Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE): protocol for a multisite prospective observational study of the causes of fever in Africa and Asia

Heidi Hopkins,1 Quique Bassat,2,3,4,5 Clare IR Chandler,6 John A Crump,7 Nicholas A Feasey,8,9 Rashida A Ferrand,1,10 Katharina Kranzer,1,10,11 David G Lalloo,12 Mayfong Mayxay,13,14 Paul N Newton,1,13,15 David Mabey,1 FIEBRE Consortium

ABSTRACT

Introduction Fever commonly leads to healthcare seeking and hospital admission in sub-Saharan Africa and Asia. There is only limited guidance for clinicians managing non-malarial fevers, which often results in inappropriate treatment for patients. Furthermore, there is little evidence for estimates of disease burden, or to guide empirical therapy, control measures, resource allocation, prioritisation of clinical diagnostics or antimicrobial stewardship. The Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE) study seeks to address these information gaps.

Methods and analysis FIEBRE investigates febrile illness in paediatric and adult outpatients and inpatients using standardised clinical, laboratory and social science protocols over a minimum 12-month period at five sites in sub-Saharan Africa and Southeastern and Southern Asia. Patients presenting with fever are enrolled and provide clinical data, pharyngeal swabs and a venous blood sample; selected participants also provide a urine sample. Laboratory assessments target infections that are treatable and/or preventable. Selected point-of-care tests, as well as blood and urine cultures and antimicrobial susceptibility testing, are performed on site. On day 28, patients provide a second venous blood sample for serology and information on clinical outcome. Further diagnostic assays are performed at international reference laboratories. Blood and pharyngeal samples from matched community controls enable calculation of AFs, and surveys of treatment seeking allow estimation of the incidence of common infections. Additional assays detect markers that may differentiate bacterial from non-bacterial causes of illness and/or prognosticate illness severity. Social science research on antimicrobial use will inform future recommendations for fever case management. Residual samples from participants are stored for future use.

Ethics and dissemination Ethics approval was obtained from all relevant institutional and national committees; written informed consent is obtained from all participants or parents/guardians. Final results will be shared with participating communities, and in open-access journals and other scientific fora. Study documents are available online (https://doi.org/10.17037/PUBS.04652739).

Strengths and limitations of this study

- Harmonised protocol at multiple sites to allow comparison of results across diverse epidemiological, geographic and cultural settings.
- Collection of data from inpatients, outpatients and community controls of all ages ≥2 months, at multiple sites across Africa and Asia, over the course of >12 months at each site to capture seasonal variation.
- Inclusion of a control group at each site to aid attribution and to allow estimation of disease incidence.
- Standardised diagnostic testing at pathogen-specific reference laboratories according to internationally accepted clinical case definitions.
- Current study limited to only five sites; protocol and supporting documents are freely available to other researchers who may wish to undertake similar work.

INTRODUCTION

Fever is one of the most common symptoms leading to healthcare seeking and hospital admission in sub-Saharan Africa and Asia.1,2 Current age-specific WHO algorithms for the primary care level provide only limited guidance to clinicians for the management of non-malarial fevers. If the malaria test is negative, the patient is classified as ‘fever: no malaria’ in the Integrated Management of Childhood Illness guidelines3 or in the Integrated Management of Adolescent and Adult Illness guidelines,4 and advice is given to ‘treat according to the apparent cause of fever.’ Many febrile illnesses present with
non-specific symptoms and signs, and the current recommendations often result in treatable diseases being left untreated or treated with inappropriate antimicrobials on the one hand and overtreatment of self-limiting conditions with antimicrobials on the other, with important implications for the development of antimicrobial resistance.\textsuperscript{5,6}

Little is currently known about the causes of fever in many low-income and middle-income countries (LMICs),\textsuperscript{7-9} so there is sparse evidence on which to base empirical treatment guidelines for febrile patients, especially in more remote areas. Some studies provide an indication of the clinical spectrum of febrile illness,\textsuperscript{10,11} but these studies were often disease specific, for example, focussing on urinary tract infections in Nigeria\textsuperscript{12} or arboviruses in Asia.\textsuperscript{13} A few studies designed to look at aetiologies of fever in given locations have been published recently.\textsuperscript{2,14-17} While the results are useful within the specific study areas, the epidemiology of infections varies in place and time, so the generalisability of single-site studies is uncertain. Furthermore, the study approaches were heterogeneous—with differences in patient age, type of health facility, seasons covered, inclusion criteria, study design, sampling techniques and pathology tests employed—making it difficult to compare findings across sites and to produce a clear picture of the most common causes of fever in each geographical setting, age group and at each level of care. In addition, there is disabling heterogeneity in eligibility criteria, case definitions, use of diagnostic tests that are not sufficiently validated or standardised and lack of control groups, preventing calculation of attributable fractions (AFs). Recently, two multisite, prospective, case–control studies demonstrated the potential of using harmonised research protocols with standardised diagnostic methods to investigate the causes of clinical syndromes with high morbidity and mortality in resource-limited settings: the Global Enteric Multicenter Study\textsuperscript{18} and the Pneumonia Aetiology Research for Child Health study\textsuperscript{19} determined the predominant infectious causes of diarrhoea and pneumonia, respectively, among children in multiple African and Asian countries.

