burden of AFDUC, reinforcing the need for better access to diagnostics, appropriate treatment and antimicrobial stewardship to improve outcomes and reduce unnecessary antibiotic use.

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Ethics approval This study involves human participants and the ANDEMIA study adheres to the tenets of the Declaration of Helsinki, as well as any national legislation and ethical standards. All participating institutions obtained approval from national ethics committees ((Côte d'Ivoire: Comité National d'Ethique des Sciences de la Vie et de la Santé de Côte d'Ivoire (105/MSHP/CNER-dk); Burkina Faso: Comité d'Ethique pour la Recherche en Santé (2017–5-057); South Africa: Faculty of Health Research Ethics Committee (Medical), University of Witwatersrand (M170403), Faculty of Health Sciences Research Ethics Committee, University of Pretoria (101/2017); Germany: Ethikkommission – Ethikausschuss am Campus Virchow-Klinikum, Charité (EA2/230/17)). Participants gave informed consent to participate in the study before taking part.

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