PARASITE-BASED DIAGNOSIS FOR MALARIA IN UGANDA: FEASIBILITY AND COST-EFFECTIVENESS

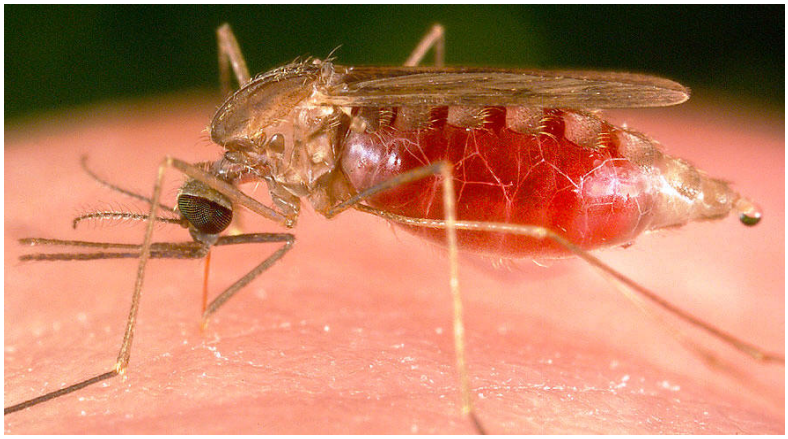
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A thesis submitted to the Directorate of Research and Graduate Training in fulfilment for the award of the degree of Doctor of Philosophy of Makerere University

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Thesis for doctoral degree (Ph.D.)
2012

Parasite-based diagnosis for malaria in Uganda: Feasibility and Cost-effectiveness



Vincent Kiirya Batwala





Negative



Positive

From School of Public Health
Makerere University, Kampala, Uganda

Parasite-based diagnosis for malaria in Uganda: Feasibility and Cost-effectiveness

Vincent Kiirya Batwala



Explanation about the cover pictures

The cover picture on top is a female *Anopheles* mosquito. This is the mosquito species that transmits malaria parasites [http://listverse.com/2007/11/15].

The cover picture on the left is a microscope traditionally used in the investigation of malaria. The current Malaria Policy recommends microscopy as the reference standard [http://www.microscope-manufacturers.com].

The cover picture on the right is the malaria rapid diagnostic test. This particular test is based on Histidine Rich Protein-2 (HRP-2) antigen specific for *Plasmodium falciparum*. If negative, only one coloured band appears in the control window "C." If positive, another coloured band appears in the test window "T."

Declaration

This is a pronouncement that this study is original and has not been submitted for any other degree award to any other university or institution of higher learning before.

…….
Vincent Kiirya Batwala

This thesis has been submitted for examination with the approval of the following supervisors:



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14th November 2012
Date

Dedication

My family and my late parents

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Original papers

This thesis is based on the following scientific articles, which are herein referred to by their Roman numerals.

I Batwala V, Magnussen P, Nuwaha F: Challenges to implementation of artemisinin combination therapy policy in Uganda. *Int Health* 2010, **2**:262-268.

II Batwala V, Magnussen P, Nuwaha F: Are rapid diagnostic tests more accurate in diagnosis of *Plasmodium falciparum* malaria compared to microscopy at rural health centres? *Malar J* 2010, **9**:349.

III Batwala V, Magnussen P, Hansen KS, Nuwaha F: Cost-effectiveness of malaria microscopy and rapid diagnostic tests versus presumptive diagnosis: implications for malaria control in Uganda. *Malar J* 2011, **10**:372.

IV Batwala V, Magnussen P, Nuwaha F: Comparative feasibility of implementing rapid diagnostic test and microscopy for parasitological diagnosis of malaria in Uganda. *Malar J* 2011, **10**:373.

V Batwala V, Magnussen P, Nuwaha F: Antibiotic use among patients with febrile illness in a low malaria endemicity setting in Uganda. *Malar J* 2011, **10**:377.

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Acronyms and Abbreviations

ACT	: Artemisinin-based Combination Therapy
AL	: Artemether-Lumefantrine
CEA	: Cost Effectiveness Analysis
CI	: Confidence Interval
CDD	: Community Drug Distributor
CQ	: Chloroquine
CRF	: Case Report Form
DNA	: Deoxyribonucleic acid
EIR	: Entomological Inoculation Rate
HBMF	: Home-Based Management of Fever
HC	: Health Centre
HIV	: Human Immunodeficiency Virus
HRP-2	: Histidine Rich Protein-2
HSD	: Health Sub District
ICCM	: Integrated Community Case Management
ICER	: Incremental Cost Effectiveness Ratio
JMS	: Joint Medical Store
NMS	: National Medical Store
NRS	: National Referral Hospital
NPV	: Negative Predictive Value
OPD	: Outpatient Department
PCR	: Polymerase Chain Reaction
Pf	: *Plasmodium falciparum*
PFP	: Private For Profit
pLDH	: parasite Lactate Dehydrogenase
PNFP	: Private Not For Profit
PPV	: Positive Predictive Value
RDT	: Rapid Diagnostic Test
RR	: Risk Ratio
RHH	: Regional Referral Hospital
SP	: Sulphadoxine-Pyrimethamine
SPSS	: Statistical Package for Social Sciences
VHT	: Village Health Team
WHO	: World Health Organisation

Operational definitions of terms

Artemisinin-based combination therapy

A combination of artemisinin or one of its derivatives with an anti-malarial of a different class

Capital cost (nonrecurring)

Inputs with useful life of more than one year

Cost-effectiveness analysis

The comparison of the relative expenditure (costs) and outcomes (effects) associated with two or more courses of action. It is typically expressed as an incremental cost-effectiveness ratio (ICER)

Health centre microscopy

Routine investigation of suspected malaria under microscope by resident health centre laboratory personnel

Likelihood ratio

The ratio of the probability of a test result in people who do have the disease to the probability in people who do not

Opportunity cost

The benefits that must be foregone by not allocating resources to the next best activity

Negative predictive value

The probability that a person does not have malaria when a negative test result is observed

Parasitaemia

Presence of malaria parasites in blood

Positive predictive value

The probability that a person is infected with malaria when a positive test result is observed

Presumptive diagnosis of malaria

Patient categorised to have malaria on basis of fever (by statement or measured) without testing blood

Rapid diagnostic test (RDT)

An antigen-based stick, cassette or card test for malaria in which a colored line in the test window indicates that *plasmodial* antigens have been detected

Sensitivity

Percent of patients with malaria infection who have a positive result in the test under evaluation, determined from the result of the reference or 'gold standard' test

Specificity

Percent of patients without malaria infection who have a negative result in the test under evaluation, determined from the result of the reference or 'gold standard' test

Test line

Line in the RDT test window displaying results of test for presence of target antigen

Uncomplicated malaria

Symptomatic infection with malaria parasitaemia without signs of severity and/or evidence of vital organ dysfunction

Unit cost

Also called average cost, computed as total cost over the number of final services

Abstract
From 2006 the first-line treatment for uncomplicated malaria in Uganda was with artemisinin-based combination therapy (ACT). In 2010, Uganda adopted the universal parasite-based diagnosis for malaria before treatment with ACT. There is paucity of information regarding use of ACT and whether it is feasible to implement parasite-based diagnosis for malaria in the country. This study aimed at describing the challenges of using ACT for treatment of malaria and to assess whether it is feasible to implement parasite-based diagnosis for malaria.

Methods: Semi-structured interviews were conducted with patients and health workers at 32 sub-County level health centres in Bushenyi (low transmission) and Iganga district (high transmission). Blood taken from 300 patients with febrile illness were tested for malaria using polymerase chain reaction, light microscopy and rapid diagnostic test (RDTs) to assess the accuracy of light microscopy and of RDT in identifying malaria infection. In a feasibility trial, 30 HCs were randomised to implement parasite-based diagnosis based on RDTs (n=10), blood microscopy (n=10) and presumptive diagnosis (n=10). Feasibility of implementing parasite-based diagnosis was assessed by comparing the cost-effectiveness of RDT and light microscopy; patient waiting time; the proportion of patients with febrile illness who received parasite-based diagnosis; and the type of treatment (either with antibiotics or anti-malarials).

Results: The challenges identified were: stock-out of ACT, continued use of non-recommended anti-malarials, understaffing and lack of parasitological diagnosis. RDT (91%) had a superior sensitivity compared to light microscopy (47.2%) in identifying malaria infection. Use of RDT for parasitological diagnosis was more cost-effective with an incremental cost-effectiveness ratio of US$ 5.0 compared to US$ 9.6 for microscopy per case correctly identified and treated. Patients were more likely to receive a parasitological diagnosis in RDT (96.6%) than with microscopy (60.9%). RDTs reduced patient waiting time compared to microscopy and were more convenient to health workers and patients. Parasite-based diagnosis was associated with a reduction in ACT prescription among patients testing negative. Prescription of anti-malarials was 100% in patients not receiving a parasitological diagnosis as it was in patients testing positive for malaria. Prescription of antibiotics was 41% among patients treated presumptively. Among patients who tested positive for malaria 23.2% were prescribed antibiotics compared to 52.1% in those who tested negative.

Conclusion: RDTs are more attractive than microscopy if parasite-based diagnosis for malaria is to be rolled out in the country. Parasite-based diagnosis reduces the prescription of anti-malarials among patients who test negative but increases the prescription of antibiotics. Measures are needed to reduce the prescription of anti-malarials among patients who test negative as well as for reducing the use of antibiotics among patients with febrile illness.

Key words: Antibiotics, Artemisinin-based Combination Therapy, Diagnostics

1 Introduction

1.1 Background

Malaria is caused by infection of red blood cells with protozoan parasites of the genus *Plasmodium*. The parasites are inoculated into the human host by a feeding female *anopheline* mosquito. The four *Plasmodium* species that infect humans are *P. falciparum*, *P. vivax, P. ovale* and *P. malariae*. Increasingly, human infections with the monkey malaria parasite *P. knowlesi* have been reported in south-east Asia [1].

Malaria poses a threat to the health of the populations, especially in tropical countries [1]. Globally, 149 – 274 million malaria cases were reported in 2010 [2], most of which were among children under five years of age. More than 90% of malaria morbidity and deaths are shouldered by low income countries of sub-Saharan Africa where *P. falciparum* is endemic [3].

Since the malaria eradication programme was abandoned by the World Health Assembly in 1969, the emphasis has been on control of the disease [4], especially on reducing mortality and morbidity by treatment of fever cases through adequate chemotherapy [5]. Thus, early diagnosis with prompt and effective anti-malarials is a cardinal feature of malaria control. Unfortunately, the widespread drug resistance in malaria parasites has hampered efforts to effective treatment [6,7]. Since 2006, most of the countries where *P. falciparum* is endemic have progressively updated treatment policies from the failing chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) to Artemisinin-based

combination therapy (ACTs) [1,3]. However, concerns about the costs and affordability are obstacles to the widespread implementation of the ACT policy [8,9].

The World Health Organisation (WHO) recommends parasitological confirmation in all patients suspected of malaria before treatment is started. Treatment solely based on clinical suspicion should only be considered when a parasitological diagnosis is not accessible [1]. However, a diagnosis of malaria in most outpatient clinics in endemic areas is based on signs and symptoms and treatment is based on this presumptive diagnosis. Due to scarce resources and trained health personnel at local level, presumptive diagnosis appears to be a realistic option. It offers the advantages of ease and speed; usually results in all patients with fever and no apparent other cause being treated for malaria. But because of considerable overlap between the signs and symptoms of malaria with those of other common illnesses, presumptive diagnosis results in misclassification of patients who do not have malaria and unnecessary treatments [10].

The consequences of misdiagnosis of malaria are felt at individual and national levels. At individual level, treating all fevers presumptively as malaria, masks underlying potentially fatal conditions [11,12]. Individuals wrongly diagnosed with malaria are exposed to unnecessary drug adverse reactions and the true cause may not be recognised or treated. This scenario leads to prolonged and worsening illness with loss of income, reduced productivity and school attendance; unnecessary purchase of drugs and other costs associated with repeated visits to health providers. Costs of preventing and treating malaria are already high for vulnerable people and these costs increase if misdiagnosis

and subsequent failure of treatment result in repeated visits to clinics and additional purchase of drugs [13].

From the public health perspective, over diagnosis of malaria results in anti-malarial drug overuse, excessive reporting of malaria cases, under-reporting of diseases that mimic malaria symptoms, increased true or perceived malaria resistance and misallocation of resources [14] with negative influence on drug budgets. Malaria misdiagnosis results in more clinic attendances, putting additional pressure on already the constrained and under-resourced health systems. In areas of high resistance, over-diagnosis of malaria may lead to misperception of the true efficacy of anti-malarial medicines when they appear to "cure" self-limiting febrile conditions mistaken for malaria, against which they are actually ineffective [15].

Attempts to improve the specificity of presumptive diagnosis by including signs and symptoms other than fever or history of fever [16] met with minimal success. The alternative approach is for each malaria treatment to be based on confirmed presence of parasites. This can be achieved either with microscopy or immunochromatography.

Microscopic examination of Giemsa stained blood smears remains the standard of malaria diagnosis [1] because it is able to differentiate and quantify malaria parasite species [17]. The detection threshold in a Giemsa-stained thick blood film under field conditions has been estimated to be 50-100 parasites per micro-litre of blood [18]. In remote settings however, with less-skilled microscopists and poor equipment, a higher

detection threshold is needed. Microscopy requires well-trained, competent personnel and rigorous maintenance of functional infrastructures in addition to effective quality control and quality assurance. The cost implications of microscopy have been discussed at National Referral Hospital level in Malawi [19], and in mobile clinics in Thailand [20], but have not extensively been empirically compared with alternative parasite-based diagnostic tools.

In the past 50 years, alternative methods of malaria diagnosis have become available for example, detection of malaria antibodies by immunofluorescence antibody assay and enzyme-linked immunosorbent assay. Later, methods to detect malaria antigens were developed. The most significant of these is the immunochromatographic assay which forms the basis for commercial malaria RDTs available today [21,22]. RDTs are devices that detect malaria parasite antigens in a small amount of blood, usually 5-15 micro-litres, by immunochromatographic assay with monoclonal antibodies directed against the parasite antigen and impregnated on a test strip. The result is shown by a coloured test line obtained in 5-20 minutes. RDTs do not require electricity, are simple to perform and easy to interpret [23]. Commercial RDTs are manufactured with different combinations of target antigens to suit the local malaria epidemiology. Histidine-Rich Protein-2 (HRP-2) is the most common malaria antigen targeted and is specific for *P. falciparum.* Parasite lactate dehydrogenase (pLDH) enzymes are the other major group of targeted antigens. Monoclonal antibodies against pLDH are available for detection of pan-malaria species (*P. falciparum* and *P. vivax*). The World Health Organisation recommends sensitivity of

95% at ≥ 100 parasites per micro-litre of blood for *P. falciparum*, with microscopy as reference standard test [24].

1.2 Global malaria diagnosis policy

Prompt and accurate diagnosis of malaria is part of effective disease management. The diagnosis of malaria is based on clinical suspicion and on the detection of parasites in blood (parasitological or confirmatory diagnosis). High test sensitivity in malaria endemic areas is particularly important for the most vulnerable population groups, such as children and the non-immune population, in whom the disease can be rapidly fatal; while high specificity will reduce unnecessary treatment with anti-malarials and improve diagnosis of other febrile illnesses in all settings. Thus, high quality malaria diagnosis is important in all settings.

The first symptoms of malaria infection are non-specific and similar to that of a minor systemic viral illness. They comprise of: headache, lassitude, fatigue, abdominal discomfort, muscle and joint aches, usually followed by fever, chills, perspiration, anorexia, vomiting and worsening malaise. Malaria is therefore, frequently over-diagnosed on the basis of symptoms alone, especially in endemic areas, because of this non-specificity of symptomatology [1].

The policy states that “in all settings, clinical suspicion of malaria should be confirmed with a parasitological diagnosis. However, in settings where parasitological diagnosis is not possible, the decision to provide anti-malarial treatment must be based on the prior

probability of the illness being malaria. Other possible causes of fever and need for alternative treatment must always be carefully considered. In children under five years of age, the strategy for Integrated Management of Childhood Illness [25] should be used to ensure full assessment and appropriate case management at the first-level health facilities."

The changing epidemiology of malaria and the introduction of ACTs have increased the urgency of improving the specificity of malaria diagnosis. Parasitological diagnosis has the following advantages: improved care in parasite-positive patients; identification of parasite-negative patients in whom another diagnosis must be sought; prevention of unnecessary use of anti-malarials, reducing frequency of adverse effects especially in those who do not need the medicines, and drug pressure selecting for resistant parasites; improved malaria case detection and reporting; and confirmation of treatment failures. The two methods in routine use for parasitological diagnosis are light microscopy and RDTs. Anti-malarial treatment should be limited to test positive cases. Negative cases should be re-assessed for other causes of fever [1].

1.3 The Uganda health care delivery system

The Uganda Health System is made up of the public and the private sectors [26]. The public sector includes all government health facilities under the Ministry of Health, health services of the Ministries of Defense (Army), Education, Internal Affairs (Police and Prisons) and Ministry of Local Government. The private health delivery system consists of private not for profit (PNFP) providers, private health practitioners, and the

traditional and complementary medicine practitioners. The provision of health services is decentralised with districts and health sub-districts (HSDs) playing a key role in the delivery and management at those levels. The system is structured into National Referral Hospitals (NRHs), Regional Referral Hospitals (RRHs), General Hospitals, then health centres (HCs) that include those at the county level (HC IV), sub county level (HC III), parish level (HC II) and lastly Village Health Teams (VHTs). This structure does not include the private health practitioners and traditional and complementary medicine practitioners.

1.3.1 Ministry of Health Headquarters and national level institutions

The core functions of the Ministry of Health headquarters are: policy analysis, formulation and dialogue; strategic planning; setting standards and quality assurance; resource mobilization; advisory; capacity development and technical support supervision; provision of nationally coordinated services including health emergency preparedness and response and epidemic prevention and control; coordination of health research; and monitoring and evaluation of the health sector performance.

Several functions have been delegated to national autonomous institutions such as Uganda Cancer Institute, Uganda Heart Institute, Uganda Blood Transfusion Services, Uganda Virus Research Institute, National Medical Stores, Central Public Health Laboratories, Professional Councils, National Drug Authority, and research institutions. The Uganda National Health Research Organisation coordinates the national health research agenda, whilst research is conducted by several institutions including the

Uganda Natural Chemotherapeutic Research Laboratory. The Health Service Commission is responsible for recruitment and deployment of personnel at central and regional referral hospital levels. In the districts, this function is carried out by the District Service Commissions. The Uganda AIDS Commission coordinates the multisectoral response to the HIV/AIDS pandemic.

1.3.2 National, Regional and General Hospitals

Hospitals provide technical back up for referral and support functions to district health services. Hospital services are provided by the public, private health providers and PNFPs. The public hospitals include: 52 general hospitals, 11 regional referral and two national referral hospitals. There are 59 PNFP and nine private for profit hospitals. All hospitals provide support supervision to lower levels and to maintain linkages with communities through Community Health Departments. All PNFP hospitals are autonomous as granted by their respective legal proprietors.

1.3.3 The District health system

District local governments plan, budget and implement health policies and health sector plans. These local governments are responsible for recruitment, deployment, development and management of human resource for district health services, development and passing of health related by-laws and monitoring of overall health sector performance. Local governments manage public general hospitals, HCs and

supervise and monitor all health activities (including those in the private sector). The public private partnership at district level is however weak.

1.3.4 The Health Sub-District system

The Health Sub-Districts (HSDs) plan, organize, budget and manage the health services at this and lower HC levels. HSDs oversee curative, preventive, promotive and rehabilitative health activities including those in the PNFPs and private for profit service providers. The headquarter of a HSD is located at the county level HC (HC IV) or a selected general hospital.

1.3.5 The sub-County Health Centre

The sub-county health centre (HC III) is an intermediate-referral unit serving a sub-county with an estimated population of 25000. It provides the following services: general outpatient, family planning, immunisation, counselling and testing of HIV, maternity and postnatal care, laboratory, inpatient care, environmental health and home visiting. They also provide support supervision of the community and parish level HCs. The staff at HC III include: two clinical officers, one nursing officer, two enrolled/registered nurses, two midwives, three nursing assistants, one health assistant, one laboratory technician, one laboratory assistant, a records officer, two night watchmen and two porters. However, in reality all these staff categories are rarely found at the HCs. A clinical officer has three years of pre-service training, certificate-holder nurses and midwives have two, and laboratory assistants have two to three, while the training for nursing assistants varies from three to nine months.

The HC III is unique in that it is placed in the middle of the patients' referral pathway (between parish HCs and county HCs). Unlike at the higher levels of care, the policy of having functional laboratories at HC III is not fully implemented. Most HCs either lack equipment and supplies or do not have laboratory personnel. In order to contribute to improvement of delivery of health services, it was considered appropriate to conduct the study at this level.

1.3.6 Parish level HCs and Village Health Teams

Parish level HCs (HC II) is the first level of interaction between the formal health sector and the communities. HC IIs provide out patient care, community outreach services and linkage with VHTs. The network of VHTs facilitates health promotion, service delivery, community participation and empowerment in access to and utilization of health services. While VHTs are supposed to play these important roles, coverage is still limited as only 31% of the districts have trained VHTs in all the villages. Attrition is high among VHTs because there is no monetary compensation for their time [26].

1.4 National malaria diagnosis policy

The main objective of malaria case management is to reduce morbidity and mortality so as to minimise related ill effects and economic losses attributed to the disease in Uganda. However, the major challenge to effective malaria case management is the emergence of parasite resistance to anti-malarial medicines. In 2000, monotherapy with chloroquine (CQ) was associated with a failure rate of 28% (in patients aged 5 years and above) to

76% (in those under-five years) [27]. In June 2000, Uganda adopted an interim policy of sulphadoxine-pyrimethamine (SP)+CQ for treatment of uncomplicated malaria. Efficacy studies on CQ+SP conducted in 2002-2004 showed a pattern of progressively increasing treatment failure [28]. In 2004, a decision was made to change the policy from CQ+SP to ACT (Artemether-Lumefantrine "AL") as first-line treatment for uncomplicated malaria. The policy specifically seeks to: ensure early diagnosis and prompt, effective treatment of malaria; and that malaria diagnosis is supported by parasitological investigations where feasible [29].

The policy states that "parasite-based diagnosis with microscopy or RDTs shall be part of malaria case management in all health facilities and at the community level:

(i) suspected malaria cases will be subjected to parasite-based diagnosis;

(ii) microscopy remains the "reference standard" for investigation of malaria at all health facilities from the level of sub-County and above;

(iii) RDTs will be used at parish-level health facilities, community and to fill the gaps at higher levels of care where microscopy is not possible; and

(iv) the type of RDTs to be deployed in the country will be guided by evidence on sensitivity, specificity, ease of use and stability in the field, as determined by the performance evaluation and pre-qualification schemes [29]."

1.5 Health care seeking behaviour and provider prescribing practices

1.5.1 Health care seeking behaviour

Prompt access to malaria diagnosis and treatment is a key component of the Roll Back Malaria Partnership's Global Malaria Action Plan to reduce deaths attributable to malaria to near-zero by 2015 [30]. Those patients presenting with severe malaria have short histories of illness, thus emphasizing the speed of disease progression if not treated promptly and effectively [31]. Studies have indicated that children who manage to reach health facilities the episode normally commences with a febrile illness 1–3 days prior to admission [32,33]. Therefore, understanding treatment-seeking behaviour should enable communities, national governments and international agencies to improve the efficacy of malaria interventions by implementing programmes tailored to specific peoples' needs.