Improved diagnosis and treatment of febrile illness matter both for the care of individual patients and for public health goals. Besides data gaps on prevalence of specific infections in febrile patients, there is very little information on incidence for many of the infections thought to be clinically important in Africa and Asia. Consequently, there is little information on which to base estimates of burden of disease or to guide empirical therapy, control measures and resource allocation.\textsuperscript{20}

In addition, the ability to differentiate between bacterial and viral infections and between broad groups of bacterial pathogens based on antimicrobial susceptibility, could have a major global impact on antimicrobial resistance by limiting the unnecessary use of antimicrobials. However, there are limited data on antimicrobial usage and how and why the frequency and appropriateness of usage vary across LMICs. There is increasing emphasis on identification and incorporation into point-of-care diagnostic tests of markers of immune and endothelial activation (hereafter ‘biomarkers’) that can distinguish between bacterial causes of fever requiring antimicrobial treatment and viral or self-limiting infections,\textsuperscript{21} or that can identify current or incipient severe illness.\textsuperscript{22,23}

The Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE) study has been designed to help address these information gaps. FIEBRE is a multisite investigation in paediatric and adult outpatients and inpatients, using standardised clinical, reference laboratory and social science protocols, in low-resource regions from which few or no data are available. FIEBRE is being conducted at five sites in sub-Saharan Africa and South-eastern and Southern Asia, and the full protocol, data collection forms, standard operating procedures and other supplementary information are freely available to researchers who may wish to conduct harmonised work at other sites (accessible on the FIEBRE study website (https://doi.org/10.17037/PUBS.04652739) or from co-investigators). This paper describes the clinical, epidemiological and laboratory activities of FIEBRE, which seek to identify infections that are treatable (eg, with specific antimicrobials) and/or preventable (eg, with vaccination or vector-control approaches), to document antimicrobial susceptibility in isolated micro-organisms and to evaluate biomarkers that may be useful in distinguishing bacterial from other causes of fever and/or in prognosis. An overview of the social science work and its relationship to the broader study is also provided, with country-specific protocols available on the FIEBRE study website (https://doi.org/10.17037/PUBS.04652739).

**METHODS AND ANALYSIS**

**Study design**

FIEBRE is a study of febrile illness in people aged 2 months and older residing at one of five sites (three sites in sub-Saharan Africa, one in South-eastern Asia and one in Southern Asia). The study’s specific objectives are listed in box 1. Patients who present with fever at the selected facilities are recruited (day 0) if they or their guardians/caregivers (in the case of minors or unconscious patients) provide written informed consent. Study staff take a targeted illness and exposure history and perform a physical examination. Nasopharyngeal and/or oropharyngeal swabs and a venous blood sample are collected from all participants; a urine sample is collected from selected participants. Tests for malaria and for HIV (at sites where HIV prevalence exceeds 1% in the general adult population, for patients not already known to be infected), serum cryptococcal antigen (CrAg) and urinary lipoarabinomannan (uLAM) detection, and blood and urine cultures are performed on site; bacteria and fungi isolated from clinical specimens are identified and tested for antimicrobial susceptibility. At day 28 after enrolment, study patients are asked to provide a further venous blood sample for serology, and clinical outcome is evaluated.
Study patients are managed by the clinical staff responsible for usual patient care at each study site, according to local standard of care. Results of diagnostic tests performed at or near the study site are provided to the clinical staff as soon as available. Other diagnostic tests are performed at internationally recognised reference laboratories (see the Specific laboratory assessments section).

Recruitment to the study is over a minimum continuous 12-month period at each site to ensure that seasonal variations in causes of fever are captured. Blood and pharyngeal samples from matched community controls enable the calculation of AFs. In addition, control participants are surveyed to obtain representative data about treatment seeking and medicine use. By combining data on causes of fever at study sites with the estimate of the proportion of patients with fever seeking care at those facilities, the incidence of common infections in the study area can be estimated, in order to contribute to efforts to define the burden and impact of infectious diseases.24-27

Social science research is conducted with purposive samples of prescribers, medicine sellers and residents in the study catchment areas in two countries, as well as with stakeholders in the wider public health community. Participant recruitment began in Zimbabwe in June 2018, in Malawi in July 2018, in Laos in October 2018 and in Mozambique in December 2018; following confirmation of funding, a fifth site is expected to begin in Bangladesh in mid-2020.