The World Health Organisation reported that approximately half of suspected malaria patients seek care outside the public sector [34]; and outside of the home [35]. People seek treatment from various sources ranging from itinerant drug sellers, local shops, drug shops, pharmacies, private clinics to health centres and hospitals [36]. Self-medication is common practice for managing most disease conditions. Community members manage malaria initially at home [37] using left over drugs previously used to treat a similar condition and then a follow-up to health facilities if the health condition does not improve. In critical situations, community members who initially seek traditional treatment resort to further management at the health facility [38-40]. People use home-made herbal preparations from different plant parts or those bought from traditional healers and shops. Generally if a patient is very ill, the public sector may be preferred

because of the presence of variety of services including laboratory and a greater range of staff.

In Uganda, most malaria patients are treated by private medical practitioners [37,41-43]. The use of traditional medicine is also common [44]. The danger of sourcing treatments from these private facilities (some of them not regulated) is inadequate doses that increase the chance of development and spread of resistance [37] to ACT. Already, the parasite is demonstrating decreased sensitivity to artemisinin in Cambodia [45]. If artemisinin-resistant strains of *P. falciparum* emerge and spread, they would weaken the last effective antimalarial we have. The reasons for seeking care outside the public services include perceived low quality of care in public health facilities, the belief that traditional remedies are effective for malaria [41,46], and unpredictable availability of drugs at those government facilities. A study in Kenya [47] though reported that patients who visited medical facilities were able to recall the medicines given to them more often than those visiting shops. In general, the factors that attract patients to providers have been cited [48-50]. Timeliness and longer operating hours are crucial in clients' choice of providers, which is typical of those with profit motives in the private sector, unlike public health centres for example in Uganda where patients do not make direct payment for medical care.

1.5.2 Prescribing practices

The prescribing practice of providers is influenced by their knowledge of the appropriate regimen for the disease condition, financial incentives, competition, perception of

patients' attitudes, and any legal or regulatory sanctions for inappropriate behaviour [36]. It has been reported that prescribing patterns are more likely to follow patient demand and expectations as well as the profit motive rather than professional principles [51], especially where patients pay for medical services. In Nigeria, the undue pressure wholesalers put to service providers to repay the cost of supplied drugs influenced the type of drugs given to patients [39]. In other settings, knowledge of some basic concepts of malaria was adequate but treatment practices were poor [52]. The social interaction between the patient and prescriber [53] has also been cited as a factor influencing prescribing practices. It was reported that for a satisfactory outcome of the consultation process, the clinician provides technically correct care, but this corresponds with the patient's expectations in order to legitimize the illness [53]. Also an earlier study [54] demonstrated that patient preference can stimulate inappropriate prescribing practice. Even in public health facilities in Uganda, the prescription practices of health staff do not comply with recommended guidelines [55]. Generally, four primary social spheres influence the prescribing practices including: initial training within a context where the importance of malaria is strongly promoted; influence of peers, conforming to perceived expectations from colleagues; pressure to conform to perceived patient preferences; and the quality of diagnostic support involving resource management, motivation and supervision [53].

1.6 Statement of the problem

The choice of first-line drug for the treatment of malaria is a key part of a national malaria control strategy, and needs to balance issues of actual and perceived efficacy,

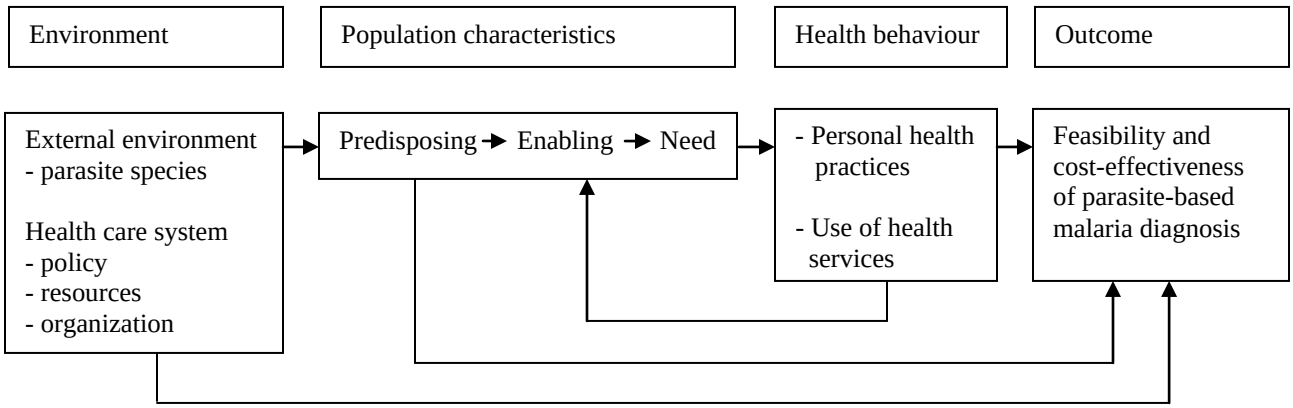
safety, simplicity of dosage and cost [36]. The choice of artemether-lumefantrine (AL) by the government of Uganda will be constrained by cost, misuse and resistance development, which pose a threat to the delivery of effective care. The Ministry of Health recommends early diagnosis and prompt effective treatment of malaria. The difficulty in making a presumptive diagnosis highlights the need for improved and feasible diagnostic tools that can be used at all points of care. In the past, high levels of malaria misdiagnosis have been tolerated because the first-line anti-malarials were inexpensive and nontoxic. AL is effective, but it is also expensive. This means that it may not be economical to treat all fevers as malaria, yet there is limited ability to improve clinical diagnosis through training or simple algorithms to guide diagnosis and treatment. This is because improving accuracy by excluding negative cases is accompanied by an increase in the number of positive cases missed [56]. This is unacceptably dangerous in rural health facilities where patients may not return if the ill health condition deteriorates. Arguably, the most ethical and cost-effective policy is to ensure that patients are tested before treatment and only true malaria cases are given AL. However, a feasible and cost-effective means of parasite-based diagnosis for malaria at primary-level health facilities is not yet identified.

1.7 Conceptual framework

The conceptual framework[1] below (Figure 1) [57] summarises the clusters of factors that influence parasite-based diagnosis of malaria at primary-level health care facilities.

[1] Adapted from that of Andersen RM (1995).

Figure 1: Factors influencing parasite-based malaria diagnosis at primary level health facilities



These factors include the environment; population characteristics; and their health behaviour. The environment includes the health care system policy regarding malaria diagnosis and treatment, available resources and its organisation. The external environment recognises the importance of parasite species that influences the type of test to be used, while treatment options impact on the cost-effectiveness of the test. The population characteristics include the predisposing factors; enabling resources; and the need for the services. The predisposing factors are the health beliefs such as attitudes and knowledge people have concerning and towards the health care system; social structure for example education and social networks; and demographics such as age of the patient. Patients aged five years and older are likely to develop low parasite density and less severe symptoms due to their immunity as a result of frequent exposure especially in high transmission settings. The enabling factors are those logistical aspects of obtaining and or providing care. They are: personal or family income, regular source of care, and the means and knowledge of accessing health services; and those related to the health unit such as availability of staff, facilities and waiting time. Waiting and travel time impact on

patient expenditures. Unlike RDT, the microscopy service is dependent on cadre-specific pre-service training, availability of that cadre at the facility, and the testing procedure requires more time all of which influence the feasibility of the diagnostic method. At the health unit, microscopy requires a specific infrastructure including space, equipment, supplies and laboratory personnel. The providers' prescription practice for example non-adherence to test results indicates lack of trust in the test. Both RDT and microscopy require good quality control. The perceived need relates to care-seeking and adherence to the given treatment, while evaluated need relates to the kind and amount of treatment provided [57]. The health behaviour includes personal practices for example self-treatment and exposure to ineffective or sub-optimal doses of anti-malarials leading to reduced levels of parasitaemia and subsequently impacting on the test performance. The use of health services is influenced by the type of service and the location of the health unit. The location of the health unit within the catchment area impacts on patient costs while the drugs used influence the overall cost-effectiveness of treatment.

1.8 Justification

The balance between the risk of inaccurate diagnosis and the use of antimalarials might be acceptable when drugs are cheap. The choice of artemether-lumefantrine (AL) however, clearly has important budgetary implications requiring a higher degree of accuracy in diagnosis. Clinical diagnosis of malaria is quick but less specific than some technological approaches such as microscopy and rapid diagnostic tests. Quite aside from the human impact of misdiagnosed febrile illness, the cost of wasted drugs on suspected cases that do not have malaria call for an assessment of more specific diagnostic

techniques and a review of the malaria treatment policy. Administering AL only to those who have malaria would improve on good clinical practice, eliminate misuse of the drug, unnecessary side effects, reduce the rate at which resistance to this drug develops and may even improve the treatment of non-malarial febrile illness. This study thus sought to provide policy makers with data on challenges to the implementation of the ACT policy; and feasibility and cost-effectiveness information on a range of diagnostic methods to enhance the use of drugs in peripheral government health centres and impact directly on treatment costs.

2 Research questions, hypothesis, aim and objectives

2.1 Research questions

1. What proportions of patients are correctly classified as having or not having malaria, using presumptive, rapid test or microscopy at government sub county level health centres?
2. Is it more cost-effective to incorporate a diagnostic test, such as a rapid test or microscopy, and then to treat only confirmed malaria diagnoses with artemether-lumefantrine, or to continue treating all patients presumptively diagnosed with malaria?
3. What is the feasibility of rapid tests or microscopy in the diagnosis of malaria?

2.2 Hypothesis

- There is no difference in feasibility and cost-effectiveness of parasite-based diagnosis for malaria using microscopy and rapid diagnostic test.

2.3 Study aim and objectives

The overall aim of the study was to describe the challenges to ACT policy implementation and to compare the feasibility and cost-effectiveness of implementing parasite-based diagnosis for malaria based on rapid diagnostic test (RDT) and light microscopy among patients with febrile illness in areas of low and high transmission intensities in Uganda where ACT (artemether-lumefantrine combination – AL) is the first-line drug.

The specific objectives were:

1. To describe the challenges to ACT policy implementation
2. To compare the accuracy of RDT, microscopy and presumptive diagnosis of malaria
3. To compare the cost-effectiveness of treating malaria with AL based on RDT, microscopy and presumptive diagnosis in different transmission settings
4. To assess the feasibility of implementing parasite-based diagnosis of malaria based on RDT and microscopy

3 Methodology

3.1 Study setting

The study was implemented in Bushenyi district in south-western Uganda and Iganga district in the south-eastern part of the country. The two districts were selected through a stratified random selection technique. Stratification was based on the level of transmission intensity. Bushenyi has low while Iganga has high malaria transmission intensity (Figure 2). The annual entomological inoculation rates are not known, but are reported to be <10 and >500 infective bites per person per year in the nearby districts of Kanungu and Tororo respectively [58]. The study was implemented at sub-County level health centres (HCs) and it commenced before the districts were partitioned. During the period of study implementation, Bushenyi was partitioned into five districts and Iganga into two districts. However, by the time the trial was closed, the level of the study HCs as well as the package of services delivered was still the same.

2.1.1 Bushenyi district

Bushenyi district lies between 0^{o}N and 0^{o}46' S; and 29^{o}41' E and 30^{o}30' E. It is bordered by Kasese district in the north, Kamwenge in northeast, Mbarara and Ibanda in the east, Rukungiri in the west, Ntungamo in the south and Democratic Republic of Congo in the northwest across Lake Edward.

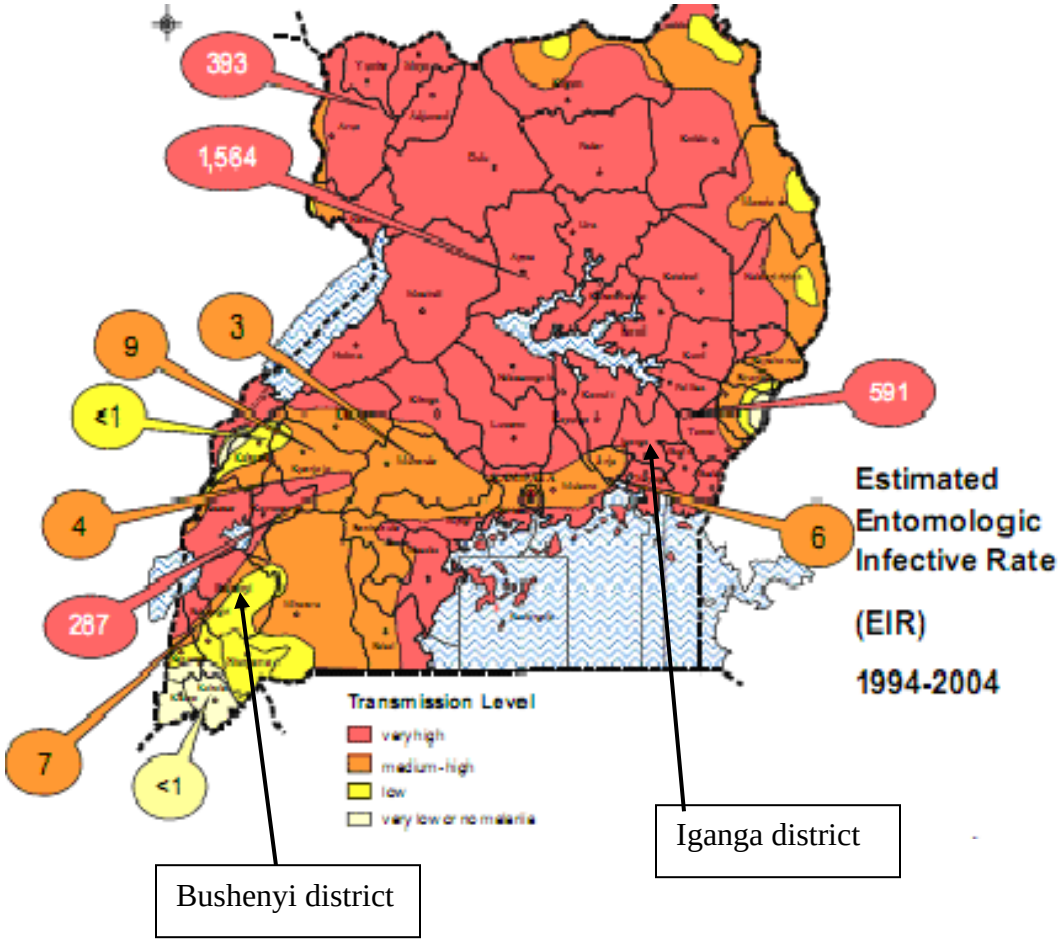


Figure 2: Levels of malaria transmission in Uganda
Source: http://www.health.go.ug/mcp/distmaps.html

With a total land area of 3,949 Km^2, the district is endowed with diverse natural resources that include arable land, forests, large lake water bodies (Edward, George and Kazinga Channel), wetlands, rivers (Kyambura, Nchwera, Kaizi and Rwempunu), Queen Elizabeth National Park and minerals. The main economic activities are semi-intensive

agriculture (growing crops and rearing animals), fishing and trade. The district is multi-ethnic with varying customs and norms. The main inhabitants are *Banyankore* and *Bakiga*.

With a population of 731,392, the district is divided into 5 counties, 27 sub-Counties, two town councils, 162 parishes and 2034 villages. The population distribution and density varies with physical geography. It is concentrated in the low-lying plateau zones of Sheema, Igara and Ruhinda; and sparse in the hilly-rough and rugged terrain of Buhweju and Bunyaruguru. The climate is relatively wet. The mean annual temperature range is 12.5^{o}-30^{o}C. Most of the district receives 1500-2000 mm of rainfall annually. The rainfall is lowest towards the rift valley.

The district is divided into 7 Health Sub-Districts (HSDs) with 87 health units. It has 3 hospitals (1 government and 2 private not for profit – PNFP); 6 county-level HCs (all government), 26 sub-County level HCs (20 government and 6 PNFP) and 52 parish level HCs (36 government and 16 PNFP). Malaria is the leading cause of morbidity in the district [59].

3.1.2 Iganga district

Iganga district is lies between longitudes 33^{o}10' E and 34^{o}0' E and latitudes 0^{o}06' N and 1^{o}12' N. It borders Mayuge district to the south, Bugiri to the southeast, Kaliro and Namutumba to the north and Jinja district to the west. It covers a total area of 1,680 Km^2.

The district is divided into three counties of Bugweri, Kigulu and Luuka; one town council, 18 sub-Counties, 112 parishes and 644 villages. The total population is 540,939. The main economic activities are agriculture and small scale trade (shop and market vendors).

The temperatures are considerably high with an annual average of 25^{o}C (23^{o}–27^{o}C). The district experiences a bimodal pattern of rainfall with the first coming between March/April and June while the second comes in September and November. The mean annual rainfall is 1,200 mm in the wetter south and 900 mm in the drier northwest. However, the rainfall pattern is complicated by the unpredicted failures in the peak months and heavy rains with hail storms in the drier months [60]. The district has one hospital, three county-level HCs and 15 sub-County level HCs (all government). There are 57 parish level HCs (37 government and 20 PNFP).

3.2 Design

This was a cluster randomised trial (Clinicaltrials.gov: NCT00565071), comprised of three malaria diagnostic arms (diagnostic method in a defined group of patients), namely presumptive diagnosis, microscopy and RDT. The study was implemented within the existing health services delivery system to reflect the real-life operational conditions. This thesis comprised of five sub-studies and is as follows:

3.2.1 Sub study I: Challenges to implementation of ACT policy

This study aimed at describing the challenges to ACT policy implementation at rural public health centres. Extra details of the outcome of this study are contained in a published paper in Annex I.

Design and data collection

In a cross sectional design, twenty government HCs at the level of sub-County in Bushenyi and twelve in Iganga were visited. The number of HCs could not be balanced because Iganga had fewer than in Bushenyi, yet three had not been clarified if they were at sub county level. The study participants included: clinical staff (clinical officers, nurses and midwives), laboratory personnel and Ministry of Health official. A semi-structured questionnaire was used for the interviews with one staff on duty and laboratory staff where available. An in-depth interview guide was used for the Ministry of Health staff. Data were collected on: staffing levels, availability of malaria treatment guidelines and drugs, personnel involved in management of malaria patients and treatment decisions. Equipment and supplies such as thermometers, microscopes; and laboratory reagents

were checked to confirm their availability. Six HCs (three per district) were randomly chosen to collect baseline patient data. A total of 613 patients clinically treated for malaria were interviewed (exit interviews) using a structured questionnaire.

Data cleaning and entry

The collected data were manually checked and cleaned. Data were double entered in EpiData 3.1 software (The EpiData Association, Odense, Denmark). The entered data sets were validated in EpiData to check for errors. Open-ended questions were coded before entry. Missing values were coded where possible and eliminated during analysis.

Quality control and data analysis

The following were done to ensure quality data: I trained research assistants; I carried out regular monitoring and supervision during the study implementation and data collection, including checking of completed data collection tools. I designed a customised data entry template with in-built consistency checks in EpiData 3.1 software. The data was analyzed in SPSS version 15.

3.2.2 Sub study II: Accuracy of malaria diagnostic methods

This was a diagnostic clinical trial (details in the published paper in Annex II) with the aim of assessing the accuracy of RDTs, microscopy and presumptive diagnosis in predicting malaria infection among patients presenting with febrile illness. The study was carried out in the six HCs (3 per district where patient interviews were conducted in sub study I).

Training of study team

The trial was preceded by training of the study team. The research team comprised of resident staff of the selected HCs (three clinical officers, three laboratory assistants, and nine nurses) and three research assistants per district. A one-week training workshop was conducted in each district. Standard operating procedures for: (1) finger prick for collection of blood, (2) thick/thin blood smear preparation, (3) staining smears, (4) blood smear reading, (5) preparation and reading of RDT, and (6) collection of blood on filter paper for polymerase chain reaction (PCR) were used in training. All members were trained in theory and practice during pilot testing of patient case report forms (CRFs). Data collection commenced after inter-reader reliability for both Paracheck and microscopy reached a very good agreement (kappa coefficient = 0.97). Paracheck was read by clinicians and laboratory personnel while blood smears were read by laboratory personnel only.

Description of study procedures

The description of the study procedures within each arm is presented below.

a) The presumptive diagnosis

In this arm, none of the HCs had functional laboratory facilities. Patients presenting with fever were enrolled to receive service without parasitological confirmation of malaria. Patients were treated with AL on the basis of signs and symptoms only. Those presenting with co-infection were assessed and managed as per the usual practice.

b) Health centre microscopy

Patients were assessed clinically and those classified as "possibly have malaria infection" were enrolled. Thick and thin blood smears were prepared by finger-prick using sterile blood lancets on separate frosted slides. Standard staining was performed using the Field's stain method. Blood films were read at the HC (magnification X1000). Each film was graded as positive (asexual malaria parasites seen) or negative (no malaria parasites seen) based on inspection of 200 fields. Patients with negative results received further assessment to decide on an appropriate treatment strategy. All slides were stored and re-read by expert microscopists.

c) The Rapid Diagnostic Test

Patients suspected to have malaria were asked to provide blood specimen for rapid testing with "*Paracheck* Pf® device (Orchid Biomedical Systems, Goa, India). *Paracheck Pf*® is based on the detection of HRP-2 produced by *P. falciparum* trophozoites and young gametocytes. The specimens were drawn by trained clinicians or laboratory assistants using a simple finger-prick. The test preparation and interpretation were done following manufacturer's instructions and standard operating procedures. The test was considered positive when the antigen line was visible in the test window, negative when only the control band was visible and invalid when the control band was not visible. Patients with negative results received further medical assessment. The choice of *Paracheck* Pf® was based on its ability to be stored at room temperature in tropical climates [61], reported low cost, high sensitivity (97%) and moderate specificity (88%) in highly controlled

research settings, reliability (kappa coefficient = 0.97), ease of use [62], and it was likely to be preferred by the Uganda Ministry of Health.

d) The Polymerase Chain Reaction (PCR) assay

Finger-prick blood was blotted on Whatman 3MM filter paper, dried in dust free area, wrapped in plastic sample bags and placed in a zip-lock bag with silica gel (Unilab Kenya Limited) to prevent DNA degradation. The PCR samples were sent in one batch to Makerere University-University of California San Francisco Molecular Research Laboratory for analysis. DNA was extracted from filter paper using the chelex method [63]. *Plasmodium* genus-specific PCR, followed by *P. falciparum* species-specific nested PCR of 18S small sub-unit ribosomal DNA was performed following a standard protocol [64]. PCR products were stained by ethidium bromide and resolved by gel electrophoresis on a 2.5% agarose gel. DNA size standards were separated alongside PCR products to allow sizing of species bands.

Upon completion of the gel electrophoresis, gels were placed in an imaging cabinet and digitally photographed under ultraviolet light. Gel images were printed and corresponding sample lanes scored visually for the presence of *P. falciparum.* Positive and negative controls were used for each round of PCR. In addition, twenty two samples were randomly selected plus eight samples that were positive by PCR only (10% of the total) for re-analysis as a quality control measure.

Sample size estimation

Using a nomogram [65], estimated malaria prevalence of 63.5% [66], *p*-value set at 0.05, with estimated sensitivity of Paracheck of 95% [67], a sample size of 150 in each district was appropriate totalling 300 patients for the two districts.

Data collection and inclusion criteria

A total of 317 patients weighing ≥5kg presenting with fever; suspected to have uncomplicated malaria infection; consenting to participate[2] and eligible to be treated with artemether-lumefantrine were enrolled. All patients (including those in the presumptive arm) provided blood samples for microscopy, RDT and on filter paper for PCR analysis. In the presumptive arm, finger-prick blood was drawn by research assistants (with laboratory training background). The RDT results were recorded on site while the slide smears were read at the HC with microscopy arm. All slides were stored special slide-boxes and re-read by expert microscopists.