Data and sample collection at the time of patient enrolment (day 0)

At patient enrolment, study staff collect basic demographic data and information on the history of the present illness. A study staff clinician performs a physical examination, including signs that may be used to calculate a
<table>
<thead>
<tr>
<th>Site-specific ethics committees*</th>
<th>Bangladesh Medical Research Council National Research Ethics Committee, Chittagong Medical College Ethical Review Committee, Oxford Tropical Research Committee†</th>
<th>National Ethics Committee for Health Research, Oxford Tropical Research Ethics Committee†</th>
<th>University of Malawi College of Medicine Research and Ethics Committee, Liverpool School of Tropical Medicine Research Ethics Committee†</th>
<th>Comité Institucional de Bioética para a Saúde do Centro de Investigação em Saúde de Manhiça, Comité Nacional de Bioética em Saúde de Moçambique</th>
<th>Medical Research Council of Zimbabwe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of health facilities where patients are recruited</td>
<td>CMCH and Bangladesh Institute of Tropical and Infectious Diseases</td>
<td>Phonhong Vientiane Provincial Hospital</td>
<td>Chikwawa District Hospital</td>
<td>Manhiça District Hospital</td>
<td>Harare Central Hospital, Chitungwiza General Hospital and three primary care clinics in Harare City</td>
</tr>
<tr>
<td>Region of country</td>
<td>Southeast</td>
<td>Northwest</td>
<td>South</td>
<td>South</td>
<td>North central</td>
</tr>
<tr>
<td>Demographic classification</td>
<td>Urban, periurban and rural</td>
<td>Periurban and rural</td>
<td>Rural</td>
<td>Rural</td>
<td>Urban</td>
</tr>
<tr>
<td>HIV epidemiology (2018 national seroprevalence among adults aged 15–49 years‡ unless otherwise indicated)</td>
<td>&lt;0.1%, no site-specific estimates available</td>
<td>0.3%, no site-specific estimates available</td>
<td>9.2%, no site-specific estimates available</td>
<td>12.6%, 39.7% among adults aged 18–47 years in Manhiça in 2012§</td>
<td>12.7%, 11.5% in Harare‡</td>
</tr>
<tr>
<td>Malaria epidemiology</td>
<td>Low transmission of <em>Plasmodium falciparum</em> and <em>P. vivax</em>, peaking from June to September; 2013–2016 average annual incidence of 4.53 per 1000 population¶; in 2019, 1.7% of CMCH febrile inpatients screened had positive malaria test**</td>
<td>Low transmission of <em>P. falciparum</em> and <em>P. vivax</em>; &lt;1% of symptomatic patients in 2008–10 had laboratory-confirmed malaria††</td>
<td>Perennial transmission of <em>P. falciparum</em>, peaking from December to May; over 12 months in 2016–2017, 12.5% of surveyed children aged &lt;5 years had symptomatic malaria‡‡</td>
<td>Perennial transmission of <em>P. falciparum</em>, with marked seasonality peaking from November to April; approximately 7% malaria prevalence in children &lt;5 years of age§§</td>
<td>No local malaria transmission; Harare health facilities may receive malaria-infected patients referred or visiting from endemic areas of Zimbabwe¶¶</td>
</tr>
</tbody>
</table>

Continued
severity score (eg, FEAST Paediatric Emergency Triage score\textsuperscript{28} and Lambarène Organ Dysfunction Score\textsuperscript{29,30} for children aged $<15$ years, and quick Sequential Organ Failure Assessment\textsuperscript{31–33} and the "universal vital assessment"\textsuperscript{34} score for older patients).

Study staff collect pharyngeal swabs and a venous blood sample from each participant using standard age-based and weight-based thresholds for blood volumes obtained.\textsuperscript{35} In addition, a urine sample is collected from patients aged $<2$ years (using clean-catch methods where possible, although this is recognised to be challenging) and from older patients who have dysuria, frequent micturition, suprapubic tenderness or costovertebral angle tenderness. Study staff prepare the samples and conduct the diagnostic tests described. All other care is provided by health facility staff according to local standards.

The FIEBRE study collects clinical samples for two purposes: for assays that are of immediate clinical benefit to patient care (malaria testing, HIV testing, serum CrAg, uLAM, and blood and urine cultures, performed at or near the clinical site) and for research purposes (serological and nucleic acid assays for pathogen-specific diagnoses, assays of immune and endothelial activation markers, and RNA analysis in a subset of participants, all of which will be done in the future at specialised laboratories).

### Data and sample collection at the time of patient follow-up (day 28)

All patients are asked to return to the study site for one follow-up visit 28 days after enrolment (acceptable range: 26–48 days, inclusive, after enrolment). At each patient’s day 28 follow-up visit, study staff record the clinical outcome of the illness (complete recovery, improvement but incomplete recovery, same as on day 0, worse than on day 0, death and loss to follow-up) and obtain a convalescent venous blood sample for paired serology and biomarker testing. In the event that a patient is lost to follow-up or deceased, information is collected from other household members where possible.

### Recruitment, data and sample collection for control participants

Interpreting the results of some serological, molecular and pharyngeal swab assays requires knowledge of background prevalence of infection or colonisation in the study population. To address this need, $\geq$600 control participants are enrolled at each study site. Control participants are community members in the study site health facilities’ catchment areas, frequency matched 1:2 (or $>1:2$, where logistically feasible) to participating outpatients by month of enrolment, age, gender and geographical location of residence to the outpatients. No controls are specifically recruited for the inpatient population, as inpatients may be referred to the participating health facilities’ catchment areas, frequency matched 1:2 (or $>1:2$, where logistically feasible) to participating outpatients by month of enrolment, age, gender and geographical location of residence to the outpatients. No controls are specifically recruited for the inpatient population, as inpatients may be referred to the participating health facilities’ catchment areas, frequency matched 1:2 (or $>1:2$, where logistically feasible) to participating outpatients by month of enrolment, age, gender and geographical location of residence to the outpatients. No controls are specifically recruited for the inpatient population, as inpatients may be referred to the participating health facilities’ catchment areas.
community health workers, where locally appropriate. Controls are recruited two times per month at each site and enrolled if they or their parents/guardians provide informed consent. The informed consent document and process for controls include an explanation that control participants are not likely to benefit directly from study participation, but that their participation may lead to better understanding of febrile illnesses in their community and others like it.

Study staff collect basic demographic data from control participants. Sample collection and diagnostic testing are identical for controls and patients, with three exceptions: blood for culture, convalescent sera and urine are not collected from controls. Venous blood is drawn from control participants using standard age-based and weight-based volume guidelines.

Healthcare utilisation survey for estimation of incidence of infections
In addition, questionnaires are administered to the community control participants in order to capture representative data about treatment seeking and antimicrobial and other medicine use. The control (or healthcare decision-maker if the participant is a child) is asked about treatment-seeking practices for each household member. This healthcare utilisation survey provides an estimate of the period prevalence of fever, as well as the proportion of individuals with fever in the community who present to the study enrolment sites for care. The fraction of people with fever presenting to a study site will be used to estimate the population-based incidence of fever overall and the incidence of specific causes of fever in the catchment area of study healthcare facilities.

Social science methods
To capture responses to febrile illness and the nature of antimicrobial use among prescribers, medicine retailers and residents, social scientists use qualitative and quantitative methods derived from medical anthropology. The first research phase involves household medicine surveys in the study catchment areas, a central feature of which is the use of ‘drug bags’ (a collection of physical examples of locally available antibiotics) that enable the production of qualitative and quantitative data about antibiotic recognition, use and access. Details of this method have been described elsewhere. The second phase is longitudinal ethnographic fieldwork, including participant observation and key informant interviews with residents, medicine retailers (pharmacists, drug shop workers and market vendors) and healthcare workers in clinics and hospitals. With prescribers and retailers, qualitative methods are complemented and contextualised by the collection of quantitative data about antimicrobial prescription, stocks and sales. Alongside ethnography, in-depth interviews with stakeholders in the wider public health community are conducted to situate local fever management and antimicrobial use within broader public and global health discourses.

Specific laboratory assessments
Laboratory assessments for detection and diagnosis of infectious causes of fever focus on those that are treatable and/or preventable (table 2). With a few exceptions, the same pathogens are sought in samples from all participants at all sites, including: blood parasites; bacterial, mycobacterial and fungal bloodstream infections; typhus group and spotted fever group Rickettsia spp; Orientia tsutsugamushi; Coxiella burnetii; Leptospira spp; Brucella spp; Borrelia spp that cause relapsing fever; Leishmania spp; and arboviruses.

Table 2 describes pathogen-based diagnostic tests that are performed at or near the point of care at each study site; these results are made available in real time to treating clinicians for use in patient care decisions. In order to standardise diagnostic testing for study results, external quality assessment of site results and further diagnostic assays for which capacity does not exist currently near the research sites are performed at internationally recognised reference laboratories (table 2). Cryopreserved samples of all microorganisms isolated in culture from blood and urine are shipped on dry ice to a reference laboratory for confirmation of identification and of antimicrobial susceptibility testing to international standards. Participants’ pharyngeal swabs and blood samples (EDTA whole blood, serum, plasma anduffy coat) are aliquoted, stored at −80°C and shipped to the collaborating reference laboratories. For each pathogen of interest, all samples from all study sites are tested at the same reference laboratory, and diagnostic strategies meet internationally accepted laboratory-based case definitions.

In addition to diagnostic testing for specific infectious agents, a set of assays is carried out to detect host (patient) biomarkers that have been identified in previous studies as potentially useful in differentiating between bacterial and non-bacterial causes of illness and/or as prognosticators of illness severity. These include C reactive protein, a triggering receptor expressed on myeloid cells (sTREM-1), angiopoietin 2, 43 44 heparin-inducing protein40 44 45 and others. The biomarkers sought prioritise assays that are most likely to lead to public health benefit in fever case management for patient populations typified by FIEBRE participants. The diagnostic and prognostic value of these biomarkers will be assessed to determine their utility alone and in combination for predicting severe outcomes, using mortality and severity scores as endpoints.