Data management

Data cleaning and entry was performed as described in sub study I. Data were analysed using STATA version 10 (Stata Corp. LP, College Station, Texas, USA). The 2-sided $\chi 2$ test was used to compare proportions. Probability values (*p*-values) were set at 0.05 level of significance and Confidence Interval (CI) was calculated at the 95% level. Socio-demographic and symptom data was presented using descriptive statistics: distribution by age, number (and percent) of positive and negative results. Performance (sensitivity,

[2] In the case of minors, assent was sought. Permission was also obtained from parents or legal guardians.

specificity, positive and negative predictive values) and likelihood ratios were displayed for each diagnostic method.

3.2.3 Sub study III: Cost-effectiveness of RDT and microscopy

The aim of this sub-study was to assess the cost-effectiveness of malaria RDT and microscopy in comparison to presumptive diagnosis as the base-case. Extra details of the outcome of this sub study are contained in a published paper in Annex III.

Laboratory procedures

Finger-prick blood was investigated in the intervention arms (microscopy and RDT). Both microscopy and RDT procedures were carried out as described in sub study II.

Data collection

Data on costs were collected in the same HCs as in sub study II. The study population included all HC staff; and all outpatients presenting with fever (by statement or measured axillary temperature $\geq 37.5^{o}$C). Of all patients enrolled a random sample of 1627 were selected to measure indirect costs. When a patient arrived at the HC, time was recorded by the research assistant on a "time sheet." The "time sheet" was then given to the patient. Thereafter, the time was recorded by clinicians and laboratory personnel at every point of service delivery. The "time sheet" was finally retained at the dispensing window. Cost data was collected from March 2010 to February 2011. These costs were collected in Uganda Shillings (USh.) and converted to United States Dollars (US$1 = USh. 2380).

Data management

Data entry and cleaning were carried out as described in sub studies I and II. Data were analysed using STATA version 10 (Stata Corp. LP, College Station, Texas, USA), Excel and TreeAge. STATA was mainly used in the descriptive analysis. Excel was used in the Step-down costing methodology. TreeAge was used for constructing and analysing the decision tree and uncertainty in the cost-effectiveness model. The cost-effectiveness analyses from the societal perspective were done using a comprehensive decision analytic modeling approach. Sensitivity analysis was performed on test validity indicators, costs of RDT and AL. The costs of AL and RDT were halved and doubled to get the lower and upper limits of the interval respectively.

Measurement of effectiveness and costing

The ultimate measure of effectiveness was the proportion of patients correctly identified and treated under each diagnostic strategy gauged against PCR as reference standard. Costs were categorised into internal (incurred by HCs) and external (those incurred by patients). Internal costs were classified into capital and recurrent costs. Recurrent costs included personnel salaries and allowances, drugs and disposables, utilities and cleaning materials. Patient costs were categorised into direct costs (out-of-pocket expenses on treatment before visiting study HCs, transport and other non-medical costs directly related to treatment seeking) and indirect costs (opportunity cost of seeking care including the time cost of waiting for treatment and travel).

Valuation of resource use and unit cost of diagnosis for each outpatient visit

The unit cost of outpatient visit in each arm was determined following a standard step-down costing method [68,69]. The ingredients approach was also employed and provided information directly measured for example "service provider effective contact time" with patients, drugs and supplies used. Further, the annualised values of buildings, furniture and equipment were estimated using a standard protocol [70] assuming a useful lifespan for these goods of 30, 10 and 7 years, respectively, and with a discount rate of 3%. Using appropriate allocation criteria, all resources identified at the HCs were allocated in steps to different cost centres until the final services in the outpatient department. The total costs of running an outpatient department were divided by the number of final services to arrive at the unit cost per service with and without parasitological confirmation of malaria. The main outcome measure was the incremental cost-effectiveness ratios (ICERs) estimated from the societal perspective.

3.2.4 Sub study IV: Feasibility of diagnostic methods

The feasibility trial was implemented in 30 sub-County government HCs (15 in each district). This trial took a cluster randomised design (HCs were the primary sampling units) to limit contamination between control and intervention group subjects and to allow service providers to operate as they would normally on a day to day basis. The details of this sub study are reported in the published paper in Annex IV.

Training

Before implementation of the study in the additional HCs, all staff members were trained in theory by re-orienting them to the malaria treatment policy. The staff in intervention arms (RDT and microscopy) received training tailored to the diagnostic method to be used. The details of the training have been described in sub study II. The staff in HCs with microscopy or RDT was instructed to treat patients according to test results. Staff in the control arm were only re-oriented to the malaria treatment guidelines but testing of patients was not performed. HC outpatient registers were modified to record additional variables such as the presenting complaints, drugs dispensed and to indicate those prescribed but out-of stock. Inter-reader reliability for both microscopy and RDT were only assessed before the commencement of data collection.

Data collection

Data collection was carried out weekly by the research assistants by retrieving information from the laboratory and outpatient registers. Regular supervision and monitoring was carried out to ensure that complete patient information was entered into the registers. From March 2010 to July 2011, a total of 102087 outpatients were enrolled in the three arms. The primary outcome measures were the proportion of patients: who received parasite-based diagnosis; with negative malaria parasite-based diagnosis who received AL and the patient waiting time measured using time-sheets described in sub study III.

Data management

Data cleaning and entry were performed as described in sub studies I-III. Before analysis in STATA version 10 (Stata Corp. LP, College Station, Texas, USA), the data was declared a cluster design using the "svyset command" with HCs as primary sampling units. The Poisson regression model was then fitted while accounting for clustering. Probability values (*p*-values) were set at 0.05 level of significance and Confidence Interval (CI) was calculated at the 95% level. Socio-demographic and symptom data was presented using descriptive statistics: distribution by age and sex, number (and percent) of patients with positive and negative results and those prescribed antimalarial drugs. Further analysis concentrated only on those patients who received antimalarial treatment.

3.2.5 Sub study V: Antibiotic use among febrile outpatients

Data collection on antibiotic treatment was carried out within the feasibility trial (sub study IV). For antibiotic use, a sub-analysis in the low transmission setting of Bushenyi district was performed. Overall, 52116 outpatients were enrolled. The data was analyzed as described in sub study IV. The primary outcome measure was the proportion of patients with febrile illness prescribed antibiotics when: not tested, they test positive and when they tested negative. The details of the outcome of this sub study are reported in the published paper in Annex V.

3.3 Ethical review

The research proposal for this PhD was reviewed by Makerere University School of Public Health Higher Degrees Research and Ethics Committee; and the Uganda National Council for Science and Technology (Ref: HS 209). All information collected was considered privileged and remained confidential. The study was registered with the Clinicaltrials.gov (NCT00565071).

3.4 Summary of methods

Table 1 below presents a summary of the number of health centres (HCs), participants, design, methods and the time frame for all sub studies.

Table 1: Participants, methods and time frame

Study	Setting	Design	Methods	Time
I	Facility-based , 32 rural HCs in Bushenyi (low transmission) and Iganga district (high transmission)	Cross sectional, descriptive	32 Semi-structured interviews with HC staff; one at Ministry of Health; 613 structured patient exit interviews at 6 randomly selected HCs (3 per district)	March-July 2007
II	Facility-based , 6 HCs in Bushenyi (low transmission) and Iganga district (high transmission)	Diagnostic clinical trial (Clinicaltrials.gov: NCT00565071)	300 patients enrolled; structured patient interviews; finger-prick blood for RDT, PCR, microscopy; accuracy of RDT, microscopy, presumptive diagnosis compared with PCR gold standard	June-July 2007
III	As study II	Cost-effectiveness randomized trial; microscopy and RDT (intervention) and presumptive diagnosis (control); HCs were primary sampling units (Clinicaltrials.gov: NCT00565071)	All clinical and non-clinical HC staff; 22052 patients enrolled; step-down costing method used; comprehensive decision analytic cost-effectiveness model developed in TreeAge and populated with costs and effectiveness data	March 2010-February 2011
IV	Facility-based , 30 HCs in Bushenyi (low transmission) and Iganga district (high transmission)	Stratified cluster randomized trial; microscopy and RDT (intervention) and presumptive diagnosis (control); HCs were the clusters and primary sampling units;10 clusters per arm (Clinicaltrials.gov: NCT00565071)	102087 patients enrolled; no sampling at enrolment; "svyset command" used in STATA to declare data cluster; primary outcomes: proportion of patients tested, proportion with negative test treated with AL, patient waiting time	March 2010-July 2011
V	Facility-based , 15 HCs in Bushenyi district (low transmission)	Stratified cluster randomized trial; microscopy and RDT (intervention) and presumptive diagnosis (control); HCs were the clusters and primary sampling units; 5 clusters per arm (Clinicaltrials.gov: NCT00565071)	52116 patients enrolled; no sampling at enrolment; "svyset command" used in STATA to declare data cluster; primary outcomes: proportion of patients prescribed antibiotics when not tested, when tested positive and when tested negative	March 2010-July 2011

AL=artemether-lumefantrine, HC=health centre, PCR=polymerase chain reaction, RDT=rapid diagnostic test

4 Results

This section integrates the results of the five papers. Additional results not included in the papers are presented here where further analyses have been made.

4.1 Sub study I: Challenges to implementation of ACT policy

4.1.1 Staffing, practices and guidelines

The level of staffing was inadequate, with less than 50% of each post filled. For example, only 27 (42.2%) of the clinical officer posts were filled. One HC had a laboratory technician. Eleven HCs (34.4%) had the laboratory assistant post filled, although only four had functioning laboratories. The diagnosis of malaria was based on signs and symptoms in 28 (87.5%) HCs. Seventeen (53.1%) did not have clinical thermometers to measure body temperature. In these centres, patient temperatures were qualitatively estimated using the back of the palm and reported either as high or normal.

All HCs had ACT treatment guidelines. Fifty three (8.6%) patients attending the four HCs with functional laboratories had their blood tested for malaria. The decision to send patients for microscopy was based on: (1) if the clinician was not sure of the diagnosis; (2) patients re-attending and not responding to initial anti-malarial treatment; (3) level of workload (number of patients) as microscopy creates delays in completing the queue; (4) patients who had never taken anti-malarial medication for the current illness; (5) patient demand for laboratory testing; (6) availability of laboratory personnel; and (7) patient

ability to pay 1000 Uganda shillings (US$ 0.59, an informal charge). Out of the 53 patients tested for malaria, 27 (50.9%) were charged for the laboratory service.

4.1.2 Availability and use of anti-malarials

Only 15 (46.9%) HCs had in stock all the four weight-specific blister packs of AL. Twenty four (75%) experienced AL stock-out during the six months preceding this study. Only 20 (62.5%) had injectable quinine in stock. For those without quinine, the average duration of stock-out was five months.

Due to erratic supply of drugs, availability especially of anti-malarials, at the health units was a prime determinant of patient attendance. Whenever new drug consignments arrived at HCs, word spread rapidly by mouth among community members who subsequently reported for treatment in large numbers. The marked increased patient attendance at HC coinciding with availability of drugs was reported in Bushenyi and Iganga.

4.1.3 Patient data

Three hundred forty one (55.6%) out of 613 reported after initiating treatment. Of these, 193 (56.6%) had used anti-malarials. The anti-malarials used included: CQ alone (115, 59.6%); SP alone (23, 11.9%); artemisinin derivatives (16, 8.3%); quinine (28, 14.5%); and CQ+SP for children under five years of age (11, 5.7%). Patients obtained anti-malarials from multiple sources (one patient trying more than one source) including: drug shops 111 (32.6%), home (leftover drugs from previous illness episodes) 88 (25.8%),

other HC 99 (29.0%) and community drug distributors 11 (3.2%). At the study HCs 560 (91.4%) patients received anti-malarial treatment.

4.2 Sub study II: Accuracy of malaria diagnostic methods

Out of 300 patients, 88(29.3%) had fever (temperature ≥37.5^{o}C). Fifty-six (18.7%) slides were positive by HC microscopy and 47(15.7%) by expert microscopy. The rapid diagnostic test (RDT) detected 110 (36.7%) positive cases and 89 (29.7%) by PCR. Out of 58 patients who had used anti-malarials, the following tested positive: HC microscopy 16 (27.6%), expert microscopy 13 (22.4%), RDT 27 (46.6%) and PCR 26 (44.8%).

4.2.1 Sensitivity, specificity, positive and negative predictive values

Basing on PCR as reference standard the overall sensitivity of presumptive diagnosis based on axillary temperature, HC microscopy, expert microscopy and RDT were: 39.3%, 47.2%, 46.1% and 91% respectively. The corresponding specificity rates were: 74.9%, 93.4%, 97.2% and 86.3% respectively (Table 2). Overall, only RDT had a NPV of >90% (95.8%), while the PPVs for all methods were <88%.

4.2.2 Likelihood ratios (LR) of malaria diagnostic methods

The likelihood ratio (+) results (Table 2) for all diagnostic methods were greater than one indicating that these results were associated with presence of malaria infection. Also the likelihood ratio (-) results for all methods were less than one and thus associated with absence of malaria infection. Expert microscopy had a likelihood ratio (+) of 16.2%,

showing the strongest evidence of presence of malaria infection; while RDT with LR(-) of 0.10% showed the strongest evidence of absence of malaria infection. However, expert microscopy had a low sensitivity, yet these expert personnel are not available at sub county level health centres.

Table 2: Sensitivity, specificity and likelihood ratios of malaria diagnostic methods

Diagnostic method	Sensitivity (%)	Specificity (%)	LR(+) %[95%CI]	LR(-) %[95%CI]
Presumptive*	39.3	74.9	1.57[1.11-2.22]	0.81[0.67-0.97]
HC microscopy	47.2	93.4	7.11[4.10-12.35]	0.57[0.46-0.69]
Expert microscopy	46.1	97.2	16.20[7.13-36.79]	0.56[0.46-0.67]
RDT	91.0	86.3	6.62[4.69-9.34	0.10[0.05-0.20]

*axillary temperature ≥37.5^{o}C, HC=health centre, LR(+) =likelihood ratio (+), LR(-) =likelihood ratio (-), RDT=rapid diagnostic test

4.3 Sub study III: Cost-effectiveness of diagnostic methods

4.3.1 Effectiveness of diagnostic methods

The effectiveness of each diagnostic strategy (number and percent patients correctly diagnosed and treated) was calculated basing on results from sub study II. RDT was the most effective with the proportion of malaria patients correctly diagnosed and treated being 87.7%, microscopy 79.7% and presumptive diagnosis 64.3%. In the high transmission area, effectiveness values were: RDT 86.0%, microscopy 70.7% and presumptive diagnosis 59.3%. The corresponding effectiveness values in the low transmission setting were 89.3%, 88.7% and 69.3% respectively.

4.3.2 Costs

The main recurrent internal cost determinants in all arms were: personnel and drugs. Cost of diagnostics was also substantial in the intervention arms. The opportunity cost (time cost of travel and waiting) was a significant patient indirect cost. Following allocation of all available resources up to the final services in the outpatient departments, the presumptive method had the lowest cost of diagnosis (US$ 0.62), RDT (US$ 1.29) and microscopy (US$ 1.53). The overall cost of treatment for malaria in the outpatient department was comprised of the cost of diagnosis, drugs dispensed and that incurred by the patient. The unit cost of treatment with AL was: presumptive US$ 3.97, RDT US$ 5.04 and microscopy US$ 5.60. The corresponding unit cost of treatment including AL, antibiotics and analgesics was US$ 5.02, US$ 6.09 and US$ 6.65 respectively. For a patient who got antibiotics and analgesics only (all patients in presumptive arm received antimalarials), the unit cost of treatment was: RDT US$ 4.71 and microscopy US$ 5.27.

4.3.3 Incremental Cost-Effectiveness Ratios (ICERs)

Using the unit cost per patient diagnosed and treated compared with the effectiveness of each diagnostic strategy, the RDT was the most cost effective with the lowest ICER US$ 5.0 compared to microscopy ICER US$ 9.61. In the high transmission setting, ICER was US$ 4.38 for RDT and US$ 12.98 for microscopy per case correctly diagnosed and treated. The corresponding ICERs in the low transmission setting were US$ 5.85 for RDT; and US$ 7.63 for microscopy. The difference in ICERs between RDT and microscopy was greater in the high transmission setting (US$ 8.9) than in the low

transmission area (US$ 1.78). At a willingness to pay of US$ 2.8, RDT remained cost effective up to a threshold value of the cost of treatment of US$ 4.7.

4.4 Sub study IV: Feasibility of diagnostic methods

4.4.1 Proficiency in conducting the test

A total of 133 HC staff (24 clinical officers, 13 nursing officers, 15 enrolled/registered nurses, 30 midwives, 36 nursing assistants and 15 laboratory assistants) were trained for the study. All cadres were experienced in testing HIV using whole blood on rapid test strips, devices or cassettes that work on the same principle as malaria RDTs. At this level of the health care services delivery system the staff in the RDT arm mentioned that one day of training was adequate. On the first day, few staff especially nursing assistants had a challenge in collecting blood for the RDT using a loop in children under five years. However, they gained adequate skills by the end of the first week. In the microscopy arm, all staff attended the practical sessions. Although they were able to prepare a thick blood smear, reading and interpretation of results was the responsibility of the laboratory personnel. Eight of the HCs (four in each district) in the microscopy arm did not have electricity. Even in the other two with electricity, black-out was frequent and therefore electricity as source of light for malaria microscopy was unreliable.

4.4.2 Duration of outpatient visit

The effective average contact time of clinicians with febrile outpatients (excluding time for investigation) was 11.4min [95%CI: 11.1-11.7], and was similar in the three arms.

The mean effective contact time for malaria investigations was significantly shorter when using RDT 7.6min [95%CI: 7.1-8.0] than microscopy 11.0min [95%CI: 10.6-11.4]. On average, outpatients spent about two and half hours to complete a HC visit. The time spent accessing services in the RDT arm 134.4min [95%CI: 123.5-145.3] was not different from that of presumptive treatment, but significantly shorter than when using microscopy 188.5min [95%CI: 173.8-203.3].

4.4.3 Diagnostic test results

Overall, 64,110 in the two intervention arms were tested. Patients were 1.59 times more likely to have a malaria test done in the RDT arm 44,565 (96.6%) compared to the microscopy arm 19,545 (60.9%) [RR: 1.59; 95%CI: 1.31-1.92]. Still patients were more likely to be tested in the RDT arm compared to microscopy arm in children under-five years of age [RR: 1.56; 95%CI: 1.20-2.02], patients five years and above [RR: 1.59; 95%CI: 1.34-1.90], patients in low transmission area [RR: 1.45; 95%CI: 1.41-1.49] and in the high transmission setting [RR: 1.71; 95%CI: 1.06-2.76].

Overall, 28,289 (44.2%) [95%CI: 30.8-57.5] had a positive test result. In the low transmission setting, the proportion of those who tested positive in the RDT arm was 3,313 (20.5%) and microscopy arm 1,548 (14.0%). The proportion of those testing positive in the RDT arm 19,414 (68.6%) in the high transmission setting was also higher than in the microscopy arm 4,014 (47.4%). Children under-five years of age more often had a positive test 10,900 (61.1%) than patients five years old and above 17,124 (37.4%) [RR: 1.62; 95%CI: 1.41-1.86]. This relationship was maintained in the low transmission

area [RR: 1.12, 95%CI: 1.05-1.21], and increased in the high transmission setting [RR: 1.29; 1.14-1.47].

4.4.4 Treatment of patients who did not receive a parasitological investigation

All patients in the control arm were treated with anti-malarials. A total of 12,527 (39.1%) in microscopy and 1,566 (3.4%) in RDT arms were not tested [RR: 11.50; 95%CI: 5.16-25.65]. Subsequently, 12,044 (96.1%) and 965 (61.6%) respectively were treated with AL.

4.4.5 Treatment of patients with positive RDT or microscopy results

All patients in microscopy and RDT arms with positive results received anti-malarial treatment. Parasitological confirmation of malaria was associated with a reduction in the prescription of AL between presumptive and RDT [RR: 0.62; 95%CI: 0.47-0.82], and between presumptive and microscopy [RR: 0.72; 95%CI: 0.60-0.86].

4.4.6 Treatment of patients with negative RDT or microscopy results

Overall, 10,558 (29.5%) patients with negative results were treated with AL. The pattern of prescribing anti-malarials among patients with negative results varied across health units from 16.7% to 42.0%. Treatment with AL in patients with negative results was also more likely in high transmission setting and varied from 20.1% to 80.9% overall; and among patients investigated under microscopy 14.9% to 63.0% as well as among children less than five years of age 26.0% to 62.7%.

4.5 Sub study V: Antibiotic use among febrile outpatients

Overall, 52116 febrile outpatients were enrolled in the low malaria transmission setting. The enrolment across diagnostic arms was as follows: presumptive 16971 (32.7%), RDT 17637 (33.8%) and microscopy 17508 (33.6%).

4.5.1 Types of antibiotics prescribed

Oral co-trimoxazole was prescribed to 11862 patients (51.0%) and amoxicillin capsules to 5986(25.8%). These two antibiotics were the most commonly prescribed. Metronidazole was prescribed to 3950 patients (17.0%), but in combination with other antibiotics. Doxycycline, erythromycin and ciprofloxacin were also prescribed, but in smaller quantities. Other drugs such as analgesics and anti-helminthics were prescribed to 49574 patients (95.2%) and 10330(19.8%) respectively.

4.5.2 Antibiotic use in patients who did not receive a parasitological test

Overall 9387 (38%) of the patients who did not receive a parasitological diagnosis were prescribed antibiotics. In the presumptive arm 7,040 patients (41.5%) were prescribed antibiotics. In the microscopy arm 6,427 (37%) did not receive a parasitological diagnosis and of these 1,537 (23.9%) were prescribed antibiotics. In the RDT arm, 1442 (8%) were not tested and of these 810 (56.2%) were prescribed antibiotics. Prescription of antibiotics was more common for children less than five years of age as compared to

those who were older. The pattern of antibiotic prescription varied across health units ranging from 29.7% to 60.8%.

4.5.3 Antibiotic use in patients with positive RDT or microscopy

Of the 3,313 patients who tested positive for malaria in the RDT arm, 854 (25.8%) were prescribed antibiotics. In the microscopy arm of 1,548 with positive results, 273 (17.6%) were prescribed antibiotics. Patients under-five years of age were more often prescribed antibiotics than in those five years and above in both RDT and microscopy arms. Prescription of antibiotics also varied between health units (ranging from 15.7% to 27.3%).

4.5.4 Antibiotic use in patients with negative RDT or microscopy

Overall, 11,658 patients (52.1%) with negative results were prescribed antibiotics. Patients with negative RDT 7,909 (61.4%) received antibiotic prescriptions more often than those with negative microscopy results 3,749 (39.3%). Again children under five years of age with negative results in both RDT and microscopy arms were more often prescribed antibiotics than the older age group. Further, antibiotic prescription varied across health units (ranging from 38.4% to 62.4%).

4.6 Summary of results for all studies

The results of the five sub studies are summarized (Table 3) below, and are presented by diagnostic arms in relation to the primary outcome measures. The confidence intervals

(CIs) were calculated at the 95% level and *p*-values were set at 0.05 level of significance. Study "I" formed the baseline and results are presented as such. The units of measurement are mainly indicated in the column of the outcome measures. In summary, the RDT showed a superior sensitivity in screening malaria infection; was more cost-effective overall, and in both low and high transmission settings; and it was more feasible to implement parasite-based diagnosis for malaria with RDT than microscopy.