Sample archive
Informed consent is sought from study participants or their parents/guardians at recruitment for the future use of their biological samples and anonymised data, including for the development and evaluation of new diagnostic tests, for example, new point-of-care diagnostic tests intended to guide the management of febrile patients and
Table 2  Pathogen-based diagnostic testing for the Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE)

<table>
<thead>
<tr>
<th>Infection or pathogen sought</th>
<th>Sample type</th>
<th>Diagnostic test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathogen-based diagnostic tests to be performed at or near the point of care</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Malaria (Plasmodium species)</em></td>
<td>EDTA whole blood</td>
<td>Antigen-detecting lateral flow malaria rapid diagnostic test (mRDT)</td>
<td>Combination test detects histidine-rich protein 2 and <em>Plasmodium</em> lactate dehydrogenase</td>
</tr>
<tr>
<td></td>
<td>Thick and thin blood smear</td>
<td>Expert light microscopy for the presence versus the absence of asexual parasites, species and density</td>
<td>For all mRDT-positive samples and 10% of mRDT-negative samples</td>
</tr>
<tr>
<td><strong>HIV</strong></td>
<td>EDTA whole blood</td>
<td>Antibody-detecting rapid tests according to national guidelines</td>
<td>Confirmatory molecular testing according to national guidelines for infants who test antibody-positive</td>
</tr>
<tr>
<td><strong>Bacteraemia and/or fungaemia</strong></td>
<td>Whole blood</td>
<td>Aerobic culture, identification and antimicrobial susceptibility testing performed for isolated microorganisms</td>
<td>Single culture bottle; blood volume of ≤10mL, weight-based volumes for small children</td>
</tr>
<tr>
<td><strong>Mycobacteraemia</strong></td>
<td>Whole blood</td>
<td>Mycobacterial culture</td>
<td>For patients aged ≥15 years who are HIV-infected and/or admitted as inpatients</td>
</tr>
<tr>
<td><strong>Mycobacterium tuberculosis</strong></td>
<td>Urine</td>
<td>Urinary lipoarabinomannan rapid test</td>
<td>For patients who are HIV-infected and/or admitted as inpatients</td>
</tr>
<tr>
<td><strong>Cryptococcus species</strong></td>
<td>Serum</td>
<td>Antigen-detecting lateral flow rapid diagnostic test</td>
<td>For patients who are HIV-infected and/or admitted as inpatients</td>
</tr>
<tr>
<td><strong>Nitrites and leucocyte esterase (evidence of urinary tract infection)</strong></td>
<td>Urine</td>
<td>Urine dipstick</td>
<td>Urine culture performed on samples positive for nitrites and/or leucocyte esterase</td>
</tr>
<tr>
<td><strong>Bacteriuria</strong></td>
<td>Urine</td>
<td>Culture, identification and antimicrobial susceptibility testing performed for isolated microorganisms</td>
<td>For samples dipstick-positive for nitrites and/or leucocyte esterase</td>
</tr>
<tr>
<td><strong>External quality assessment of diagnostic results obtained at or near the point of care, to be performed at internationally recognised reference laboratories</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Malaria (Plasmodium species)</em></td>
<td>Thick and thin blood smear</td>
<td>Expert light microscopy for the presence versus the absence of asexual parasites, species and density</td>
<td>Randomly selected sample of 10% of microscopy-positive and 10% of microscopy-negative smears from each site</td>
</tr>
<tr>
<td>Bacteria and fungi isolated from blood and urine at sites</td>
<td>Cryopreserved isolates</td>
<td>MALDI-TOF MS for identification and drug susceptibility testing to EUCAST standards</td>
<td>–</td>
</tr>
<tr>
<td>Mycobacteria isolated from blood at sites</td>
<td>Cryopreserved isolates</td>
<td>Identification using subculture and molecular testing, drug susceptibility testing depending on organisms identified</td>
<td>–</td>
</tr>
<tr>
<td><strong>Pathogen-based diagnostic tests to be performed at internationally recognised reference laboratories</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Borrelia</em> species (louse-borne and tick-borne relapsing fevers)</td>
<td>Thick and thin blood smear</td>
<td>Expert light microscopy</td>
<td>Random 10% sample of all smears from each site; if positives are identified, a larger proportion are to be read</td>
</tr>
</tbody>
</table>