Table 3: Summary of results

Study	Outcome measures	Presumptive	RDT	Microscopy	RR[95%CI]	*p*-value
I	***Baseline in all*** HC: location; availability of staff, treatment guidelines and antimalarials; use of antimalarials before reporting to HCs; proportion of patients tested; antimalarial treatment	- maps of HC locations were drawn - each post at HC was <50% filled - all HCs had ACT treatment guidelines - 17(53.1%) HCs had no clinical thermometers - only 15(46.9%) stocked all 4 ACT blister packs - 193/613 patients used anti-malarials before coming - only 53(8.6%) patients were tested at HCs - majority, 560(91.4%) were treated for malaria			N/C	N/C
II	Sensitivity %[95%CI]	39.3[29.1-50.3]	91.0[83.1-96.0]	47.2[36.5-58.1]	N/C	N/C
	Specificity %[95%CI]	74.9[68.5-80.6]	86.3[80.9-90.6]	93.4[89.1-96.3]		
	LR (+) %[95%CI]	1.57[1.11-2.22]	6.62[4.69-9.34]	7.11[4.10-12.35]		
	LR (-) %[95%CI]	0.81[0.67-0.97]	0.01[0.05-0.20]	0.57[0.46-0.69]		
III	Effectiveness* (%)	64.3	87.7	79.7	N/C	N/C
	ICER (in US$), overall	Base case	5.0	9.6		
	ICER (in US$) low transmission	Base case	5.85	7.63		
	ICER (in US$) high transmission	Base case	4.38	12.98		
IV	Proportion patients tested n(%)	N/A	44565(96.6)	19545(60.9)	1.58[1.28-1.95]	<0.0001
	Proportion treated with AL when not tested n(%)	23804(99.7)	965(61.6)	12044(96.1)	N/C	N/C
	Proportion treated with AL when negative** n(%)	N/A	5045(23.2)	5438(38.9)	1.84[1.06-3.19]	<0.0001
	Overall mean visit time at HC (minutes) [95%CI]	143[131-156]	134[123-145]	188[173-203]	N/C	N/C
V	Proportion given antibiotics when not tested n(%)	7040(41.5)	810(56.2)	1537(23.9)	N/C	N/C
	Proportion given antibiotics when positive*** n(%)	N/A	854(25.8)	273(17.6)	1.46[1.26-1.69]	<0.0001
	Proportion given antibiotics when negative*** n(%)	N/A	7909(61.6)	3749(39.4)	1.57[1.41-1.73]	<0.0001

* proportion of patients correctly identified (true positive + true negative) and treated; ** adjusted for clustering, age and transmission intensity; ***adjusted for clustering and age CI=confidence interval; ICER=incremental cost-effectiveness ratio; HC=health centre; LR=likelihood ratio; N/A=not applicable; N/C=not calculated; RDT=rapid diagnostic test; RR= risk ratio

5 Discussion

5.1 General discussion of results

Universal parasitological testing and treatment of test positive patients with ACTs are critical components of the latest international recommendations for malaria case-management [1]. However, the success of the implementation of the new case-management policy is dependent upon a series of factors of which availability of commodities at health facilities and case management practices are of vital importance to ensure cost-benefit of the diagnostics and ACT based case-management strategies [71-73]. This study identified lack of parasitological diagnosis, problems with human resources, stock-out of ACTs in HCs and the continued use of non-recommended anti-malarials as challenges to the ACT treatment policy.

Although the stock-out of ACTs is a health systems issue resulting from inadequate ordering, distribution and supply [74], stock-out in HCs was related to increased utilisation of outpatient services, and inability to make a parasitological confirmation of malaria. This study reported that with availability of anti-malarials at HCs, word spread rapidly among community members who turned up in large numbers. This increased attendance with majority of patients not being tested impacted on drug stocks. Another study in Uganda reported that only 34% public sector facilities stocked all tablet packs of artemether-lumefantrine (AL) [75]. AL stock-outs were also reported in Kenya [76] and in Zambia [77]. In this study, parasitological-based diagnosis significantly reduced anti-malarial prescription in both RDT and microscopy arms compared to presumptive treatment. This implies that with the widespread implementation of parasite-based

malaria diagnosis, and if clinicians adhere to test results would save anti-malarial drugs at the primary level health facilities.

The health policy recommends having a functioning laboratory at all sub-county government HCs. However, there was a shortage of staff with only 34% of the laboratory assistant posts filled. Further, a laboratory at this level of the health care delivery system serves about 25,000 people, implying that there is one microscope for this population. The microscope is used for other investigations than malaria. In addition, the laboratory personnel perform HIV, pregnancy, syphilis and typhoid tests. Due to patient load, 20-30 malaria slides can be examined satisfactorily per day. Lack of adequate laboratory services was a weakness that people exploited by collecting anti-malarials even when they were not truly sick. With the evidence that RDTs are more accurate, more feasible and more cost-effective than microscopy it is realistic to scale-up use of RDTs rather than microscopy. Furthermore, with clinical staff other than laboratory personnel performing the RDT implies that testing for malaria is not interrupted even when the laboratory personnel are away on leave (a form of task-shifting).

The current malaria policy provides guidance on therapy through the public and probably private not for profit (PNFP) facilities, but it is silent regarding the private for profit (PFP) sector. As elsewhere, the PFP sector serves a major part of the population seeking care [37,78,79]. The current study observed that 31.5% patients reported after they had used anti-malarials (including CQ and SP) which were not first-line drugs for treatment of uncomplicated malaria. These anti-malarials were mainly sourced from the PFP sector

including private clinics, pharmacies and drug shops. One study [79] observed that drug shops were an important source of health care that is more accessible in terms of convenience, cost, time spent, social proximity, better quality in terms of services and reliability of stocks than public health care facilities, and that the services were guided by responsiveness to client demands. There is no doubt that to make the greatest impact on current use of anti-malarials, testing needs to be available at all points where patients seek treatment. However, the style of service provision in the PFP sector where patients demand particular treatments (in drug shops for example) contrasts with the conventional clinical practice. Therefore, interventions to shift to RDT-based malaria treatment might require different approaches than those for the public health sector [79], particularly in raising awareness in communities of the multitude of causes and management for malaria-like illness. Due to the profits anticipated to be accrued from the sale of drugs, further research is needed regarding integration of RDTs in the PFP sector, other than drug shops and in incentivising the providers to adhere to test results.

It is reported here that RDTs were more feasible and more cost-effective at sub-county level primary care facilities in both low and high transmission settings in Uganda. However, these findings may not be applicable to lower levels of care such as the Village Health Teams (VHTs) that are currently implementing the Integrated Community Case Management (ICCM) strategy. Under this strategy, the VHTs are expected to use RDTs as a diagnostic tool for malaria in newborn and children under-five years of age at the community level. Although the potential for community health workers to use RDTs in Zambia were reportedly promising [80], and likely to be acceptable in Uganda [81], data

on economic evaluation regarding use of RDTs by VHTs, adherence to test results and the impact of RDTs on work load and retention of VHTs is not available.

The prescription of anti-malarials to patients with negative results was widespread in all transmission settings. Prescription of anti-malarials to patients with negative results is driven by the ingrained belief that a patient may still have malaria even in a setting of a negative blood smear, a position which was promoted by national [82] and international guidelines [83]. An earlier study [84] however, reported that withholding anti-malarial therapy in febrile children with negative blood smears in a malaria meso-endemic setting of Uganda was safe. In Tanzania, withholding anti-malarial treatment in children with fever who had a negative RDT result in a setting highly endemic for malaria was also safe and did not expose children to an increased risk of complications [85]. These findings support that in the era of expensive ACTs, directing resources towards improving diagnostic and treatment practices may provide a cost-effective measure for promoting rational use of anti-malarial therapy and may result in overall better health outcome, which in turn could support the credibility of the recommended treatment policies. Prescribing practice is therefore central in adherence to test results that should be a target for future interventions.

This study reported that in the low transmission setting, 25.8% and 17.6% patients with positive RDT and microscopy results respectively were treated with antibiotics. If parasite prevalence in the population is low, a diagnostic test is relevant. In high transmission settings detecting the presence of parasites can be misleading and may

divert the attention of clinicians from other diagnoses if they do not conduct an appropriate clinical examination. In such settings, clinical skills are irreplaceable, in order to differentiate malaria from other causes of fever, such as benign viral infection or potentially dangerous conditions, which can all be present with the malaria parasite co-existing only as a "commensal" or silent undesirable guest [86]. These rates of antibiotic treatment in patients with malaria positive test results reported here are high. In febrile outpatients, antibiotics should be used after meticulous assessment; otherwise there is a high risk of evolution and spread of resistance (because all human beings harbour commensal bacteria which can evolve resistance to antibiotics, and become pathogenic). It is thus essential that primary health care providers be availed with simple guidelines to enhance rational use of antibiotics among febrile patients.

The current study findings therefore, emphasize that as malaria infection reduces due to intensified control strategies such as: indoor residual spraying; use of mosquito bed nets and or preventive therapy; parasite-based diagnosis is not only more cost-effective, but is also necessary for good clinical practice.

The RDTs and laboratory supplies were in stock throughout the study implementation period. However, one constraint of this study was that AL got out of stock in the setting of high transmission intensity. This scenario impacted on outpatient attendance, and clinicians appeared to prescribe anti-malarials for patients to buy in the PFP sector but gave out antibiotics more often, a picture that distorted the true antibiotic prescription rates across diagnostic methods. Therefore, the clinicians' response to test results

regarding antibiotic prescription was fully analysed only in the low transmission setting. Further, sub study I (the baseline) had minimal qualitative input for the subsequent quantitative findings in sub studies II-V.

This trial was comprised of several sub-studies and the data collection process lasted for over one and half years. Because the study was integrated within the districts health services delivery system, most procedures were inclined to the resident clinical and laboratory staff. One challenge was that some staff were transferred while others left for further professional training. This necessitated re-training of the new staff posted by the district authorities. During the course of the trial, Bushenyi was partitioned into five districts while Iganga was partitioned into two districts. As the political environment in this country continues to evolve, further partitioning of the districts is anticipated. Although partitioning did not affect the study, description of the setting in the final report was rather challenging. Further, because of the longitudinal design of this study, some adjustments were made in the malaria treatment policy during the study implementation period (in the year 2010) and effected before the study closed. The step-down cost allocation methodology [68,69] adopted by sub study III was unique in that it enabled the inclusion of all resources available at the health centres. This study was the first to use this kind of costing in this setting. However other than medical records, it was challenging to search some financial related source documents (capital goods and some recurrent expenditures for example). It is crucial that health personnel at this level of care also get trained in financial records management.

5.2 Methodological considerations

5.2.1 Internal validity

This refers to the degree to which conclusions drawn from the study population relate to the source population. Assessing internal validity requires an evaluation of bias, confounding and chance which could distort the interpretation of the study findings.

5.2.2 Bias

This is the systematic deviation of results from the truth. The main biases of concern in this study were: information bias and selection bias.

Information bias

In order to prevent flaws in collecting information, the research team underwent rigorous training including theory and practice during pilot testing of study tools. Study tools were tested in two districts with comparable malaria transmission levels for their accuracy and reliability in collecting the required information. Thereafter, the research team was involved in refining the tools. In sub studies II-V, standard operating procedures for: finger prick for collection of blood; thick/thin blood smear preparation; staining smears; blood smear reading; and preparation and reading of RDT were used in training. The standard operating procedure for collecting blood samples on filter paper for PCR was used in sub study II only. The thermometers for measuring axillary temperature were new and were tested during training before being used on the study participants.

As a quality control measure and to avoid misclassification of test results, in sub study II data collection commenced after inter-reader reliability for both RDT and microscopy reached a very good agreement (kappa coefficient = 0.97). In addition, microscopists were blinded from RDT results; all malaria slides were double read by two expert microscopists; blood spots on filter paper for PCR were air-dried in dust free area on site, wrapped in plastic sample bags and placed in a zip-lock bag with silica gel to prevent DNA degradation; positive and negative controls were used for each round of PCR assay in addition to the 10% of the PCR samples that were randomly selected for re-analysis. PCR assays were carried out in a recognized laboratory "Makerere University-University of California San Francisco Molecular Research Laboratory." In sub studies II-V, blood slides were stained on site. Blood smears for sub study II were stored in special slide boxes and transported for reading by expert microscopists. Recall bias in relation to the drugs used for the current illness before coming to the study HCs was not a challenge as the mean duration of illness was 4.0 days for patients under-five years and 5.9 days for those five years and older. In sub study III, the Step-down cost allocation approach ensures that all available resources are included and recognizes the contribution of the administrative and support departments to the management of malaria patients in the outpatient departments. Further, the comprehensive decision analytic modelling approach ensured that all the computed costs and effectiveness information were used to populate the model. In all sub studies, close supervision and monitoring were done during collection and entry of data. But before entry, the filled data collection tools were manually checked and cleaned. Although the data were double entered in a customized entry template with in-built consistency checks in EpiData, validation of data sets were

done to check for entry errors before being exported to the analysis software. In sub studies IV-V, data were declared cluster design using the “svyset” command with HC as sampling units to cater for the clustering effects.

Selection bias

This bias occurs due to a flaw in the selection process of study units or subjects where a subset of the data is systematically excluded due to a particular attribute producing distorted results. In sub study I, all HCs at sub country level were included; and the 6/32 HCs where patient interviews were carried out were randomly selected. In addition, the 613 patients enrolled in the exit interviews were randomly selected and none refused to participate. In sub study II 5/317, about 1.6% of the total enrolled patients left before interview due to unknown reasons. This proportion is too small and unlikely to impact the reported findings in a significant way. In sub studies III-V, clusters (HCs as the primary sampling units) were randomised to the three diagnostic arms. This process was not influenced by the investigator. In these studies, all patients suspected to have malaria and eligible to be treated with artemether-lumefantrine (the recommended first-line drug for uncomplicated malaria) were enrolled and thus eliminating the probability of selection bias.

5.2.3 Confounding

Confounding is a distortion (inaccuracy) in the estimated measure of association that occurs when the primary exposure of interest is mixed up with some other factor that is associated with the outcome. At the design stage, confounding was controlled by

restricting participants using an inclusion criteria “patients weighing ≥5kg; suspected to have uncomplicated malaria infection; and eligible to be treated with artemether-lumefantrine – the first-line drug for uncomplicated malaria in Uganda.” In addition, randomisation of HCs to the three diagnostic arms in sub studies III-V was carried out so that the distribution of known and unknown confounding variables was similar in both the intervention and comparison groups. During analysis, control of confounding in sub studies II-V was achieved through stratification by malaria transmission levels, age and HC. Further, age and transmission intensity were adjusted in the Poisson regression model in sub studies IV and V.

5.2.4 Chance (or random error)

Many epidemiological studies are based on samples drawn from larger populations. The use of samples inevitably introduces uncertainty into the results. Any sample will, by chance, differ at least a little from its parent population. Therefore, even after biases and confounding have been taken into account, study estimates may result by chance and not represent the ultimate true values. An indication of the potential for such chance effects is provided by statistical analysis. In here, the probability values or *p*-values “the probability that the result found (or a more extreme one) could have been produced just by chance if there is no underlying association in the population” and confidence intervals are presented. The *p*-values were set at 0.05 level of significance and confidence intervals were calculated at the 95% level. The calculation of sample sizes using standard methods in sub studies I-II ensured enough study power and therefore my ability to rule out chance as a possible explanation for the findings. In sub studies III-V, the very large

sample sizes (range 22,052 – 102,087) even improved the precision of the estimates yielding narrow confidence intervals and thus making chance the unlikely explanation for the findings. In sub study III, robustness in the cost-effectiveness model was tested through sensitivity analysis in TreeAge software on the variables that were uncertain and prone to change over time.

5.2.5 External validity

External validity concerns the extent to which the results of a study can be generalized. This thesis is strategically positioned in an important area focussing on improving the management of febrile illness in Uganda, where these illnesses are the major killers. The accuracy and feasibility of different diagnostic methods were tested that is critical in reducing severe forms of malaria and other febrile illnesses. In this country, the laboratory is one of the areas usually not getting much attention. Thus, this study underscores the importance of distinguishing when and where to use other drugs like antibiotics in the presence or absence of positive parasitological evidence. The studies constituting this thesis were carried out in both high and low malaria transmission areas; were integrated within the existing healthcare delivery system to reflect the real-life operational conditions; and thus making results more relevant to both types of transmission settings. The studies are very inclusive and involved both males and females with age range of three months to 95 years. These are facility-based studies at primary level of care, highlighting the interface between the healthcare delivery system and the demand for services from the community, and thus strengthening the primary health care concept. The technology used was not only microscopy, but also RDTs that are based on

the detection of histidine rich protein-2 produced by *Plasmodium falciparum* and PCR that detects ribosomal DNA with the high quality control exhibited. Ethical procedures were adhered to; and the methods of randomisation and comparisons bring out strong evidence that is very useful for policy makers and programme implementers. Therefore, these findings could be generalised to the general population in settings with similar malaria transmission levels.

6 Recommendations for policy, practice and research

6.1 Policy recommendations

This study showed that the continued treatment of every patient with fever as malaria, problems with human resources, stock-out of ACTs in HCs and use of non-recommended anti-malarials are barriers to the universal parasitological testing and the ACT malaria case management policy. Assiduous prescription of anti-malarials disregarding negative results is likely to impact on the cost-effectiveness of the diagnostic methods, clinical care of patients as well as increasing the costs of diagnosis and that of the overall treatment. Furthermore if negative patients continue receiving anti-malarials, health workers are more likely not to see the need for parasite-based diagnosis and may not be motivated to implement the policy. The low sensitivity of microscopy reported here is an indicator that the quality of malaria case diagnosis greatly needs to be improved. The time cost with subsequent loss of income for patients was greater with microscopy because the time taken to diagnose a case and produce results was much longer compared with other diagnostic methods. The antibiotic prescription among febrile patients was widespread and has the potential to emasculate the good clinical practice and to erode the financial savings anticipated to be accrued from the widespread implementation of the universal ‘test and treat’ malaria strategy. RDTs demonstrated a superior accuracy and were found to be more feasible and more cost-effective. These findings therefore reinforce the malaria treatment policy adjustments made to rollout RDTs during the time when this study was being implemented.

There is need to supply adequate quantities of ACTs to all first line care facilities including the private sector and to train providers in this sector on the implementation of the ACT policy; withdraw ineffective anti-malarials from the market and to strengthen frontline health facilities to carry out investigations for malaria, and regular in-service training so that providers adhere to policy guidelines. Also there is need to increase access to parasite-based diagnosis where microscopy is currently used. In order to fully harness the benefits of parasitological confirmation of malaria, it is necessary to reduce the prescription of anti-malarials in negative as well as antibiotic treatment in febrile patients. Because RDTs demonstrated a superior accuracy, are more cost-effective and more feasible to implement, there is need to consider scaling up use of RDT rather than microscopy. However, as national and international drive to reduce the cost of RDTs and ACTs yield results, a contingency plan regarding malaria diagnosis is realistic because presumptive treatment is likely to become more attractive. Furthermore, there is need to sensitize health service users about the benefits of appropriate malaria diagnosis and treatment.

6.2 Recommendations for clinical practice

High test sensitivity in malaria diagnosis is important especially in settings where *P. falciparum* is endemic because this species contributes majority of malaria-related morbidity and mortality. Those infected with *P. falciparum* are likely to suffer poor outcomes if they are not appropriately managed. The HRP2-based RDT demonstrated a superior sensitivity and it was more feasible to implement parasite-based diagnosis for uncomplicated malaria using this method than with microscopy. Therefore RDT should

be integrated into routine management of febrile patients. However, it is vital to reduce prescription of anti-malarials in negative as well as antibiotics to febrile patients in general.

6.3 Recommendations for further research

- In this study, it was planned to have three diagnostic arms (presumptive, RDT and microscopy). In order to fully understand the provider behaviour regarding the use of these diagnostic methods, further research should incorporate an arm of RDT plus microscopy.
- This study presents data on parasite-based diagnosis at sub-county level government health facilities in Uganda. However, these findings may not be applicable to lower levels of care such as the VHTs that are currently implementing the ICCM strategy. More data on the feasibility of parasite-based diagnosis of malaria in ICCM by VHTs; and the impact RDTs have on VHTs' workload and retention of VHTs is warranted.
- This study was implemented in government health centres where patients do not make direct payment for medical services. Due to profits anticipated to be accrued from the full management of patients in the private sector including the retailing of drugs, further research is needed regarding integration of RDTs in the provision of services in PFP sector that might involve incentivising providers to adhere to test results.
- There is need to assess antibiotic prescription practice of providers in response to test results in a setting of high malaria transmission intensity.

- Before the study commenced, large quantities of RDTs were delivered to the Districts Medical Stores. These tests were in-stock throughout the implementation period. In real life however, requisitions are made though the pull-system (unlike drugs that are on the push-system) from National Medical Stores. This system is prone to stock outs due to supply chain problems and inability to quantify needs. It is thus important that with RDTs being rolled-out, an innovation is needed so as to document and track RDT stocks for example through the use of Short text Message Service (SMS) technology.
- In all five sub studies, the proportion of women attending health centres was approximately 60%. Further analysis indicated that the proportion of males ≥18 years of age attending health centres was 39%. Information is needed on the health care seeking practices of males in these rural settings.

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Annex: Papers (I-V)

I

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Challenges to implementation of artemisinin combination therapy policy in Uganda

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ABSTRACT

Uganda launched an artemisinin combination therapy (ACT) policy in 2006, using artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria, but insufficient information is available regarding its implementation. Semi-structured interviews were conducted with key personnel: 32 clinical and four laboratory staff from 32 health centres (HCs) in Bushenyi and Iganga districts and the Ministry of Health. Structured interviews with 613 patients receiving malaria treatment at six randomly chosen HCs were held. Data were collected on availability of antimalarials, treatment guidelines, staffing and malaria treatment decisions. Posts for clinical staff were inadequately filled. Only 15 (46.9%) HCs stocked AL for all weight categories. Nationwide, AL was out-of-stock March–July 2007. Twenty-one (65.6%) HCs stocked chloroquine. Out of 193 patients, 177 (91.7%) used antimalarials other than AL before coming to HCs. The unrecommended antimalarials were mainly sourced from the private for profit (PFP) sector yet there were no guidelines regarding provision of AL in the PFP sector. Only 53/613 (8.6%) patients were examined for parasites and only 8 (15.1%) had a positive blood slide. The majority of the patients attending HCs (560; 91.4%) received antimalarials but only 323 (57.7%) received AL. In order to improve the implementation of the current policy, AL should be availed in adequate amounts at all points of care including the PFP sector; non-recommended drugs should be withdrawn from the market and it should be ensured that malaria is confirmed by laboratory diagnosis. Study registration: Clinicaltrials.gov NCT00565071.

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1. Introduction

In recent years, the threat posed by failing, but inexpensive, antimalarial monotherapy such as chloroquine (CQ), sulphadoxine-pyrimethamine (SP) or in combination led to an international effort to replace these drugs with relatively more expensive artemisinin combination therapies (ACTs) for the treatment of uncomplicated malaria.[1,2] In 2002, the treatment policy in Uganda was changed from CQ to a combination of CQ + SP. This policy was short-lived due to an observed increase in resistance to 16% for SP alone and 12% to CQ + SP by 2004.[3] This observation coincided with the World Health Organization (WHO) recommendation to treat uncomplicated malaria using ACT. The Uganda government again changed the first-line drug from CQ + SP to artemether-lumefantrine (AL) in 2005.[4] The current malaria treatment policy further states that artesunate + amodiaquine is the alternative that can be used in situations where AL is not available. Oral quinine is the second-line drug to be given if AL has failed or when it is contra-indicated. Parenteral quinine is recommended for

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severe and complicated malaria, while SP is for intermittent preventive treatment during pregnancy. For malaria in pregnancy, quinine is the recommended treatment.

AL is a co-formulation containing 20 mg artemether and 120 mg lumefantrine per tablet. It is supplied by government through the drugs and supply delivery chain from the National Medical Stores (NMS). Patients access these drugs at no cost. AL is distributed as packaged in four different weight-specific categories: 5–14 kg, 15–24 kg, 25–34 kg and ≥35 kg.[5] It is a three-day course of oral treatment with tablets taken twelve-hourly.

Several studies have reported on the difficulties facing changes in national antimalarial drug policy such as conflict of interest of pharmaceutical companies, delays in release of funds, complex drug ordering[6–8] and non adherence of health workers to treatment guidelines.[9–11] Thus, understanding the key factors that facilitate or undermine policy implementation in various contexts is critical for guiding resource allocation. In this paper, we describe the challenges to ACT policy implementation at rural public health centres in Bushenyi and Iganga districts of Uganda. This data is part of the ongoing trial assessing the cost-effectiveness of managing malaria with AL using various diagnostic techniques.

2. Materials and Methods

2.1. Type of study, population and setting

The study design was cross sectional and data was collected from September 2006 to February 2007. Thereafter, drug stock status was monitored monthly until July 2007. Information was collected from rural health workers at public health centres at sub-county level (HC III), outpatients attending these units and at the Ministry of Health level. The study was carried out in Bushenyi district located in south-western Uganda and Iganga district in the eastern part of the country. The two districts were selected because they experience different malaria transmission intensities.

Bushenyi district: Bushenyi has a population of 731 392. The district is divided into seven health service zones (Health Sub-Districts or HSDs) with four hospitals, six health centres (HC) at county level (HC IV), 20 public HC III and 56 health centres at parish level (HC II). The district experiences low and unstable malaria transmission with people of all ages being at risk. It is epidemic-prone, with occasional malaria outbreaks occurring shortly after the rains.[12] Before the change to the current malaria policy, Bushenyi had a strong home-based management of fever (HBMF) strategy that was being implemented by 4034 community drug distributors (CDDs). HOMAPAK (CQ + SP) was used in HBMF for children under five years of age.

Iganga district: Iganga has a total population of 540 939. The district is divided into four HSDs. It has one public hospital, three HC IV, 15 HC III and 57 HC II. The district experiences very high malaria transmission intensity. Malaria is the leading cause of morbidity and outpatient attendance for all age-groups.[13] Before the current malaria treatment policy, Iganga trained 1396 CDDs but they were not active due to lack of HOMAPAK.

2.2. Health Centre grade III

HC III is an intermediate-referral unit serving a sub-county with an estimated population of 25 000. It provides the following services: general outpatient, family planning, immunisation, counselling and testing of HIV, maternity and postnatal care, laboratory, inpatient care, environmental health and home visiting to review the progress of critically ill patients. Ideally it is manned by two clinical officers (medical assistants), one nursing officer, two enrolled/registered nurses, two midwives, three nursing assistants, one health assistant, one laboratory technician, one laboratory assistant, a records officer, two night watchmen and two porters. However, all these staff categories are rarely found at the HCs. Clinical officers have three years of pre-service training, certificate-holder nurses and midwives have two, while the training for nursing assistants varies from three to nine months.

Requisition for AL is made by HC staff employing the 'pull' system. This is a demand-based system where quantification of drugs is based on the number of patients seen and the diagnoses made in the previous month. Drugs are delivered by NMS to the district headquarters from where they are collected by the HCs. The duration from requisition to notification of drug availability at NMS is unpredictable. However, if some of the requested drugs are not available, a certificate of non-availability is issued permitting the HC to purchase elsewhere.

The policy was changed in 2005, but it was launched on 25 April 2006. Prior to the distribution of AL, malaria case management guidelines were developed,[14] and wall charts were prepared to serve as job aids.[7,15] In-service training for health workers was conducted starting with 'training of trainers' (that included senior clinicians) at the national level, who in turn facilitated the training at the districts. The training sessions for lower cadres of staff were conducted at their health units. AL was then distributed to public health units.

As a quality control measure in the delivery of services, sub-county HCs are visited and supervised by staff from the county-level HC, termed 'support supervision'. At the end of the visit, a technical support supervision report is made and a meeting held with staff to discuss the findings plus giving suggestions for improvement.

The HC III is unique in that it is placed in the middle of the patients' referral pathway (between parish HCs and county HCs). Unlike at the higher levels of care, the policy of having functional laboratories at HC III is not fully implemented. Most HCs either lack equipment and supplies or do not have laboratory personnel. In order to contribute to improvement of delivery of health services, it was considered appropriate to conduct the study at this level.

2.3. Data collection

A one-week training workshop for the research team was conducted in each district. All members underwent rigorous training including theory and practice during pilot testing of the questionnaires. The questionnaires were tested in three HCs in Mbarara and three in Mayuge districts for their accuracy and reliability in collecting the required

Table 1
Posted staff at sub-County public health centres compared with the recommended staffing.

Cadre	Health centres with stated cadre posted					Recommended staffing	Total filled post in 32 HCs	Total[b] recommended in 32 HCs	% posts filled
	0	1	2	3	4				
Clinical officer	6	25	1			2	27	64	42
Nursing officer	17	14	1[a]			1	15	32	47
Enrolled/Registered nurse	18	13	1			2	15	64	23
Midwife	3	25	3		1[a]	2	31	64	48
Nursing assistant	4	15	8	4	1[a]	3	43	96	45
Laboratory technician	31	1				1	1	32	3
Laboratory assistant	21	11				1	11	32	34
Health assistant	6	25	1[a]			1	26	32	81
Records assistant	17	15				1	15	32	47
Night watchman	6	22	4			2	30	128	23.4
Porter		22	9	1[a]		2	42	64	65

[a] Excess staff were excluded in computation of percentages.
[b] Total recommended posts = 32 health centres **x** recommended staffing for that post.

information. Thereafter, the research team was involved in refining the questionnaires. The malaria transmission intensities in these districts are similar to Bushenyi and Iganga respectively.

The study participants included: clinical staff (clinical officers, nurses and midwives), laboratory personnel and Ministry of Health officials. All 20 sub-county public HCs in Bushenyi and 12 in Iganga were visited. On the day of visit, one of the available clinical staff was interviewed using a semi-structured questionnaire. Data were collected on: staffing levels, availability of malaria treatment guidelines and drugs, personnel involved in management of malaria patients and treatment decisions. Equipment and supplies such as thermometers, microscopes and laboratory reagents were checked to confirm their availability. The staff in the four HCs with functioning laboratories were also interviewed. Six HCs (three per district) were randomly chosen to collect baseline patient data. Six hundred and thirteen patients clinically treated for malaria were interviewed (exit interviews). This sample size was estimated using a standard formula[16] assuming 50% suspected malaria cases received AL with 90% power and 95% Confidence Interval. Patient interviews were conducted using a structured questionnaire and collected data on use of antimalarials before reporting to the HCs, sources of drugs used, and on the services received at the health units.

2.4. Data management

All questionnaires were manually checked for completeness and the semi-structured interviews were coded before entry. All quantitative data was then entered in EpiData version 3.1 software (The EpiData Association, Odense, Denmark), and exported to SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) for analysis using descriptive statistics. The qualitative data was analysed manually and common themes developed. The outcomes were: availability of staff, antimalarials, treatment guidelines, use of antimalarials before reporting to HC, proportion of patients receiving parasitological confirmation of malaria and treatment decisions by staff.

3. Results

Thirty two interviews were conducted with the clinical staff (one per HC), four laboratory staff and one at the Ministry of Health. Six hundred and thirteen patient interviews were also conducted after receiving the services.

3.1. Staffing, practices and guidelines

Only 27 (42%) of the clinical officer posts were filled (Table 1). Six HCs were headed by Enrolled Nurses. In all health units, the clinical staff including midwives and nursing assistants managed malaria patients. In some centres, nursing assistants managed clinics without support of more qualified staff. Only one HC had a laboratory technician–this was the referral facility for trypanosomiasis located in Iganga district. Eleven HCs (34%) had the laboratory assistant post filled, although only four had functioning laboratories.

All HCs had AL malaria case management guidelines displayed in the consultation rooms. The diagnosis of malaria was based on signs and symptoms in 28 (88%) HCs. However, 17 (53%) did not have thermometers to measure body temperature. In these centres, patient temperatures were qualitatively estimated using the back of the palm and reported either as high or normal.

Only 53 (8.6%) patients attending the four HCs with functional laboratories had their blood tested for malaria. Of these, 8 (15%) had a positive blood slide. The decision to send the patient for microscopy was based on: (1) if the clinician was not sure of the diagnosis, (2) patients had not responded to initial antimalarial treatment, (3) level of workload (number of patients) as microscopy creates some delays, (4) patients that had never taken antimalarial medication for the current illness, (5) a patient demand for laboratory testing, (6) availability of laboratory personnel and (7) patient ability to pay 1000 Uganda shillings (US$ 0.59). Out of the 53 patients tested for malaria, 27 (51%) were charged 1000 Uganda shillings (US$ 0.59) each for laboratory service.

Table 2
Percent sub-County public health centres with antimalarial drugs in-stock.

Antimalarial	Bushenyi *n* (%)	Iganga *n* (%)	Total *n* (%)
AL (for all weight categories) in stock	9 (45)	6 (50)	15 (47)
AL for 5-14 kg in-stock	20 (100)	10 (83)	30 (94)
AL for 15-24 kg in-stock	16 (80)	7 (58)	23 (72)
AL for 25-34 kg in-stock	12 (60)	6 (50)	18 (56)
AL for >34 kg in-stock	11(55)	11 (92)	22 (69)
Quinine (injectable formulation)	15 (75)	5 (42)	20 (63)
Chloroquine	17 (85)	4 (33)	21(66)
Artesunate-Amodiaquine	0	0	0
Sulphadoxine/pyremethamine	0	0	0

AL = Artemether-Lumefantrine.

3.2. Availability and use of antimalarials

Only 15 (47%) of the HCs had AL in stock for all weight-specific categories (Table 2). Of the different weights, 30 (94%) had AL stock for children weighing 5–14 kg; 23 (72%) stocked AL for 15–24 kg; 18 (56%) for 25–34 kg; and 22 (69%) for $\geq$ 35 kg. Twenty four (75%) of the HCs experienced AL stock-out during the six months preceding this study. There was a nationwide AL stock-out from March to July 2007.

All HCs stocked neither SP nor artesunate-amodiaquine. Only 20 (63%) had injectable quinine in stock. For those without quinine, the average duration of stock-out was five months. Twenty one (66%) stocked CQ.

HCs followed the demand-driven system in making requisitions for AL. NMS however continued to supply CQ together with AL. The presence of both antimalarials in stock complicated the treatment decisions. Table 3 shows the decisions by clinicians to dispense either AL or CQ when both were in-stock. CQ was reserved for pregnant women in first trimester, children who were not vomiting but suspected to have malaria, patients with a negative blood slide and those who wanted to keep antimalarials for future use. The need to get CQ off the shelves also influenced its prescription. The HC staff also reported that AL was reserved for patients not responding to CQ or CQ/SP, allergy to CQ and those with positive blood slide.

Due to the erratic supply of drugs, availability, especially of antimalarials, at the health unit was a prime determinant of patient attendance. Whenever new drug consignments arrived at HCs, word spread rapidly among community members and many people reported to health units for treatment. 'Information about drug availability was shared during community gatherings such as local council meetings and funeral services' (Clinical Officer, Nambale HC III, Iganga). The marked increased patient attendance at HC coinciding with availability of drugs was also reported by health personnel in Bushenyi HCs.

3.3. Patient data

Of the 613 patients clinically diagnosed as having malaria, 360 (58.7%) were female and 194 (31.6%) were children below five years of age. Table 4 shows that there were significantly more children below five years attending HCs in Iganga than in Bushenyi ($P < 0.0001$). The fewer children attending HCs in Bushenyi was attributed to the strong HBMF strategy that was implemented through CDDs. The majority (341, 55.6%) of the patients reported after initiating treatment with a range of medications. Of these, 193 (56.6%) had used antimalarials including: CQ alone (115, 59.6%), SP alone (23, 11.9%), artemisinin derivatives (16, 8.3%), quinine (28, 14.5%) and CQ + SP combination for children under five years of age (11, 5.7%). Patients who used CQ before reporting to HCs were more likely to be in Iganga ($P < 0.0001$). Ten patients used herbs that they believed cured malaria. Patients obtained antimalarials from multiple sources (one patient trying more than one source) including: drug shops 111 (32.6%), home (leftover drugs from previous illness episodes) 88 (25.8%), other HC 99 (29.0%) and CDDs 11 (3.2%).

At the study HCs 560 (91.4%) patients received antimalarial treatment. This included 323 (57.7%) AL, 159 (28.4%) CQ monotherapy and 78 (13.9%) CQ + SP for children under five years. Clinicians in Iganga were more likely to prescribe AL compared to their counterparts in Bushenyi

Table 3
Factors considered in dispensing AL or CQ if both are in stock at sub-County public health centres.

Criteria for giving CQ if AL is also in stock	Criteria for giving AL if CQ is also in stock
• Pregnant women in first trimester	• Those who have already used CQ for current illness
• Children because they do not complain about bitterness	• If the patient asks for AL
• If blood smear result is negative	• If the patient suffered from malaria less than three months ago
• If patient had malaria three or more months ago	• If the patient has been on CQ or CQ/SP and not improving
• If clinician detects that the patient just wants to keep drugs at home	• Patient is allergic or complains of itching while on CQ treatment
• If patient has no complaint about CQ	• Because parasites in adults are resistant
• If the patient had no previous malaria treatment for the current complaint	• Patient is re-attending at the centre
• If the patient refuses AL because the tablets are too many	• Patient has severe symptoms
• If it is uncomplicated malaria	• Patient has positive blood smear
• Sickle cell patient with malaria infection	• If the patient is about 60yrs or above

AL = Artemether-Lumefantrine, CQ = Chloroquine.

Table 4
Patient data stratified by District.

Variable	Bushenyi *n* (%)	Iganga *n* (%)	*P*-value[a]
Female	176 (56.4)	184 (61.1)	0.251
<5 years of age	70 (22.4)	124 (41.2)	0.000[b]
Got some treatment before coming	167 (53.5)	174 (57.8)	0.292
Got malaria drug before coming			
AL	3 (1)	13 (4.3)	NC
Chloroquine	29 (9.3)	86 (28.6)	0.000 [b]
Quinine	21 (6.7)	7 (2.3)	NC
Sulfadixine/Pyrimethamine	10 (3.2)	13 (4.3)	NC
Source of drugs			
Home (left over from previous treatment)	40 (24)	48 (27.6)	0.460
Drug shops	53 (31.7)	58 (33.3)	0.871
Community Drug Distributors	11 (3.2)	0	NC
Another health centre	13 (4.2)	86 (49.4)	0.000 [b]
Prescribed AL at study health centre	136 (42)	187 (58)	0.000 [b]

AL = Artemether-Lumefantrine.
[a] Two-sided Fisher's Exact Test.
[b] Statistically significant, NC = Not calculated.

($P < 0.0001$). Out of 323 treated with AL, 25 (7.7%) and 16 (5.0%) were given quinine or CQ injections respectively as starting dose although they were not vomiting. Persistent vomiting is one of the danger signs of severe malaria where patients cannot tolerate oral medication and therefore merit parenteral treatment.

4. Discussion

The decision to change the antimalarial treatment policy and the subsequent implementation of the policy has been challenging. The major challenges identified in this study regarding use of AL for every patient of non-severe malaria were: stock-outs in HCs, continued use of non-recommended antimalarials, lack of parasitological diagnosis and problems with human resources.

Less than 50% of the HCs had AL for all weight-specific categories in stock. Several factors contributed to the stock-out of AL. At the national level, stock-out was related to delayed release of procurement funds, bureaucracy in the procurement process, limited stocks at NMS and erratic delivery schedules. The Malaria Control Programme at the Ministry of Health confirmed that the delay in release of procurement funds directly impacts onto the AL stocks (personal communication). NMS was supposed to deliver drugs quarterly to district medical stores. In some quarters however, drugs were not delivered prompting HCs to make alternative collection arrangements. Even after quantification following guidelines by HCs, NMS issued fewer quantities of the drugs requested. As discussed elsewhere,[17] this stock-out crisis is a health systems issue resulting from inadequate ordering, distribution and supply. Because AL comes in four weight-specific packs, NMS is managing the supply of four products and not one.

Stock-out in HCs was related to increased utilisation of outpatient services, and inability to make a parasitological confirmation of malaria. Clinical management of all fevers as malaria was the norm even with these expensive drugs. At the same time, utilisation of outpatient services in HCs had increased since the abolition of user fees in 2000.[18] In another study in Uganda it was reported that in the public sector, ACT stock-outs was an obstacle to antimalarial treatments with only 34% of the health units stocking all tablet packs of AL; ACTs are often unavailable 33% of the time.[19] AL stock-outs have also been reported in Kenya.[20] In Zambia, health units had AL stock-outs for 30% of the year.[21]

Due to frequent stock-outs (especially tablets for patients weighing $\geq$14 kg) and because the strength of 20 mg artemether and 120 mg lumefantrine is the same across the weight-specific categories, clinicians coped with the shortage by combining the children's blister packs to treat adults. Although this practice led to treatment of those patients in need instead of sending them away without treatment, it impacted the drug stocks for the young children. In the whole country, there was a general stock-out of AL from March to July 2007, in addition to HCs experiencing stock-outs within the six months preceding this study. AL stocks have not stabilised to date. In order to maintain adequate stocks, the procurement system and stock management information system need to be strengthened.[17]

Unfortunately, none of the HCs stocked artesunate-amodiaquine (the alternative first-line drug) although it is manufactured by a local company. Artesunate-amodiaquine is available in private for profit (PFP) drug outlets. A memorandum of understanding needs to be drawn between government and the local manufacturing company to supply artesunate-amodiaquine so as to bridge the gap whenever AL stock-out is anticipated.

The current malaria policy provides guidance on malaria therapy and simplifies treatment decisions by specifying which drugs are to be used for uncomplicated malaria. This policy includes provision of antimalarials through the public and private not for profit facilities, but it is silent regarding the PFP sector. As elsewhere, the PFP sector serves a major part of the population seeking care.[22–24] The current study reports that 55.6% patients came to HCs after initiating treatment, with 56.6% using antimalarials that included CQ, SP, quinine and branded artemisinin derivatives. The non first-line antimalarials were sourced from the PFP sector mainly from private clinics, pharmacies and drug shops where they are normally accessed without prescription.

However from the outset, the PFP sector was excluded in the new ACT malaria treatment policy, yet as evidenced from this study many patients with malaria continue to seek care from this sector. Thus, measures are required to subsidise and scale up the availability of the recommended antimalarials to include the PFP sector and to train providers in this sector on the implementation of ACT policy.[9,11]

In HCs, clinicians administered CQ or quinine injections as a starting dose followed by AL. None of the patients given injections had danger signs of severe malaria such as persistent vomiting that would merit parenteral treatment. This clearly demonstrated non-adherence to malaria treatment guidelines. Furthermore, clinicians prescribed CQ monotherapy, although the first-line regimen had been changed to CQ + SP in 2002 and from 2005 to AL. CQ was supplied by NMS in addition to donations from health partners. CQ is imported by private pharmaceutical companies, as well as being manufactured locally. The continued availability of non-recommended antimalarials is a setback to AL policy implementation.[19] Our findings are in agreement with another study,[25] which reported that AL was more likely to be prescribed in the absence of CQ. A study in Kenya[26] also reported that presence of amodiaquine + SP in health units was an impediment to AL implementation. Earlier observations[27] have shown that the probable result of continued use of ineffective drugs such as CQ are increases in malaria-associated morbidity and mortality, expansion of malaria in new areas and outbreaks in areas of unstable malaria. Strategies are needed to suspend the local manufacturing and importation of ineffective drugs and to systematically withdraw those in circulation without creating a vacuum. Furthermore, there is need to conduct regular refresher training for clinicians so that they adhere to the policy guidelines.

We report here the shortage of clinical staff and laboratory personnel at HC III. Due to under-staffing, nursing assistants managed outpatients' clinics without the support of more qualified staff. Additionally, those in charge of health units were frequently unavailable, reportedly attending workshops. The turnover of staff was also high with no immediate replacement due to public service recruitment restrictions.

The health policy recommends having a laboratory with a functioning microscope at all sub-count HCs. However, a HC at this level serves about 25 000 people, implying that there is one microscope for this population. The microscope is also used for investigations other than malaria. Furthermore, in the four HCs where the laboratory was operational, the personnel also performed HIV, pregnancy and syphilis tests. We have reported that these laboratories were charging fees from patients which were a barrier for those who could not afford the service. Lack of adequate laboratory services was a weakness that some people exploited by collecting antimalarials even when they were not sick. In order to focus the new policy, current recommendations of presumptive diagnosis and subsequent management of fever with antimalarials needs to be adjusted. Guidelines for parasitological confirmation are needed so that all patients are tested and AL is prescribed to only those with confirmed malaria.[28] Malaria rapid diagnostic tests could be considered in these rural HCs where microscopy is a challenge.[29,30]

This study shows the major challenges to use of ACTs in Uganda that are related to drug stock outs, non availability of ACTs in the private for profit sector, continued use of ineffective antimalarials and treatment of every patient with fever as malaria. In order to overcome these challenges there is need to supply adequate quantities of ACTs to all first line care facilities including the PFP sector, withdraw ineffective antimalarials from the market and to strengthen the health centres to carry out laboratory diagnosis of malaria.

Authors' contributions: All authors conceived and designed the study; VB and FN collected, analysed, interpreted the data and drafted the manuscript; PM critically revised the manuscript. All authors read and approved the final manuscript and are guarantors of the paper.

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MALARIA JOURNAL

RESEARCH Open Access

Are rapid diagnostic tests more accurate in diagnosis of *plasmodium falciparum* malaria compared to microscopy at rural health centres?

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Abstract

Background: Prompt, accurate diagnosis and treatment with artemisinin combination therapy remains vital to current malaria control. Blood film microscopy the current standard test for diagnosis of malaria has several limitations that necessitate field evaluation of alternative diagnostic methods especially in low income countries of sub-Saharan Africa where malaria is endemic.

Methods: The accuracy of axillary temperature, health centre (HC) microscopy, expert microscopy and a HRP2-based rapid diagnostic test (Paracheck) was compared in predicting malaria infection using polymerase chain reaction (PCR) as the gold standard. Three hundred patients with a clinical suspicion of malaria based on fever and or history of fever from a low and high transmission setting in Uganda were consecutively enrolled and provided blood samples for all tests. Accuracy of each test was calculated overall with 95% confidence interval and then adjusted for age-groups and level of transmission intensity using a stratified analysis. The endpoints were: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). This study is registered with Clinicaltrials.gov, NCT00565071.

Results: Of the 300 patients, 88(29.3%) had fever, 56(18.7%) were positive by HC microscopy, 47(15.7%) by expert microscopy, 110(36.7%) by Paracheck and 89(29.7%) by PCR. The overall sensitivity >90% was only shown by Paracheck 91.0% [95%CI: 83.1-96.0]. The sensitivity of expert microscopy was 46%, similar to HC microscopy. The superior sensitivity of Paracheck compared to microscopy was maintained when data was stratified for transmission intensity and age. The overall specificity rates were: Paracheck 86.3% [95%CI: 80.9-90.6], HC microscopy 93.4% [95% CI: 89.1-96.3] and expert microscopy 97.2% [95%CI: 93.9-98.9]. The NPV >90% was shown by Paracheck 95.8% [95% CI: 91.9-98.2]. The overall PPV was <88% for all methods.

Conclusion: The HRP2-based RDT has shown superior sensitivity compared to microscopy in diagnosis of malaria and may be more suitable for screening of malaria infection.

Background

The World Health Organization (WHO) recommends prompt parasitological confirmation by microscopy or rapid diagnostic test (RDTs) for all patients with suspected malaria before treatment is started [1,2]. Treatment solely on the basis of clinical suspicion should be considered only when a parasitological and or RDT diagnosis is not accessible [1-3]. Parasitological based diagnosis of malaria is currently of global public health priority as use of more expensive and limited supply antimalarials increases [4-6], malaria infection and disease become rarer with increasing malaria control strategies [7] and for good clinical practice [3].