Continued
### Table 2  Continued

<table>
<thead>
<tr>
<th>Infection or pathogen sought</th>
<th>Sample type</th>
<th>Diagnostic test</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arboviruses: chikungunya, dengue, Japanese encephalitis, o’nyong ’nyong, Zika</td>
<td>Serum</td>
<td>Africa-specific or Asia-specific IgG ELISA and qPCR, microneutralisation for samples positive by ELISA</td>
<td>A proportion of African samples to be tested for Japanese encephalitis virus, and a proportion of Asian samples to be tested for o’nyong ’nyong virus; if positives are identified, a larger proportion of samples are to be tested</td>
</tr>
<tr>
<td><strong>Brucella species (brucellosis)</strong></td>
<td>Serum</td>
<td>Brucella IgM EIA, <em>Brucella</em> microagglutination test for samples positive by EIA</td>
<td>Convalescent sera screened for exposure using EIA; positives tested by IgM EIA and microagglutination on acute and convalescent sera</td>
</tr>
<tr>
<td><strong>Leptospira species (leptospirosis)</strong></td>
<td>Serum</td>
<td>Microagglutination test</td>
<td>–</td>
</tr>
<tr>
<td><strong>Rickettsiae: Orientia species, Rickettsia (lymph group and spotted fever group), Coxiella burnetii</strong></td>
<td>Serum and buffy coat</td>
<td>IgG and IgM IFA; qPCR for samples positive by serological screen</td>
<td>Buffy coat is preferred sample for <em>Orientia</em> and rickettsial species detection, serum to be used for <em>C. burnetii</em></td>
</tr>
<tr>
<td>Visceral leishmaniasis</td>
<td>Serum</td>
<td>Direct agglutination test</td>
<td>–</td>
</tr>
<tr>
<td><strong>Histoplasma species (histoplasmosis)</strong></td>
<td>Serum</td>
<td>Histoplasma EIA††</td>
<td>–</td>
</tr>
<tr>
<td>Respiratory pathogens: influenza A and B, respiratory syncytial virus†</td>
<td>Serum and buffy coat</td>
<td>Luminex respiratory panel</td>
<td>–</td>
</tr>
<tr>
<td>Paediatric viraemia and/or bacteraemia‡</td>
<td>EDTA whole blood</td>
<td>PCR</td>
<td>(Details to be determined)</td>
</tr>
</tbody>
</table>

*At sites where HIV prevalence is >1% in the general adult population.*

†The Luminex respiratory panel also detects adenovirus, parainfluenza viruses 1–4, enterovirus, rhinovirus, B virus, coronaviruses (229E, OC43, HKu1 and NL63), metapneumovirus, bocavirus, *Legionella pneumophila*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

‡To be performed on samples from children aged <5 years, from whom blood volumes will not be adequate for all serology tests listed.

EIA, enzyme immunoassay; EUCAST, European Committee of Antimicrobial Susceptibility Testing; IFA, immunofluorescence assay; MALDI-TOF MS, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry; mRDT, malaria rapid diagnostic test; qPCR, quantitative PCR.
assays detecting host transcriptomic signatures of specific infections. Residual blood and pharyngeal samples from participants are stored and monitored in a central laboratory facility. Access to the samples follows a formal process of application and requires approval from both the FIEBRE consortium and an independent committee including senior scientists as well as lay members.

Data sharing
Anonymised data outputs are shared on institutional data repositories. All data releases are assigned persistent interoperable digital object identifier (DOI) numbers (ISO 26324). Nucleic acid sequences and associated datasets will be released on relevant data archives (e.g., EMBL-ENA and GENBANK). Data outputs which reasonably, ethically and legally can be shared will be released on open-attribute ShareAlike licenses, such as the Creative Commons Attribution-ShareAlike V.2.0 Generic (CC BY-SA V.2.0).

Sample size considerations
Any single pathogen or fever aetiology is likely to be rare in the study populations. The prevalence of respiratory viruses (e.g., influenza and respiratory syncytial virus) detected by PCR, and of baseline seropositivity to other pathogens, will be compared between cases and controls. The prevalence of seropositivity to common causes of fever is assumed to be approximately 5% in the general population. To identify causes of fever, a sample size of 600 patients per group will enable estimation of the prevalence of an infection whose true prevalence is 5%, with a precision of ±1.7% with 95% confidence, and to estimate the prevalence of an infection whose true prevalence is 1%, with a precision of ±0.8%. A sample of 600 outpatients and 300 controls will provide >90% power to show a significant difference between a prevalence of 12% in cases and 5% in controls. Therefore, 600 febrile patients are to be enrolled in each of four analysis groups (children aged ≥2 months to <15 years, and patients aged ≥15 years, with stratified enrolment so that within each of the two age groups, approximately half are inpatients and half are outpatients), for a total of 2400 patients per site, plus 300 controls in each of the two age groups at each site (total 600). Site-specific recruitment strategies allow enrolment of a representative sample of patients presenting over the course of the study at each site. The social science research will involve 100–150 participants per site, with the sample size determined by data saturation.

Data analysis plan for primary outcomes
To determine the treatable and/or preventable causes of fever in the study population, the AF will be calculated for each pathogen or group of pathogens. This will be done separately for each site and age stratum. For each group of patients, the OR for the association between each pathogen and fever will be calculated, using logistic regression, by comparing cases and controls. Strata based on geographical location and season will be defined at each site, and the analysis will be adjusted for age, sex and stratum. A weighted analysis will be performed to reflect the relative frequency of fevers that present in each season. For pathogen A, the AF (AF_A) will then be calculated as AF_A = p_A(1-OR_A), where p_A is the proportion of cases with pathogen A, and OR_A is the OR for the association between presence of pathogen A and being a case. CIs will be calculated using bootstrap methods.

To determine the prevalence and spectrum of antimicrobial resistance among bacterial pathogens identified in the study, at each site, the proportion of bacterial pathogens with antimicrobial resistance defined by standardised criteria will be calculated. The proportion of common organisms demonstrating resistance to a standard panel of antimicrobials will be reported.