In most malaria endemic countries of sub-Saharan Africa, the current standard for laboratory confirmation of a clinical malaria diagnosis is a peripheral blood film, examined microscopically. However, microscopic based diagnosis of malaria is labour-intensive requiring trained staff and quality equipment attributes that are scarce in resource-poor settings [8,9]. Thus, most clinicians often rely on clinical signs and symptoms for diagnosis of malaria, even when slide microscopy is available [10,11].

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Besides, when anti-malarials were relatively cheap, presumptive treatment of all fever cases was deemed more cost-effective [12].

Uganda piloted the histidine-rich protein II (HRP2)-based RDT (Paracheck) and rolled it out as an instrument of choice for parasite-based malaria diagnosis and patient management in six districts in the first phase in 2008. Paracheck was distributed to parish-level and to selected sub-county health centres (HCs) without laboratory infrastructure. RDTs have been out of stock for twelve months. Currently, there are 955 sub-county and 2,008 parish-level HCs in 112 districts in the country. Depending on availability of RDTs, scaling up to additional 22 districts is planned for January 2011.

Some data suggest that RDTs may not be very sensitive especially in varying transmission intensities [13,14]. However, most evaluations of RDTs have used expert microscopy as gold standard [15-17]. Since both microscopy and RDTs have limitations in identifying malaria infection [13,18,19], there is need to use a more accurate gold standard (such as PCR) in assessing the accuracies of these diagnostic tests. The aim of this study was to compare the diagnostic accuracy of a histidine-based RDT (Paracheck) to that of HC microscopy, expert microscopy and presumptive diagnostic method in diagnosis of malaria in rural HCs of Uganda, with PCR as gold standard.

Methods

Study setting

Data was collected from June to July 2007 in three randomly selected sub-county level government HCs in Bushenyi and three in Iganga districts of Uganda. Bushenyi is categorized as low and Iganga as high malaria transmission intensity settings. The annual entomological inoculation rates are not known, but are reported to be <10 and >500 infective bites per person per year in the neighboring districts of Kanungu and Tororo respectively [20]. The detailed description of the study sites has already been published [6].

A sub-county level HC laboratory is manned by one laboratory assistant who has two years of pre-service training. The laboratory personnel perform all investigations requested by the clinician. For malaria, at least one out of ten slides is stored daily for quality control. The district laboratory-focal person (who is also the in-charge of the district hospital laboratory) performs quarterly technical support supervision to HC laboratories. During supervision the microscope, stains, staining of slides and reporting are checked. The supervisor performs fresh films with the laboratory assistant. Where necessary, an immediate feedback is given, but also takes a percentage of the slides for further examination. The external quality assurance is coordinated at the national level. As contribution to improvement in the delivery of services, the study provided HC laboratories with new binocular microscopes, reagents and supplies.

Sample size estimation

Using a nomogram [21], estimated malaria prevalence of 63.5% [22], p-value set at 0.05, with estimated sensitivity of Paracheck of 95% [23], a sample size of 150 in each district was appropriate totalling 300 patients for the two districts.

Participants and eligibility

All male and female outpatients attending study centres with clinical suspicion of uncomplicated malaria based on fever and or history of fever within the previous 48 hours were eligible for inclusion in the study. Lack of consent and incomplete data constituted the exclusion criteria.

Training of study team

The research team comprised of staff of the selected HCs (three clinical officers, three laboratory assistants, nine nurses) and three research assistants per district. A one-week residential training workshop was conducted in each district. Standard operating procedures for: 1) finger prick for collection of blood, 2) thick/thin blood smear preparation, 3) staining smears, 4) blood smear reading, 5) preparation and reading of Paracheck, and 6) collection of blood on filter paper for PCR were used in training. All members were trained in theory and practice during pilot testing of the patient case report forms (CRFs). Data collection commenced after inter-reader reliability for Paracheck reached a very good agreement (kappa coefficient = 0.97).

Design and patient enrolment

This was a diagnostic clinical trial. The diagnosis of malaria was made in the outpatient department by the attending HC clinicians. Only those eligible were informed about the study. Those who gave consent were consecutively enrolled. Medical history, socio-economic and demographic data were recorded on CRFs. Figure 1 shows the trial profile.

Description of laboratory procedures

Malaria microscopy

Blood for thick and thin smears, RDT and PCR were collected from the same finger-prick. Duplicate thick and thin smears were prepared on separate frosted slides bearing the patient's identification number. Standard staining was made using the "Field's stain" method. This method provides a readable film within few minutes compared to "Giemsa stain." The Field's stain was the only re-agent available in HCs and to which the laboratory assistants

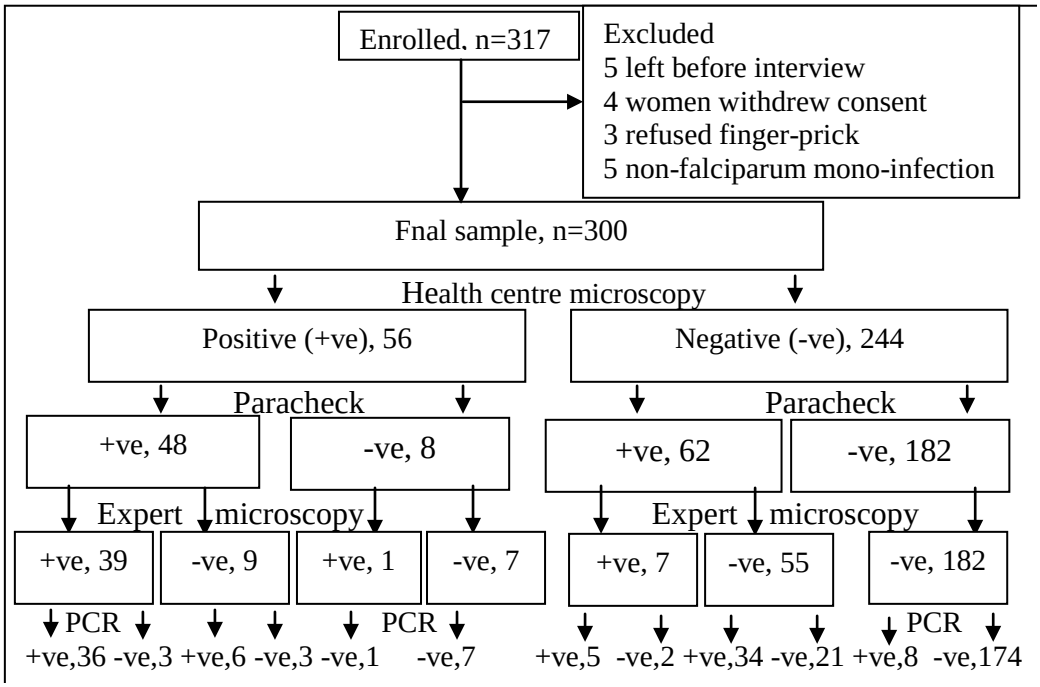


Figure 1 Trial profile.

were familiar. Blood slides were read at the HCs (magnification x1,000) under natural light (none of the study HCs had electricity). Each film was graded as positive (asexual malaria parasites seen) or negative (no malaria parasites seen) based on the inspection of 200 fields of the thick smear. The parasite density was estimated assuming 8,000 white blood cells/μl [24]. The thin smear was useful in species identification. The laboratory assistants were blinded of the RDT results. All slides were stored in secured slide-boxes and read by an expert microscopist at Mbarara Regional Referral/University Teaching Hospital located in western Uganda. For quality control, all slides were re-read by another expert microscopist at Makerere University School of Public Health in Kampala. The expert microscopist was blind to HC microscopy and RDT results. The readings of the expert microscopist and that of the quality control microscopist were discrepant in seven slides. In these cases, the judgement of another senior laboratory technician was final.

All HC laboratory personnel had ≥4 years of work experience at the study HCs. The expert microscopist was a senior laboratory technician with eight years of work/research experience at Mbarara Regional Referral/University teaching hospital. Quality control was performed by senior laboratory personnel with over nine years of work/research experience.

Paracheck Pf

The choice of Paracheck Pf device (Orchid Biomedical Systems, Goa, India) was based on its stability [3], low cost, reported high sensitivity (97%) and moderate specificity (88%) in controlled trials, ease of use [16] and it was likely to be preferred by the Uganda Ministry of Health. About 5μl of blood was drawn by the laboratory assistant using a loop provided with the device. The test preparation and interpretation was done following manufacturer's instructions. Each test was read by two trained nurses. The nurses were not aware of the microscopy results. The test was considered positive when the antigen and control lines were visible in their respective windows, negative when only the control band was visible and invalid when the control band was not visible. Faint test lines were considered positive. The readings were discrepant in two faint test lines, where the judgement of a third trained nurse was final.

The PCR assay

The *Plasmodium falciparum* species-specific nested PCR was preferred because this species contributes majority of malaria morbidity in Uganda. Those infected with *P. falciparum* are likely to suffer poor outcomes if they are not appropriately managed. Finger-prick blood was blotted on Whatman 3 MM filter paper, dried in dust free area, wrapped inplastic sample bags and placed in a zip-lock bag with silica gel to prevent DNA degradation. The samples were sent to Makerere University-University of California San Francisco Molecular Research Laboratory http://muucsf.org/index.html located in Mulago National Referral Hospital in Kampala for analysis. DNA was extracted from filter paper using the chelex method

[25]. *Plasmodium* genus-specific PCR, followed by *P. falciparum* species-specific nested PCR of 18 S small subunit ribosomal DNA was performed following a standard protocol [26]. PCR products were stained by ethidium bromide and resolved by gel electrophoresis on a 2.5% agarose gel. DNA size standards were separated alongside PCR products to allow sizing of species bands. Upon completion of the gel electrophoresis, gels were placed in a gel imaging cabinet and digitally photographed under ultraviolet light. Gel images were printed and corresponding sample lanes scored visually for the presence of *P. falciparum*. Positive and negative controls were used for each round of PCR. In addition, twenty two samples were randomly selected plus eight samples that were positive by PCR only (10% of the total) for re-analysis as a quality control measure. Quality assurance is performed by the University of California San Francisco, USA.

Patient management

The field microscopy results and that of Paracheck were forwarded to the clinician to guide on the treatment decision. All patients with positive test results (slide or Paracheck) were immediately treated with artemether-lumefantrine (the current first-line anti-malarial) on the same day of visit. Patients with negative results received further assessment and an appropriate treatment strategy was given.

Statistical analysis

Data was double-entered and validated in EpiData version 3.1 software (The EpiData Association, Odense, Denmark) and analysed using Stata version 10 (Stata Corp, Lakeway, College Station, Tx, USA). The sensitivity, specificity, PPV and NPV of HC microscopy, expert microscopy, axillary temperature diagnosis and RDT were determined with PCR as gold standard. A sub-analysis with expert microscopy as gold standard was performed to compare the results with that of PCR. Sensitivity was calculated as the proportion of positive test results obtained among samples containing malaria parasites by PCR, specificity as the proportion of negative test results obtained among samples whose PCR results were negative. PPVs and NPVs were calculated as the proportion of true-positive results among all positively reacting samples and as the proportion of true negative results among all negatively reacting samples, respectively. Accuracy of each test was calculated overall with 95% confidence interval (CI) and then adjusted for age-groups and level of transmission intensity using a stratified analysis. Because PCR was *P. falciparum* species-specific, and yet Paracheck only detects *P. falciparum*, five non-falciparum mono-infections (three *Plasmodium malariae* and two *Plasmodium ovale*) were excluded in the analysis.

Ethical approval

The study was approved by Makerere University School of Public Health Institutional Review Board; and the Uganda National Council for Science and Technology (Ref: HS 209). Written informed consent was sought from participants (or parents/legal guardians for minors) at the time of interview. The study is registered with the Clinicaltrials.gov (NCT00565071).

Results

Socio-demographic profile of subjects

Three hundred seventeen patients were enrolled from June to July 2007. Five patients left before interview, four female patients withdrew consent because they wanted to consult their husbands, three feared the finger-prick and it was not possible to get blood specimens while five had non-*falciparum* mono-infection. The final sample was 300 patients. All were rural and majority were peasants. Their mean age was 17.1 years (range three months to 88 years). Those under five years of age were 117 (39.1%) while 175 (58.5%) were below 14 years. Females constituted 191(63.7%) of the sample. One hundred thirty four patients (44.7%) slept under insecticide treated mosquito net a night prior to enrolment into the study (Table 1).

The mean duration of illness before reporting to study HCs was 4.0 days [95%CI: 3.3-4.7] for children under-five years and 5.9 days [95%CI: 4.5-7.4] for those ≥5 years. At least 170(56.6%) had used some medications before reporting to study HCs. These included antimalarials 58(34%), analgesics 125(73.5%) and antibiotics 43 (25.3%). Some patients used combinations of medicines.

Overall results of diagnostic techniques

Out of 300 patients, 88(29.3%) had fever (temperature ≥37.5°C) with a mean of 38.3°C. Fifty-six (18.7%) slides were positive by HC microscopy and 47(15.7%) by expert microscopy (Table 1). The geometric mean of asexual parasitaemia was 111/μl. Paracheck detected 110 (36.7%) positive cases and 89 (29.7%) by PCR. The PCR gave positive results in eight patients who were negative with microscopy and Paracheck. Out of 58 patients who had used anti-malarials, the following tested positive: HC microscopy 16 (27.6%), expert microscopy 13 (22.4%), Paracheck 27 (46.6%) and PCR 26 (44.8%). Their geometric mean of asexual parasitaemia was 96.4/μl.

Sensitivity and specificity of diagnostic techniques

Basing on PCR as gold standard (Table 2) the overall sensitivity of presumptive diagnosis based on axillary temperature, HC microscopy, expert microscopy and Paracheck were: 39.3%, 47.2%, 46.1% and 91% respectively. The corresponding specificity rates were: 74.9%, 93.4%, 97.2% and 86.3% respectively. With a sub-analysis

Table 1 Selected characteristics of study participants

Selected variable	Overall n(%)	Low transmission n(%)	High transmission n(%)	*p*-value (2-sided)
Proportion <5 years of age	117 (39.1)	52 (34.7)	65 (43.3)	0.124
Sleeps under mosquito net	134 (44.7)	78 (52)	56 (37.3)	0.015
Used anti-malarial prior to visiting study health centre	58 (34.1)	20 (20.6)	38 (52.1)	0.000
Axillary temperature ≥37.5°C	88 (29.3)	40 (26.7)	48 (32.0)	0.375
Health centre microscopy slide positive	56 (18.7)	11 (7.3)	45 (30.0)	0.000
Expert microscopy slide positive	47 (15.7)	5 (3.3)	42 (28.0)	0.000
Paracheck positive	110 (36.7)	22 (14.7)	88 (58.7)	0.000
PCR positive	89 (29.7)	12 (8.0)	77 (51.3)	0.000

PCR = Polymerase Chain Reaction

using expert microscopy as gold standard, the overall sensitivity for presumptive diagnosis based on axillary temperature, HC microscopy and Paracheck were: 42.6%, 85.1% and 97.9% respectively. The corresponding specificity rates were: 73.1%, 93.7% and 74.7% respectively.

In the low transmission setting, sensitivity of Paracheck was 75%. The sensitivity of presumptive diagnosis based on axillary temperature, HC- and expert microscopy was very low (25% for each). Unlike HC microscopy 94.2% [95%CI: 88.9-97.5], the specificity of expert microscopy 98.6% [95%CI: 94.9-99.8] was significantly higher than that of Paracheck 90.6% [95%CI: 84.4-94.5], $p = 0.004$.

In the high transmission setting, the sensitivity of Paracheck was 93.5% [95%CI: 85.5-97.9] and significantly higher than that of other diagnostic methods ($p < 0.001$ for each comparison). Its specificity (78.1%) was significantly lower than that of HC microscopy 91.8% [95%CI: 83-96.9] ($p < 0.001$) or expert microscopy 94.5% [95%CI: 86.6-98.5] ($p < 0.001$), but similar to presumptive diagnosis based axillary temperature.

With regard to age, the sensitivity of Paracheck was significantly higher than that of other techniques and was excellent in children <5 years of age 97.7% [95%CI: 88-99.9] compared to those ≥5 years 83.7% [95%CI: 69.3-93.2]. The specificity of Paracheck in children <5 years was 79.5% [95%CI: 68.4-88.0] while it was 89.9% [95%CI: 83.6-94.3] in those ≥5 years. The specificity of HC microscopy 95.7% [95%CI: 90.8-98.4] in patients ≥5 years was not different from that of expert microscopy 98.6% [95%CI: 94.9-99.8]. In addition, the specificity of HC microscopy 89.0% [95%CI: 79.5-95.1] in children <5 years was not statistically different from that of expert microscopy 94.5% [95%CI: 86.6-98.5].

Positive and negative predictive values

Overall, only Paracheck had a NPV of >90%, while the PPVs for all methods were <88%. In the low transmission setting, PPV was low for all diagnostic methods: axillary temperature (7.5%), HC microscopy (27.3%), expert microscopy (60%) and Paracheck (40.9%). In the high transmission areas, the PPV for presumptive diagnosis (66.7%) was significantly lower than for other diagnostic methods. In addition, the PPV for expert microscopy 90.5% [95%CI: 77.4-97.3], HC microscopy 86.7% [95%CI: 73.2-94.9] and Paracheck 81.8% [95%CI: 72.2-89.2] were statistically not different. The NPV for Paracheck 97.7% [95%CI: 93.3-99.5] in low transmission and 91.9% [95%CI: 82.3-97.3] in high transmission were significantly higher than that of other methods. Only Paracheck had a NPV >90% in both age-groups, being

Table 2 Overall sensitivity, specificity, PPV and NPV of malaria diagnostic methods with PCR as gold standard

Diagnostic techniques	Sensitivity %(number/total) [95%CI]	Specificity %(number/total) [95%CI]	PPV %(number/total) [95%CI]	NPV %(number/total) [95%CI]
Axillary temperature ≥37.5°C	39.3 (35/89) [29.1-50.3]	74.9 (158/211) [68.5-80.6]	39.8 (35/88) [29.5-50.8]	74.5 (158/211) [68.1-80.2]
Health centremicroscopy	47.2 (42/89) [36.5-58.1]	93.4 (197/211) [89.1-96.3]	75.0 (42/56) [61.6-85.6]	80.7 (197/244) [75.2-85.5]
Expert microscopy	46.1 (41/89) [35.4-57.0]	97.2 (205/211) [93.9-98.9]	87.2 (41/47) [74.3-95.2]	81.0 (205/253) [75.6-85.7]
Paracheck	91.0 (81/89) [83.1-96.0]	86.3 (182/211) [80.9-90.6]	73.6 (81/110) [64.4-81.6]	95.8 (182/190) [91.9-98.2]

PPV = Positive Predictive Value, NPV = Negative Predictive Value, 95%CI = 95% Confidence Interval

94.7% [95%CI: 89.3-97.8] in patients ≥5 years and 98.3% [95%CI: 90.9-100] in those <5 years.

Discussion

The accuracy of clinical diagnosis routinely practiced at rural HCs, health centre microscopy, expert microscopy and Paracheck was compared in patients with uncomplicated malaria. The diagnostic accuracy of these methods was measured against PCR as gold standard. Some studies have however reported on the accuracy of RDTs using expert microscopy as gold standard [15-17]. For a balanced comparison, a sub-analysis using expert microscopy as gold standard was also performed.

If expert microscopy was the gold standard, the overall sensitivity was consistently high. The sensitivity (97.9%), specificity (74.7%) and NPV (99.5%) of Paracheck were similar to that reported elsewhere [13,16,17,27]. When PCR was used as gold standard, the sensitivity of Paracheck was 91%, specificity 86.4% and NPV 95.8%. The sensitivity of expert microscopy (46.1%) was unacceptably low and similar to that of HC microscopy. A number of factors might have contributed to the low sensitivity of microscopy including the inherent limitations of microscopy [19], existence of low density infections and inappropriate use of anti-malarials [28] resulting into low parasitaemia. The low sensitivity of microscopy demonstrated here is an eye-opener to yet another limitation not only to the use of malaria microscopy as gold standard in research but also to interpretation of results in routine patient care. Indeed this finding substantiates the clinicians' concerns that influence them specifically not to adhere to negative malaria microscopy results [10,11]. Although low parasitaemic patients are less at risk from severe clinical malaria episodes, they perpetuate parasite transmission, and are still a public health concern [23]. For confident diagnosis of malaria in a routine outpatient practice, a sensitivity of >90% is critical [29] and this was only achieved by Paracheck.

This study reports that 37% and 47% patients who were negative by HC microscopy and expert microscopy respectively were confirmed to have malaria by PCR. These rates are slightly lower than that of another study [12], which reported that 67% of patients, who had been classified as negative by expert microscopy, were actually positive by PCR. On the other hand, the rapid test identified positive cases in excess of the gold standard, likely to be patients with persistently circulating antigen due to prior use of anti-malarials. Microscopy techniques fell short of the required critical level of sensitivity with the potential consequences of missing infections in individuals who might even have had low immunity. Paracheck detected majority of malaria cases but also led to treatment of a small percentage of patients without malaria infection.

Eight patients were negative with microscopy and Paracheck, but positive with PCR. Even a repeat of the analysis during quality control, the eight samples remained positive. An earlier investigation [30] into the disappearance of *P. falciparum* during treatment found that PCR remained positive for a median of 144 hours. In another study [31] PCR detected *P. falciparum* DNA from circulating nonviable parasites after successful treatment. However, HRP2-based RDTs remain positive after treatment, and the HRP2 signal is of no value during the first week of treatment [32]. Therefore, the eight patients with positive PCR and negative HRP2-based RDT reported here may represent *P. falciparum* with an HRP2 gene deletion or reduced HRP2 expression [18], and such patients never give a positive result with these tests [33].

The overall specificity of Paracheck was lower than that of HC- and expert microscopy. This pattern was also shown when data was adjusted by transmission intensity and age-groups. The low specificity rates have been attributed to persistent antigenaemia even after successful treatment in some reports [18,23,27,34], which is an inherent weakness in HRP2-based tests. In the current study however, out of the 27 patients who had prior use of antimalarials and were positive by Paracheck, only one was declared negative by PCR. It is likely that although they had used anti-malarials, they were still infected with malaria parasites.

Uganda has adopted RDT as a method for parasitological diagnosis of malaria in addition to microscopy [35]. RDTs are rolled out in lower level HCs where microscopy services are not functional or not available. The low sensitivity of HC microscopy reported here is an indicator that the quality of malaria case diagnosis greatly needs to be improved. This might involve strengthening HCs through in-service training, being equipped with adequate malaria diagnostic supplies, improved technical laboratory support supervision, and external quality assurance. Due to patient load (one microscope serving 25,000 people at sub-county level HC) and with laboratory investigations other than malaria being requested, 20-30 malaria slides can be examined satisfactorily per day. Therefore, many patients are likely to be treated presumptively. If a steady supply of RDTs is guaranteed, the distribution should be extended to all lower level facilities. In addition, it is vital to routinely evaluate the performance of RDTs as they are being rolled out in the country. Although microscopy has limitations [19,36] plus the low sensitivity reported here, it is useful in estimating the level of parasitaemia in a blood film as well as for detecting non-*falciparum* infections [35].

The PCR assay was used as the gold standard because it has the ability to detect malaria parasites in patients with low levels of parasitaemia. Infections with ≤5 parasites per μl can be detected with 100% sensitivity and equal specificity [26]. However, PCR also has limitations. PCR might give false negative results if samples containing the parasites fail to amplify because the target sequence recognized by oligonucleotide primers is absent or because it is present but inaccessible. Absence of the target sequence may be due to deletion/mutation of sequence homologous to the primers, degradation of DNA during sample preparation and storage. Alternatively, if the correct target sequence is present, amplification may fail due to inhibition of PCR by sample components. Also, target DNA may not be accessible because of inadequate cellular lysis, or the target sequence copy number may be too low for amplicons to be detected under conditions used. False positive PCR results might arise from carry over during sample processing [37]. The urgency and importance of obtaining results quickly for patients with suspected malaria limits the usefulness of PCR in routine clinical practice. Furthermore, in malaria endemic areas, limited financial resources, persistent sub-clinical parasitaemia and inadequate laboratory infrastructures in remote settings preclude PCR as a diagnostic method. Nonetheless, PCR remains a reference tool both clinically and in research [38].

In estimation of the sample size with the aid of nomogram [21], the prevalence of 63.5% was used. This study reports the overall prevalence to be 36.7%. With all other assumptions remaining constant, and using a nomogram, the prevalence of 63.5% gives the same sample size as 36.7%. Therefore, the power of the study was not affected.

Conclusion

High sensitivity of malaria diagnosis is important in all settings, and essential for the most vulnerable population groups in which malaria infection produces an acute illness that can rapidly progress to death. The HRP2-based test demonstrated a superior sensitivity compared to microscopy and presumptive methods in the diagnosis of uncomplicated malaria in remote health facilities. Based on the current findings, the HRP2-baed RDT may be more suitable for screening of malaria infection in routine practice in primary health care centres.

Conflicts of interests

The authors declare that they have no competing interests.

Authors' contributions

All authors conceived and designed the study; VB and FN collected, analysed, interpreted the data and drafted the manuscript; PM critically revised the manuscript. All authors read and approved the final manuscript.

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MALARIA JOURNAL

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Cost-effectiveness of malaria microscopy and rapid diagnostic tests *versus* presumptive diagnosis: implications for malaria control in Uganda

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Abstract

Background: Current Uganda National Malaria treatment guidelines recommend parasitological confirmation either by microscopy or rapid diagnostic test (RDT) before treatment with artemether-lumefantrine (AL). However, the cost-effectiveness of these strategies has not been assessed at rural operational primary care centres.

Methods: Three health centres (HCs) were randomized to three diagnostic arms (microscopy, RDT and presumptive diagnosis) in a district of low and another of high malaria transmission intensities in Uganda. Some 22,052 patients presenting with fever at outpatients departments were enrolled from March 2010 to February 2011. Of these, a random sample of 1,627 was selected to measure additional socio-economic characteristics. Costing was performed following the standard step-down cost allocation and the ingredients approach. Effectiveness was measured as the number and proportion of patients correctly diagnosed and treated. Incremental Cost-Effectiveness Ratios (ICERs) were estimated from the societal perspective (http://Clinicaltrials.gov, NCT00565071).

Results: Overall RDT was most cost-effective with lowest ICER US$5.0 compared to microscopy US$9.61 per case correctly diagnosed and treated. In the high transmission setting, ICER was US$4.38 for RDT and US$12.98 for microscopy. The corresponding ICERs in the low transmission setting were US$5.85 and US$7.63 respectively. The difference in ICERs between RDT and microscopy was greater in the high transmission area (US$8.9) than in low transmission setting (US$1.78). At a willingness to pay of US$2.8, RDT remained cost effective up to a threshold value of the cost of treatment of US$4.7.

Conclusion: RDT was cost effective in both low and high transmission settings. With a global campaign to reduce the costs of AL and RDT, the Malaria Control Programme and stakeholders need a strategy for malaria diagnosis because as the cost of AL decreases, presumptive treatment is likely to become more attractive.

Background

The replacement of conventional anti-malarial drugs with artemisinin-based combination therapy (ACT) for treatment of uncomplicated malaria stimulated the interest in reassessing the diagnostic practices in malaria-endemic countries in sub-Saharan Africa [1-3]. Clinical (presumptive) diagnosis of malaria, the current diagnostic strategy in remote settings leads to considerable drug expenditure [4,5] on inappropriate treatment of non-parasitaemic patients [6-8]. The World Health Organization and the Uganda national guidelines [9,10] recommend parasitological confirmation of malaria either by microscopy or rapid diagnostic tests (RDT) before treatment is started.

In rural health centres (HCs) routine malaria microscopy if available, is often of limited quality [11,12]. In view of the limitations of microscopy, Uganda commenced the rollout of malaria RDTs (primarily histidine-rich protein II [HRP2] based tests) in parish and sub-county level HCs with no laboratory infrastructure,

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as a means of targeting ACT use. The rollout was phased commencing with six districts in 2008. The exercise is however stalled due to stock-out of RDTs from end of 2009. With availability of RDTs, scaling up is planned to an additional 22 districts. Currently, there are 112 districts in the country.

There is considerable debate regarding the cost-effectiveness of routine use of RDTs as an integral part of deploying ACTs. On the one hand, some studies conducted elsewhere reported that replacement of malaria microscopy with RDTs would increase the provider costs [2,13]. On the other hand, relative to microscopy, the use of RDTs was likely to be cost-effective [3,14-16]. In the face of the dynamic market, this debate necessitated an empirical economic evaluation of the available malaria diagnostic techniques in the local setting. The current study assessed the cost effectiveness of treating malaria with artemether-lumefantrine (AL) based on microscopy, RDT or presumptive diagnosis at sub-county public HCs from the societal perspective.

Methods

Study setting

The study was carried out in six sub-county level government HCs, three in Bushenyi and three in Iganga districts of Uganda. Bushenyi district is located in south-western Uganda, 310 km from the capital city Kampala. HCs in Bushenyi are located at an altitude of 1,744-1,962 m above sea level. The climate is relatively wet with annual rainfall of 800-2,000 mm, and annual temperature range of 12.5°-30°C. The substantial part of Bushenyi is of hilly-rough and rugged terrain with the topography dominated by undulating hills. Iganga district is located in eastern Uganda. The land surface is mainly flat. HCs in Iganga are located at an altitude of 1,059-1,119 m above sea level. The temperatures range from 23°-27°C with annual rainfall of 900-1,200 mm. The Uganda government categorizes Bushenyi as low and Iganga as high malaria transmission intensity settings. Since 2001, patients do not pay for medical services at public HCs. Bushenyi and Iganga were not among the districts in the first phase of RDT rollout. However, RDTs were introduced in the "RDT arm" by this trial in 2007. Additional description of the study setting was published elsewhere [17].

Study design and population

The study was a randomized cost-effectiveness trial (http://Clinicaltrials.gov: NCT00565071). In each district, HCs were randomized to three diagnostic arms (microscopy, RDT and presumptive diagnosis). The study population included HC clinical and laboratory staff and all outpatients presenting with fever (by statement or measured axillary temperature ≥37.5°C). Of these, a random sample of 1,627 outpatients was chosen to measure selected socio-economic data. The study was integrated within the existing district health services delivery system to reflect the real-life operational conditions.

Laboratory procedures

The detailed description of the laboratory procedures and subsequently the performance (sensitivity and specificity) of the three diagnostic strategies has been published [12]. Briefly, finger-prick blood specimens were drawn from a sample of 300 outpatients attending HCs stated above. They were then processed following standard operating procedures. The validity indicators were gauged against polymerase chain reaction as reference standard.

Collection of cost data

Cost data was categorized into internal costs (public provider costs) and external costs (patient costs). Data were collected from March 2010 to February 2011 as detailed below.

Collection of internal costs

Real-life facility cost data were collected covering a period of 12 months in which 22,052 malaria visits were made. The data included recurrent costs (personnel, stationery, utilities, medicines, clinical and laboratory supplies) and capital costs (buildings, equipment and furniture). Financial reports were reviewed and interviews were conducted with staff to ascertain additional resources used such as the primary health care funds and those likely to be donations. Costs were collected in Uganda shillings (Ush) and converted to US dollars (exchange rate US$1 = Ush2,380, February 3rd 2011).

Health centre personnel costs

The effective contact time with those seeking care was one of the main input parameters for personnel costs. Contact time was recorded for a random sample of 1,627 outpatients. When a patient arrived at the HC, time was recorded by the research assistant on a "time sheet." The "time sheet" was then given to the patient. Thereafter, the time was recorded by clinicians and laboratory personnel for every service provided. The "time sheet" was finally retained at the dispensing window. For the laboratory personnel, the effective contact time was comprised of: drawing a sample from the patient, slide preparation, scanning the 200 film fields until declaring a slide negative and reporting of results. With regard to RDT, effective contact time was comprised of: drawing blood samples from patients, applying samples onto the test, test reading and reporting of results. The outpatient clinics at sub-county HCs run

for eight hours from Monday to Saturday and about five hours on Sunday. Therefore, HC staff work 68 hours (4,080 minutes) weekly. Assuming a year of 52 weeks; HC staff work 48 weeks, less four weeks of annual leave. The HC staff were also interviewed regarding their time allocation for other services. The time used for administration was documented separately by the research assistants based at the HCs. Personnel monthly salaries were recorded from their pay-slips.

Drugs, laboratory supplies and RDT

Artemether-lumefantrine was costed as it is distributed in four different fixed-dose weight-specific packs (35 kg and above, 25-34.9 kg, 15-24.9 kg and 5-14.9 kg). The costing of non-malaria treatment was based on "per tablet" or "per capsule" of prescribed drugs to make up a dose in each of the weight categories. All laboratory supplies used for the 12 months were documented. The cost of RDT (Paracheck, Orchid Biomedical Systems, Goa, India) was US$0.84 as per Joint Medical Stores price catalogue. Medicines and supplies used were recorded from primary source documents including dispensary records, outpatient registers, laboratory records and stock cards. The cost of medicines and supplies were obtained from Uganda National Medical Stores and Joint Medical Stores delivery reports kept at the HCs. Medicines and disposables that were supplied but not used during the study period were excluded.

Utilities and stationery

Utilities mainly included coffee/tea for staff, water and fuel for lighting. None of the six HCs had electricity at the time of implementation of this study. The water source was either bore-hole or rain-harvested. The quantity of water used per week in litres was annualized and converted into National Water and Sewerage Corporation (NWSC) units. Costing of water was performed basing on NWSC institutional/government rate [18]. The costs of fuel, sugar and coffee/tea were obtained from receipts and HC reports. The health management information system registers, laboratory registers and stock cards were mainly supplied by National Medical Stores and the costs were obtained from delivery reports. The costs of additional stationery purchased were estimated from market prices.

Capital costs

Capital goods were those items with a useful lifespan of more than one year (buildings, equipment and furniture). Inventories of capital goods were generated and grouped within their locations (rooms) in the HC. HC building plans were obtained from the district engineers' office to determine the construction costs and relative contribution of area coverage to outpatient case management. The costs of renovations and repairs (if any) within the 12 months of the study were also collected.

Collection of patient (external) costs

Patient costs were episode-related expenditures. A sample of 1,627 outpatients was enrolled for collection of external costs. On arrival at the HC, the time was recorded on a "time sheet" but patients were also asked to state the estimated time of departure from home. Patients were systematically tracked until departure from the HC. The aim of tracking patients was to estimate the time spent accessing services. Exit interviews were carried out and information recorded on study questionnaire. The patient travel time was equal to arrival time minus the estimated time of departure from home. It was assumed that patients used a similar time for the return journey. The duration of interviews (in minutes) was eliminated during analysis. Patient costs were categorized into direct (out-of-pocket expenses on transport and other incidentals related to treatment seeking) and indirect (lost income due to medical care seeking, including travel and waiting time). Direct patient costs were valued according to reported expenditures. Because majority of the patients were rural and mainly peasants, lost earnings due to care-seeking were valued using the Uganda Ministry of Public Service minimum wage "for unskilled labour" salary scale U8 "entry point for support staff mainly attendants" which is approximately US$55.9 per month employing standard methods [19].

Valuation of resource use and unit cost of diagnosis for each outpatient visit

The unit cost of diagnosis for each outpatient visit in each arm was determined following a standard step-down cost allocation method [20,21] of all available HC resources. The ingredients technique was also employed and provided data directly measured for example "service provider effective contact time" with patients, quantity and costs of drugs and supplies used. Further, the annualized values of buildings, furniture and equipment were estimated using a standard procedure [21] assuming a useful lifespan for these goods of 30, 10 and seven years respectively and with a discount rate of 3%. Three major cost centres (overhead, support and the final services) were identified. Resources (Table 1) were allocated in three steps separately for each HC and later aggregated. In the first step, the total cost of running each HC were allocated to the three cost centres. Personnel salaries were allocated using the measured staff time. Laboratory supplies, cleaning materials, drugs and disposables were proportioned or fully allocated to the relevant cost centres. Capital costs and stationery were

Table 1 Aggregate resources (US$) at two health centres per arm and allocation criteria

Recurrent costs	Presumptive	RDT	Microscopy	Allocation criteria
Salaries	24955	28374	40001[§]	Measured staff time
Drugs and disposables	13757	14177	18521	To relevant cost centre
Stationery	311	393	432	Estimated use
Utilities	459	393	565	Relative to size of space
Laboratory and RDT supplies	N/A	4221	1874	To relevant cost centre
Cleaning materials	340	306	331	To relevant cost centre
Capital costs*				
Building**	3749	5973	7497	Actual use
Equipment	402	498	767	Actual use
Furniture	144	149	216	Actual use
Total	44,117	54,484	70,204	

N/A = not applicable, RDT = rapid diagnostic test

[§]includes salaries of laboratory personnel

*goods with useful lifespan of more than one year, costs annualised at 3% discount rate

** includes sits in the waiting area made of fixed concrete

allocated basing on actual or estimated use. In the second step, overhead costs were allocated to the support and final services centre. In the third step, the total costs of running a HC were subsequently allocated to the level performing the individual final services, such as the outpatient department (OPD) using an allocation criterion that reflected the actual resources used. The total costs of running an OPD were divided by the number of final services to arrive at the unit cost per service with and without parasitological confirmation of malaria.

The cost effectiveness analysis (CEA) model

In order to determine the cost effectiveness, a decision tree in TreeAge was used with a patient cohort presenting at the outpatient department of a rural HC. Patients entered the model at the time when the attending clinicians suspected uncomplicated malaria. Once in the model, patients were investigated using microscopy, RDT or presumptive diagnosis. Effectiveness values of the strategies (Table 2) and costs of treating an outpatient (provider and patient costs combined) were used to populate the model. Because there is normally over prescription of analgesics, it was assumed that a true parasitaemic patient getting AL was also likely to get an analgesic. It was also assumed that a patient with negative test result got antibiotic and analgesic treatments.

Table 2 Comparison of effectiveness of the three diagnostic strategies

Test results[ψ]	Diagnostic strategy		
	Presumptive	Microscopy	RDT
True positive[a]	35	42	81
False positive	53	14	29
False negative	54	47	8
True negative[b]	158	197	182
Total[c]	300	300	300
Number correctly diagnosed[d]	193	239	263
Proportion correctly diagnosed (%)[e]	64.3	79.7	87.7

[ψ]gauged against polymerase chain reaction as reference standard, RDT = rapid diagnostic test, d = (a+b), e = (d/c)*100

Statistical analysis

Data was double-entered and validated in EpiData version 3.1 software (The EpiData Association, Odense, Denmark) and analysed in Stata version 10 (Stata Corp, College Station, Tx, USA), TreeAge and MS-Excel. STATA was mainly used in the descriptive analysis. Excel was used in costing especially Step-down accounting. TreeAge was used for constructing and analysing the decision tree and uncertainty in the cost-effectiveness model. The p-values were calculated at the 0.05 level of significance and Confidence Interval (CI) was set at 95%.

Sensitivity analysis

Sensitivity analysis was conducted in TreeAge on the variables that were uncertain and prone to change over time. These included prevalence of malaria, costs of RDT and AL. Sensitivity and specificity [12] were also examined. The cost of AL and RDT were halved and doubled to get the lower and upper limits of the interval respectively.

Outcome measures

The primary endpoints were: 1) effectiveness measured as the number and proportion of patients correctly diagnosed (true positive + true negative) and treated; and 2) incremental cost per additional case correctly diagnosed and treated (incremental cost effectiveness ratio - ICER)

defined as the change in costs over the change in effectiveness of moving from the presumptive strategy (the base case) to the next best alternative.

Ethical approval

The study was approved by Makerere University School of Public Health Higher Degrees Research and Ethics Committee; and the Uganda National Council for Science and Technology (Ref: HS 209). The study was registered with the http://Clinicaltrials.gov (NCT00565071).

Results

Effectiveness of the three diagnostic strategies

Effectiveness of each diagnostic strategy was calculated based on a recent publication [12] from the same trial preceding this paper. Malaria RDT was the most effective with the number of cases correctly diagnosed and treated being 263, followed by microscopy: 239 and presumptive diagnosis: 193 (Table 2). The corresponding proportions of patients correctly diagnosed and treated were 87.7%, 79.7% and 64.3% respectively. Routine use of RDT or microscopy would result into an additional 36.3% and 23.8% respectively of patients correctly diagnosed and treated in comparison to the presumptive technique.

In the high transmission area, effectiveness values were: RDT 86.0%, microscopy 70.7% and presumptive diagnosis 59.3%. The corresponding effectiveness values in the low transmission setting were 89.3%, 88.7% and 69.3% respectively.

Analysis of internal costs

The unit cost of diagnosis in United States dollars (US$) in each arm was determined using the number of final services in the outpatient department. Overall, 22,052 malaria visits were made over the 12 months of data collection period. The costs for each strategy were allocated using the step-down technique to the level of an individual attending the OPD. The cost analysis for each strategy is presented in Table 3 and briefly described as follows:

The presumptive strategy

The overall cost allocation for the outpatient services in the presumptive arm was US$18,807 (Table 3). There was no parasitological confirmation of malaria in this arm. Therefore, the total allocation for clinical diagnosis of malaria was equal to the total for malaria management in the OPD (US$10,242) minus that of drugs and disposables (US$3,762) which was equal to US$6,480. The aggregate number of malaria visits in the two HCs constituting the presumptive arm was 10,446. Therefore, the unit cost of presumptive diagnosis was US$6,480/10,446 which is equal to US$0.62.

Rapid diagnostic test strategy (RDT)

In the RDT arm, the total cost of running an OPD was US$25,437 (Table 3). Out of this, US$12,100 (47.6%) was allocated to management of malaria, of which US $5,227 was for diagnostic services. The cost of RDTs was the major determinant constituting 74.6% of the amount allocated to diagnostic services. The aggregate number of malaria RDTs performed in the two HCs in this arm was 4,039. Therefore, the unit cost of diagnosis was US$1.29, which was lower than that for HC microscopy.

The microscopy strategy

The total cost of running an OPD was US$31,759, with US$16,553 (52.1%) allocated to malaria management of which US$9,515 (57.5%) was to diagnostic services. Laboratory supplies and equipment accounted for 17.9% while salary constituted 56.7% of the diagnostic services. The number of malaria investigations done over the period of study was 6,219 giving a unit cost of diagnosis of US$1.53.

Drug costs

The unit cost of AL fixed-dose weight-specific pack decreased with the weight of the patient. However, the average cost of AL dose was US$1.38. The average cost of antibiotic/analgesic dose was US$1.05 and the pattern across the weight-specific groups was similar to that of AL.

Table 3 Aggregate and unit cost of diagnosis by diagnostic strategy (US$)

	Presumptive	Microscopy	RDT
Total cost allocation for OPD services	18807	31759	25437
Number of final services at OPD	20103	24864	18480
Total cost allocation for management of malaria at OPD	10242	16553	8512
Number of OPD malaria visits made in the 12 months[a]	10446	7560	4046
Total cost allocation for OPD malaria diagnostic services[b]	6480	9515	5227
Number of OPD malaria tests done[c]	N/A	6219	4039
Unit cost per malaria test[d]	N/A	1.53	1.29
Unit cost of presumptive diagnosis[e]	0.62	N/A	N/A

RDT = rapid diagnostic test, OPD = outpatient department, N/A = not applicable, d = (b/c), e = (b/a)

External costs (patient costs)

In public HCs, patients do not pay for medical services. Therefore, patient costs relate to direct out-of-pocket expense on the episode prior to visiting the HC, transport and other non-medical incidentals (external direct costs); and the indirect cost of travel and waiting time (external indirect costs).

External indirect costs

The mean distance from home to the HC was 5.7 km [5%CI: 2.9-8.4]. Overall, HC staff in the presumptive arm tended to report to work in late morning after patients had arrived resulting into an extended visiting time. The mean travel time was 139.5 minutes [95%CI: 131.1-147.9], but was not different between arms (Table 4). The time spent accessing services when using RDT 134.4 minutes [95%CI: 123.5-145.3] was not different from that of presumptive treatment, but significantly shorter than that of microscopy 188.5 minutes [95%CI: 173.8-203.3]. The travel time and the overall time for the HC visit were converted into a single monetary measure "opportunity cost." This implies that on average, the time cost due to absence from work as a result of suffering from suspected or confirmed malaria was US$1.59. The time cost with subsequent loss of income was likely to be greater in the microscopy arm (US $1.68) compared to RDT US$1.45, $p < 0.001$, or presumptive-based treatment US$0.80, $p = 0.005$ because the time taken to diagnose a case and produce results was much longer in the microscopy arm.

External direct costs

The mean monthly household income was US$19.8 [95%CI: 15.0-24.5] (Table 4), and was similar in the three arms. The mean expenditure on treating the episode prior to visiting the study HC was US$0.40 [95% CI: 0.34-0.46], still not significantly different between the study arms. Patients in the low transmission setting significantly incurred direct expense on transport US $0.65 [95%CI: 0.55-0.76], compared to those in the high transmission area US$0.31 [95%CI: 0.23-0.38]. Expenditure on transport was not significantly different between arms. The mean supplementary non-medical out-of-pocket expenditure during the HC visit was US$0.2.

Treatment cost from the societal perspective

The total cost of treatment of an outpatient with suspected or confirmed malaria was comprised of the cost of diagnosis, drugs dispensed and patient costs. The unit cost of treatment was cheapest in the presumptive arm (Table 5), followed by that in the RDT arm while microscopy was the most expensive.

Determining the incremental cost effectiveness ratio (ICER)

Using the unit cost per patient correctly diagnosed and treated compared with the effectiveness of each strategy, overall RDT was the most cost effective (Figure 1) with the lowest ICER US$5.0 compared to microscopy US $9.61 (Table 6). In the high transmission setting, the ICER was US$4.38 for RDT and US$12.98 for microscopy. The corresponding ICERs in the low transmission setting were US$5.85 for RDT and US$7.63 for microscopy respectively. The difference in ICERs between RDT and microscopy was greater in the high transmission setting (US$8.9) than in the low transmission area (US$1.78).

Sensitivity analysis

Overall, a reduction in the cost of AL and RDT was associated with improvement in the cost effectiveness of RDT and microscopy. An increase in malaria prevalence was associated with an improvement in the cost effectiveness of RDT. At a willingness to pay of US$2.8, the RDT remained cost effective up to a threshold value of the cost of treatment of US$4.7.