To generate data on incidence of specific infections among study participants and contribute to estimates of disease burden, for an area with known or estimated population size, responses to the healthcare utilisation survey questions will be used to estimate r, the proportion of fevers for which treatment was sought at the study facility. The total number of cases seen at the study site from the defined population will be multiplied by 1/r to obtain the total cases in a year from the defined population. This will be divided by the size of the population to estimate incidence. Incidence of fever caused by specific pathogens will be calculated by multiplying the incidence by the AF for that pathogen.

To assess the performance and potential utility of biomarker assays to guide fever case management in the study areas, each biomarker and biomarker combinations will be compared with mortality and severity scores calculated using clinical data and with pathogen-specific diagnoses.

To generate data to support the development of new fever case management algorithms which may be evaluated in future studies, the association between the presence of pathogens with predefined clinical and other variables will be examined, and social science research findings will shape recommendations. Social science data will be analysed iteratively as themes emerge and are followed up during ethnographic fieldwork. Patterns in the data will be interrogated in consultation with wider social theory, building on our reviews and analyses of ‘the social’ in fever case management and antimicrobial resistance.

Ethics and dissemination
Ethics approval of the study protocol was obtained from all relevant institutional and national committees (table 1). Written informed consent was obtained from all participants, or their parents/guardians, for study participation and for future use of biological samples. No individual participant identities will be used in any reports or publications resulting from the study.

Before beginning study activities at each site, meetings are held with community leaders and representatives of the public, and with staff at participating health facilities.
to provide information about the aims of the study and the methods to be used. When final results are available, feedback and dissemination meetings will be held at each site both for healthcare staff and for the communities who participated in the study.

Investigators and study staff engage with national and international networks to ensure that researchers, public health advocates and policy makers at various levels are aware of the study. The study protocol, standard operating procedures, data collection tools and other study documents are freely available on request from investigators and at the FIEBRE study website (DOI: https://doi.org/10.17037/PUBS.04652739). Press releases and website updates publicise study progress. Study results will be prepared for publication in open-access peer-reviewed journals, and presented at national and international scientific conferences as soon as possible after study completion.

**Patient and public involvement**

Patients were not directly involved in the development of the research questions, the design of this study or the conduct of the study. The FIEBRE study does include substantial interaction with communities in the study areas to promote awareness and acceptance of patient recruitment at health facilities, and to encourage participation as community controls and in social science activities. Study results will be disseminated in participating communities at each site through the collaborating research group’s public engagement teams and community advisory groups, using locally appropriate strategies. Community feedback, as well as findings from social science research, will be incorporated into future recommendations for improved fever case management.

**DISCUSSION**

The FIEBRE study is designed to investigate causes of febrile illness and antimicrobial resistance at multiple sites in Africa and Asia, where currently there is little evidence and very limited diagnostic capacity to guide fever case management. FIEBRE focuses on detecting infections that are treatable (eg, with specific antimicrobials) and/or preventable (eg, with vaccination or vector-control approaches). Across all sites, the study uses a common design, selection criteria, case definitions, laboratory procedures and analysis plan. This harmonised approach will generate reliable and comparable data that can contribute to updated recommendations on the clinical management and prevention of febrile illnesses, adapted to local contexts. In addition, due to the inclusion of community control participants, the study will provide data to support reliable estimates of the incidence and, in turn, burden of disease.

This study provides a unique opportunity to collect and store biomedical samples with data from a large and well-characterised group of febrile patients and controls from representative settings in Africa and Asia. The samples will be useful for identification of novel diagnostic targets and to guide prioritisation for the development and evaluation of new point-of-care diagnostic tests intended to guide the management of febrile patients. New tests could include those that predict severity of illness, detect specific infections, and/or differentiate between bacterial and viral infections to help guide antimicrobial therapy, identified as high priority at a WHO meeting of experts convened in 2015.\(^\text{21}\)

It is anticipated that data generated by FIEBRE on causes of febrile illness and antimicrobial susceptibility, alongside the social science work on the role of antimicrobials in fever case management, will be incorporated into new diagnostic strategies and case management guidelines which can then be evaluated and optimised in various contexts.

**Author affiliations**

1Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK
2ISGlobal, Hospital Clinic - Universitat de Barcelona, Barcelona, Spain
3Centro de Investigación en Saúde de Manhiça (CISM), Maputo, Mozambique
4ICREA, Pg. Lluís Companys 23, Barcelona, Spain
5Pediatric Infectious Diseases Unit, Pediatrics Department, Hospital Sant Joan de Déu (University of Barcelona), Barcelona, Spain
6Department of Global Health and Development, London School of Hygiene & Tropical Medicine, London, UK
7Centre for International Health, University of Otago, Dunedin, New Zealand
8Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
9Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi
10Biomedical Research and Training Institute, Harare, Zimbabwe
11National and Supranational Reference Center for Mycobacteria, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
12Liverpool School of Tropical Medicine, Liverpool, UK
13Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Mahosot Hospital, Vientiane, Lao People’s Democratic Republic
14Institute of Research and Education Development, University of Health Sciences, Ministry of Health, Vientiane, Lao People’s Democratic Republic
15Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK

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**FIEBRE consortium and co-authors** FIEBRE Consortium: Benjamin Amos (Independent consultant, Falmouth, Cornwall, UK), David Bell (Independent consultant, Issaquah, Washington, USA), Stuart D Blacksell (Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Mahosot Hospital, Vientiane, Lao PDR), Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK, Mahidol-Oxford Tropical Medicine Research Unit (MORU), Mahidol University, Bangkok, Thailand), John Bradley (Medical Research Council Tropical Epidemiology Group, LSHTM, London, UK), Vilada Chansamouth (Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Mahosot Hospital, Vientiane, Lao PDR), Matveio Chimenya (Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi), Scott B Craig (WHO/OIC Centre for Leptospirosis Reference and Research, Forensic & Scientific Services, Health

Support Queensland, Brisbane, Australia), David AB Dance (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK; Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Mahosot Hospital, Vientiane, Lao PDR; Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK), Ethel Davay (Biomedical Research and Training Institute, Harare, Zimbabwe), Xavier de Lamballerie (Unité des Virus Emergents (UVE), Aix Marseille Univ, IRD 190, INSERM 1207, HU Méditerranée Infection, 13005, Marseille, France), Justin Dixon (Department of Global Health and Development, LSHTM, London, UK), Audrey Dubot-Pérède (Unité des Virus Emergents (UVE), Aix Marseille Univ, IRD 190, INSERM 1207, HU Méditerranée Infection, 13005, Marseille, France), Michelle M Durkin (MirVista Diagnostics, Indianapolis, Indiana, USA), Colin Fink (Micropathology Ltd, Coventry, UK), Felicity C Fitzgerald (Biomedical Research and Training Institute, Harare, Zimbabwe; University College of London Great Ormond Street Institute of Child Health, London, UK), Stephen R Graves (Australian Rickettsial Reference Laboratory, University Hospital Geelong, Geelong, Australia), Edward W Green, Kate A Haigh (Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK; Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi), Becca L HANDLEY, Martin L Hibberd (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK), E. Ollie Jones (National Leprosy Elimination Programme, Department of Health and Development, LSHTM, London, UK), Jayne Jones (Clinical Diagnostic Parasitology Laboratory, Liverpool School of Tropical Medicine, Liverpool, UK), Kevin C Kain (Tropical Disease Unit, Department of Medicine, UHN-Toronto General Hospital, University of Toronto, Toronto, Ontario, Canada), Pankaj Lal (Liverpool Clinical Laboratories, Liverpool, UK), Sham Lal (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK), Yovel Lubell (Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK), Mahidol-Oxford Tropical Medicine Research Unit (MORU), Mahidol University, Bangkok, Thailand), Teysgen Marias (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK), Florence P Maurer (Superseded Membership Centre for Mycobacteria Research Center Belininz Lung Center, Borstel, Germany), Ioana D Olaru (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK; Biomedical Research and Training Institute, Harare, Zimbabwe), Christopher M Parry (Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK; Institute of Infectious and Global Health, University of Liverpool, UK; School of Tropical Medicine and Global Health, Nagasaki University, Japan), Chrsity h Roberts (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK), John Stenos (Australian Rickettsial Reference Laboratory, University Hospital Geelong, Geelong, Australia), Roni Proctor, Nelson Tembo (Centre de Recherche en Santé de Maniço (CISM), Maputo, Mozambique), James E Ussher (Southern Community Laboratories, Dunedin, New Zealand; Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand), Marta Valente (ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain; Centro de Investigación en Salud de Manchíça (CISM), Maputo, Mozambique), Pi Vitorino (Centro de Investigação en Saúde de Manchíça (CISM), Maputo, Mozambique), Marie-A Voice (Micropathology Ltd, Coventry, UK), L Joseph Wheat (MirVista Diagnostics, Indianapolis, Indiana, USA), Shummay Yeung (Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine (LSHTM), London, UK).

Contributors All coauthors contributed to the design and development of Febrile Illness Evaluation in a Broad Range of Endemicities protocol V.1.0 and/or subsequent amendments. DB, MC, JC, ED, RF, EWO, KAH, HH, KK, DGL, DM, MM, PNU, TD, NT, MV, PV and SY contributed expertise for the clinical, epidemiological and diagnostic activities. CHR and SL led the development of data management processes. BLH and TM contributed laboratory expertise. CIRC, JD and CH led development of social science activities. BA, DB, SD, SBC, DABO, XL, ADP, MM, CF, SRG, MHJ, JJ, KCK, PL, YL, FPM, CMP, JS, EEU, MAV and LJW contributed specialty expertise for laboratory and diagnostic activities. JB drafted the analysis plan and performed the sample size calculations. HH drafted the manuscript. All authors critically revised the manuscript for important intellectual content, and read and approved the final paper. HH is guarantor of the paper.

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ORCID ID Heidi Hopkins http://orcid.org/0000-0003-1076-6758

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