Discussion

Following the change in the malaria treatment policy in 2005/2006, the Uganda government commenced the rollout of RDTs in 2008 to target AL to only parasitaemic patients. However, as the health system has struggled with stock-out of RDTs since 2009, the price of AL has decreased from US$2.4 in the year 2004/2005

Table 4 Mean direct expenditure and lost income while seeking care (US$) at government health centres

Variable	Overall Mean[95%CI]	Presumptive Mean[95%CI]	RDT Mean[95%CI]	Microscopy Mean[95%CI]
Monthly household income	19.8[15.0-24.5]	21.4[11-31.8]	16.5[9.4-23.6]	21.3[14.9-27.7]
Medication prior to visiting HC	0.40[0.34-0.46]	0.44[0.32-0.56]	0.40[0.27-0.52]	0.34[0.30-0.44]
Expense on transport	0.43[0.35-0.50]	0.43[0.18-0.69]	0.39[0.26-0.52]	0.44[0.34-0.54]
Out-of-pocket (other non-medical) expense	0.20[0.11-0.29]	0.30[0.06-0.66]	0.13[0.03-0.22]	0.23[0.12-0.34]
Opportunity cost (travel and waiting)	1.59[1.53-1.66]	0.80[0.71-0.94]*	1.45[1.35-1.50]	1.68[1.57-1.79]

*only travel time converted into money, CI = Confidence Interval, HC = health centre, RDT = rapid diagnostic test

Table 5 Unit cost of outpatient treatment by diagnostic arm (US$)

Cost category	Presumptive	RDT	Microscopy
Unit cost of diagnosis[a]	0.62	1.29	1.53
Mean cost of AL only per visit[b]	1.38	1.38	1.38
Mean cost of antibiotics/analgesics per visit[c]	1.05	1.05	1.05
Patient costs per visit (mean)[d]	1.97	2.37	2.69
Unit cost of OPD treatment visit with AL only[e]	3.97	5.04	5.60
Unit cost of OPD treatment visit with AL+antibiotic/analgesic[f]	5.02	6.09	6.65
Unit cost of OPD treatment visit with antibiotic/analgesic[g]	3.64	4.71	5.27

AL = artemether-lumefantrine, OPD = outpatient department, RDT = rapid diagnostic test, e = (a+b+d), f = (a+b+c+d), g = (a+c+d)

[2,3,15,16,22,23] to the current average of US$1.38 due to the global campaign to reduce costs and presence of generic products in the market. It was therefore imperative to institute an empirical economic evaluation of the three malaria diagnostic strategies in remote primary care centres in a health system where patients do not pay for medical services.

It is reported here that diagnosing malaria based on signs and symptoms alone (presumptive diagnosis) had the lowest cost (US$0.62). The clinician time was a major recurrent input that determined the cost of presumptive diagnosis. However, the effective clinician contact time was similar in the three arms. Also, the unit cost of outpatient treatment was lowest in the presumptive arm whether the patient got AL alone or in combination with antibiotics. In this analysis, the presumptive method was the base-case because documentation of fever or history of fever has traditionally been considered sufficient evidence for prescribing anti-malarial therapy in rural primary level HCs in Uganda as elsewhere in sub-Saharan Africa. Even when microscopy is available, not all patients get the service, yet clinicians often prescribe anti-malarial treatment to persons with negative microscopy results [7,24,25]. Presumptive diagnosis was the least effective. However, effectiveness of all methods was inversely related to transmission intensity. This observation was also reported in Tanzania [2].

Overall, the RDT technique was the most effective. This finding is supported by comparable studies carried out in other settings [2,15,16], although effectiveness in these studies was determined against microscopy as reference standard. An earlier publication [12] from the current trial reported that expert microscopy "would be gold standard" performed poorly. Here, effectiveness is defined as the proportion of patients correctly diagnosed (true positive + true negative) and treated. Use of expert microscopy as gold standard would erroneously increase the sensitivity and specificity resulting into high effectiveness values. In the current paper therefore, effectiveness was determined with PCR as the reference standard.

The major parameter that determined the cost of RDT-based patient management was cost of the test supplemented by the personnel cost for giving the service. The current price of RDT (US$0.84) at point of use is still high, although it has decreased over time.

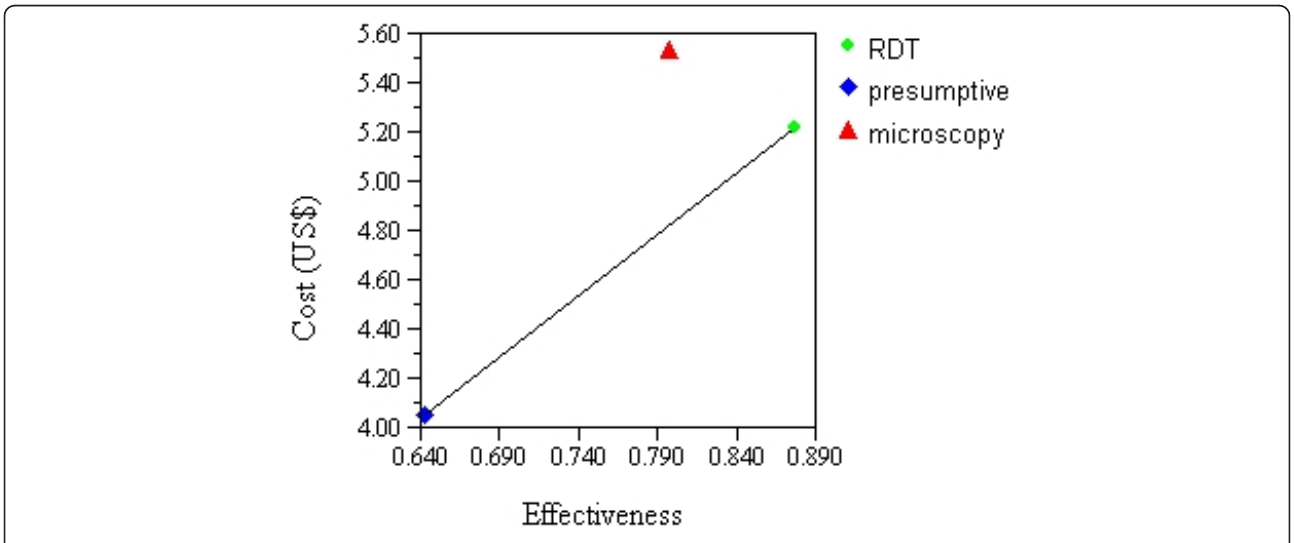


Figure 1 Cost effectiveness of three malaria diagnostic strategies.

Table 6 Cost effectiveness of three malaria diagnostic strategies text report

Strategy	Cost (US$)	Incremental Cost (US$)	Effectiveness	Incremental Effectiveness	Average Cost/Effectiveness ratio	ICER
Presumptive	4.04		0.643		6.28	
RDT	5.22	1.17	0.877	0.234	5.95	5.0
Microscopy	5.53	1.48	0.797	0.154	6.94	9.61 (Dominated)

Dominance Report:
The strategy "microscopy" is dominated by "RDT".

ICER = incremental cost-effectiveness ratio, RDT = rapid diagnostic test

Previous reports have used RDT costs ranging from US $1.30 to US$1.50 [15,16]. In the current paper, the use of RDT increased the cost of diagnosis by US$ 0.67 (about 108% increase) in comparison with the presumptive technique. An increase in provider cost with introduction of RDTs as a diagnostic service was also reported in Tanzania [2,13]. This increase is however likely to be offset by its superior effectiveness if clinicians adhere to test results. The cost of radical drug treatment normally occurred with all strategies. However, the unit cost of outpatient treatment visit was lower than that of microscopy but higher than in the presumptive method.

Microscopy was less effective compared to RDT. The cost of microscopy diagnostic service was however higher (US$1.53) than that of RDT (US$1.29). In malaria microscopy, the major cost input was the personnel salary constituting 57.5% while laboratory supplies and equipment constituted only 17.9% of the diagnostic service. Laboratory-related capital costs (laboratory space, equipment, etc) were additional costs which were not inputs in the RDT or presumptive arms. This paper focussed on the evaluation of the cost effectiveness of the three strategies at point of care in rural settings. A study in Thailand that extended the analysis to include the pre-service training reported microscopy to be even more expensive [26]. In the Thailand study, however, the presumptive arm was non-existent while blood smears were drawn from patients and transported to static centres for microscopic investigations constraining appropriate comparison with the current findings.

With regard to external costs, the opportunity cost of travel was not significantly different between study arms. The opportunity cost of waiting for treatments especially test results was the major external indirect cost more so in the microscopy arm. The direct cost of transport was not different between study arms, but it was significantly higher in the setting of low transmission due to the hilly difficult terrain. Overall, these external costs were a major part of the unit cost per patient correctly treated and on ICERs.

Few comparable empirical cost-effectiveness analyses done in Africa were available at the time of writing this paper. However, a full comparison with the current findings was difficult. The study in Zambia [15] examined the cost effectiveness of the three strategies from the narrow provider perspective, and was carried out in sentinel sites, which in reality did not represent typical rural HCs. In Tanzania [2], the cost effectiveness of RDT was only compared to microscopy, the presumptive arm was missing. In addition, the study was performed in hospital settings where patient volumes would not be comparable to those attending HCs. On the other hand, in Nigeria [16], patients five years and older paid for the medical services, which impacted on their direct expenditure. Further, the Nigerian study used a different measure of effectiveness and patients were treated with dihydroxy-artemisinin/piperaquine, while costing was based on AL. At the time these studies were conducted, the costs of AL and RDT were higher than the current rates. However other than the study in Tanzania [2], all support the current findings that RDT is the most cost effective.

In all scenarios in the sensitivity analysis, RDT maintained its superior cost effectiveness compared to presumptive diagnosis and microscopy. With the global campaign to reduce the prices of ACT and RDTs, policy makers need to re-think and make contingency plans regarding malaria diagnosis. As the cost of AL decreases, presumptive treatment is likely to become more attractive. This scenario is likely to mirror the era of chloroquine or chloroquine/sulphadoxine-pyrimethamine as first-line drugs that were cheap. Therefore, with falling prices of AL, measures need to be put in place to sensitize the health service users about the benefits of appropriate malaria diagnosis and treatment.

Conclusion

RDT was the most cost effective. However, with the reduction in the cost of RDT and AL, the Malaria Control Programme and stakeholders need a contingency plan regarding malaria diagnosis. Further, there is need to sensitize health service users about the benefits of appropriate malaria diagnosis.

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Authors' contributions
All authors conceived and designed the study; VB and FN collected, analysed, interpreted the data and drafted the manuscript; PM and KSH critically revised the manuscript and performed further analysis where necessary. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Comparative feasibility of implementing rapid diagnostic test and microscopy for parasitological diagnosis of malaria in Uganda

Vincent Batwala[1,3*], Pascal Magnussen[2] and Fred Nuwaha[3]

Abstract

Background: In Uganda, parasite-based diagnosis is recommended for every patient suspected to have malaria before prescribing anti-malarials. However, the majority of patients are still treated presumptively especially in low-level health units. The feasibility of implementing parasite-based diagnosis for uncomplicated malaria in rural health centres (HCs) was investigated with a view to recommending measures for scaling up the policy.

Methods: Thirty HCs were randomized to implement parasite-based diagnosis based on rapid diagnostic tests [RDTs] (n = 10), blood microscopy (n = 10) and presumptive diagnosis (control arm) (n = 10). Feasibility was assessed by comparing the proportion of patients who received parasite-based diagnosis; with a positive malaria parasite-based diagnosis who received artemether-lumefantrine (AL); with a negative malaria parasite-based diagnosis who received AL; and patient waiting time. Clinicaltrials.gov: NCT00565071.

Results: 102, 087 outpatients were enrolled. Patients were more likely to be tested in the RDT 44, 565 (96.6%) than in microscopy arm 19, 545 (60.9%) [RR: 1.59]. RDTs reduced patient waiting time compared to microscopy and were more convenient to health workers and patients. Majority 23, 804 (99.7%) in presumptive arm were prescribed AL. All (100%) of patients who tested positive for malaria in RDT and microscopy arms were prescribed anti-malarials. Parasitological-based diagnosis significantly reduced AL prescription in RDT arm [RR: 0.62] and microscopy arm [RR: 0.72] compared to presumptive treatment. Among patients not tested in the two intervention arms, 12, 044 (96.1%) in microscopy and 965 (61.6%) in RDT arm were treated with AL [RR: 1.56]. Overall 10, 558 (29.4%) with negative results [5, 110 (23.4%) in RDT and 5, 448 (39.0%) in microscopy arms] were prescribed AL.

Conclusion: It was more feasible to implement parasite-based diagnosis for malaria using RDT than with microscopy. A high proportion of patients with negative malaria results are still prescribed anti-malarials. There is need to increase access to parasite-based diagnosis where microscopy is used. In order to fully harness the benefits of parasitological confirmation of malaria, it is necessary to reduce the prescription of anti-malarials in negative patients.

Background

Malaria presents a diagnostic challenge in most endemic countries in sub-Saharan Africa, yet early diagnosis and appropriate treatment is a basic tenet of current malaria policy [1]. In sub-Saharan Africa, documentation of fever or history of fever has traditionally been considered sufficient evidence for prescribing anti-malarial therapy [2-4]. This presumptive diagnostic technique is inaccurate [5-8] and results in over diagnosis and over treatment. Further, because of the inaccuracies associated with presumptive treatment, decline in the proportion of fevers attributable to malaria, and use of expensive anti-malarials such as artemisinin-based combination therapy (ACT) [4,9-11], the World Health Organization (WHO) [1] now recommends parasite-based confirmation of parasitaemia before prescription of ACT.

The role of laboratory diagnosis of malaria is primarily to support clinical care [12], and the current reference standard for confirmatory presence of parasitaemia is

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microscopy. However, implementation of routine malaria microscopy is a challenge. Maintaining good quality, effective microscopy service requires an organized health system infrastructure, including the provision of high-quality supplies and reagents, presence of satisfactory microscopes, availability of microscopists, maintenance and technical competence of microscopists, an adequate workplace environment and the ability to prepare usable blood films. Field microscopy where established, often falls short of these requirements [13-15]. In Uganda, there is shortage of staff, especially laboratory personnel to perform microscopy [16].

Because parasite detection is performed by someone other than the prescriber, there is a tendency to ignore microscopy results provided by the laboratory [2-4]. Yet health workers' adherence to diagnostic and treatment guidelines is a critical aspect in determining effective implementation of malaria case management policies [17,18]. To put testing and clinical decisions in the hands of the prescriber, the use of malaria rapid diagnostic tests (RDTs) has been encouraged [1,7,10,15] as an integral part of widespread deployment of ACT. In line with international recommendations, Uganda introduced artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria in 2006, and commenced a phased role-out of a histidine rich protein-2 (HRP-2)-based RDT as an alternative to microscopy in primary level health centres (HCs) in 2008. However, quality parasite-based diagnosis remains unavailable to most outpatients presenting with febrile illness.

Although the sensitivity and specificity of malaria microscopy and RDTs have been assessed in different studies [8,19] and the feasibility of RDT in other settings [20], there has been no study gauging the feasibility of the use of these diagnostic strategies and whether the use of parasite-based diagnosis changes prescription of AL in rural settings in Uganda. The key question is "given the current standing at sub-county HCs and available resources, is it possible to test every patient presenting with febrile illness before treatment? Therefore the current study compared the feasibility of these malaria diagnostic techniques in rural government HCs located within areas of varying transmission intensities in Uganda. The main outcome measures were: proportion of febrile outpatients receiving a malaria test, proportion of those with negative results treated with AL and waiting time.

Methods

Study design

This stratified cluster randomized trial (Clinicaltrials.gov: NCT00565071) was implemented in 15 out of 20 sub-county government HCs randomly selected in a district of low and 15 in a district of high malaria transmission intensity from March 2010 to July 2011. Stratification was based on transmission intensity. HCs were the primary sampling units. In each district, HCs were randomized to two intervention arms (microscopy and RDT) and control arm (presumptive diagnosis), resulting into five HCs per arm. This trial took a cluster design to avoid contamination between control and intervention group subjects and to allow service providers to operate as they would normally on a day-to-day basis.

Setting

The trial was carried out in Bushenyi and Iganga districts in Uganda, and commenced before the two districts were partitioned. However, partitioning did not affect the delivery of health services by the time the study was closed. Bushenyi district has a population of 731, 392. The district experiences low and unstable malaria transmission, with people of all ages being at risk. It is epidemic-prone, with occasional malaria outbreaks occurring shortly after the rains. Iganga district has a total population of 540, 939 with 15 HCs at sub-county level. The district experiences very high malaria transmission intensity. Malaria is the leading cause of morbidity and outpatient attendance for all age groups. Additional description of the study setting has been published elsewhere [16].

Study procedures

Randomization of health centres to the three arms

Health centres were allocated to the three diagnostic arms following simple randomization. In Bushenyi district, three of the five HCs randomized to the microscopy arm were already offering the service. The study strengthened the laboratory of Kabira HC with a microscope and supplies. In Iganga district, two HCs were offering microscopy services but the study strengthened the laboratory for one (Nambale HC). In the other three HCs, the posts for laboratory personnel were already filled although the laboratories were not functional. Therefore, the district provided microscopes and laboratory supplies. In the RDT arm, three selected HCs in Bushenyi district and two in Iganga had microscopy services. However, RDTs were introduced and both clinicians and laboratory personnel were trained. They were informed during training that their HCs had been randomized to use RDTs. However, they were free to test patients with microscopy if needed. In the presumptive arm although one HC in Bushenyi and two in Iganga had the laboratory assistants post filled, the laboratories were not functional. They either had no space or laboratory equipment and supplies.

Delivery of RDTs and artemether-lumefantrine (AL)

Before the study commenced RDTs, AL, laboratory reagents and supplies from the National Medical Stores were delivered to the districts medical stores. This was

meant to integrate the study into the district health services delivery system. It was also a strategy for continuity of services from the time of closing the study. RDTs were packaged in cartons of 1, 000 tests. In each carton, there were 40 boxes of 25 tests. Also in the box were 25 alcohol swabs, lancets and a 10 ml bottle of clearing buffer. Each sachet of RDT contained the test device, a loop for collecting blood and a descant. The study HCs were to make requisitions for AL, RDTs, and laboratory supplies together with antibiotics, analgesics and sundries following the usual guidelines. HCs opened up stock cards for RDTs that were updated regularly.

Training of staff

Clinicians (clinical officers, nurses/midwives and nursing assistants) and laboratory assistants received a one-day refresher training on-site. The training and subsequent study procedures were a scale-up of the activities performed during the assessment of the accuracy of these diagnostic techniques [8]. All staff members were trained in theory by re-orienting them to the malaria treatment policy. In RDT arm, the staff were in addition trained in 1) finger prick for collection of blood and 2) preparation and reading of *Paracheck®*. In the microscopy arm, members were in addition trained in: 1) finger prick for collection of blood, 2) thick/thin blood smear preparation, 3) staining smears, and 4) blood smear reading. In the microscopy and RDT arms, data collection commenced after inter-reader reliability reached a very good agreement (kappa coefficient = 0.97). The staff in HCs with microscopy or RDT were instructed to treat patients who have positive results with anti-malarials. Those with negative results were to receive alternative medications after further assessment. Staff in the control arm were only re-oriented to the current malaria treatment guidelines. HC outpatient registers were modified to record additional variables such as the presenting complaints, drugs dispensed and to indicate those prescribed but out-of-stock. The trained staff were charged with training those that were off-duty on the day of training. Further clarifications were provided during supervision. Supervision by the study team and the district laboratory focal persons was carried out weekly during the first two months and monthly thereafter. The supervisory visits were aimed at troubleshooting problems related to the skills of performing an RDT and interpretation of results, quality of the test, quality of blood smears, reagents, status of the microscopes and recording of results. In addition to monthly support supervision, the district laboratory focal person provided further visits during the routine quarterly schedules.

Description of the diagnostic arms

Presumptive (control) arm

Patients presenting with fever (by statement or measured) were enrolled to receive service without parasitological confirmation of malaria. Patients were treated on the basis of signs and symptoms only.

Microscopy arm

Patients were enrolled on the basis of fever (by statement or measured). Microscopy was performed by laboratory assistants. These laboratory personnel have two to three years of pre-service training. Thick and thin blood smears were prepared by finger-prick using sterile blood lancets on separate frosted slides. Standard staining was performed using the Field's stain method. Laboratory assistants were only familiar with this staining technique. Blood films were read at magnification x1, 000. Each film was graded as positive (asexual malaria parasites seen) or negative (no malaria parasites seen) based on inspection of 200 fields. Microscopy test results were recorded in the laboratory registers. Patients received the results and treatment on the same day of visit.

Rapid diagnostic test arm

Patients presenting with fever (by statement or measured) underwent rapid testing with the "*Paracheck®*" device (Orchid Biomedical Systems, Goa, India). *Paracheck Pf®* is based on the detection of histidine rich protein-2 (Pf HRP-2) produced by *Plasmodium falciparum* trophozoites and young gametocytes. The specimens were drawn by trained clinicians or laboratory assistants using a simple finger-prick. The test preparation and interpretation were done following manufacturer's instructions and standard operating procedures prepared for this study. The test was considered positive when the antigen line was visible in the test window, negative when only the control band was visible. RDT results were recorded in the outpatient registers. Also patients received the results and treatment on that day of visit.

Duration of outpatient visit

The duration of outpatient visit was assessed in six HCs using a random sample of 1627 consenting patients. When a patient arrived at the HC, time was recorded by the research assistant on a "time sheet." The "time sheet" was then given to the patient. Thereafter, the time was recorded by clinicians and laboratory personnel at every point of service delivery. The "time sheet" was finally retained at the dispensing window. For the laboratory personnel, the effective contact time was comprised of: drawing a sample from the patient, slide preparation, scanning the 200 film fields until declaring a slide negative and reporting of results. With regard to RDT, the effective contact time was comprised of: drawing blood samples from patients, applying samples onto the test, test reading and reporting of results.

Overall data collection

Data collection was carried out weekly by the research assistants by extracting information from the laboratory and outpatient registers from March 2010 to July 2011.

Statistical analysis

The collected data were manually checked and cleaned. Data were double entered by two trained database assistants in a customized entry template with in-built consistency checks in EpiData 3.1 software (The EpiData Association, Odense, Denmark). The two data sets were validated to check for entry errors. Before analysis in Stata version 10 (Stata Corp LP, College Station, Texas, USA), the data was declared a cluster design using the "svyset command" with HCs as primary sampling units. Further, the Poisson regression model was fitted while accounting for clustering. Probability values (*p*-values) were set at 0.05 and confidence intervals (CI) were calculated at the 95% level. Socio-demographic and symptom data was presented using descriptive statistics: distribution by age, number and percent of patients with positive and negative results by transmission setting and diagnostic method and prescribed drugs.

Ethical considerations

The study was approved by Makerere University School of Public Health Higher Degrees Research and Ethics Committee; and the Uganda National Council for Science and Technology (Ref: HS 209). The study was registered with the Clinicaltrials.gov (NCT00565071).

Results

Description of the sample

The study was carried out in 30 sub-county level government HCs. Each diagnostic arm had five HCs located in an area of low malaria transmission and another five in a setting of high transmission intensity. Overall, 102, 087 outpatients presenting with fever were enrolled. This included 52, 116 (51%) in low and 49, 971 (49%) in high transmission settings. Those enrolled in the presumptive arm were 23, 884, RDT 46, 131 and microscopy arm 32, 072 (Figure 1). Of the patients enrolled 59, 876 (58.7%) were females and 26, 421 (25.9%) were children under five years of age (Table 1). The median age in years for children under-five was 1.7 [inter-quartile range one to three years] and for those five years and above was 22 [inter-quartile range 13-35 years].

Proficiency in conducting the test

One hundred and thirty three HC staff were trained for the study. The staff included: 24 clinical officers, 13 nursing officers, 15 enrolled/registered nurses, 30 midwives, 36 nursing assistants and 15 laboratory assistants. Clinical officers have three years of pre-service training; nurses/midwives have two, laboratory assistants two to three years while nursing assistants have six to nine months. Laboratory assistants had two to six years of work experience. All cadres of staff had attended training in human immunodeficiency virus (HIV) counselling and testing. Therefore, they were experienced in testing HIV using whole blood on rapid test strips, devices or cassettes that work on the same principle as malaria RDTs. At this level of the health care services delivery system the staff in the RDT arm mentioned that one day of training is adequate, mainly focusing on practical (preparation of test and interpretation of results). On

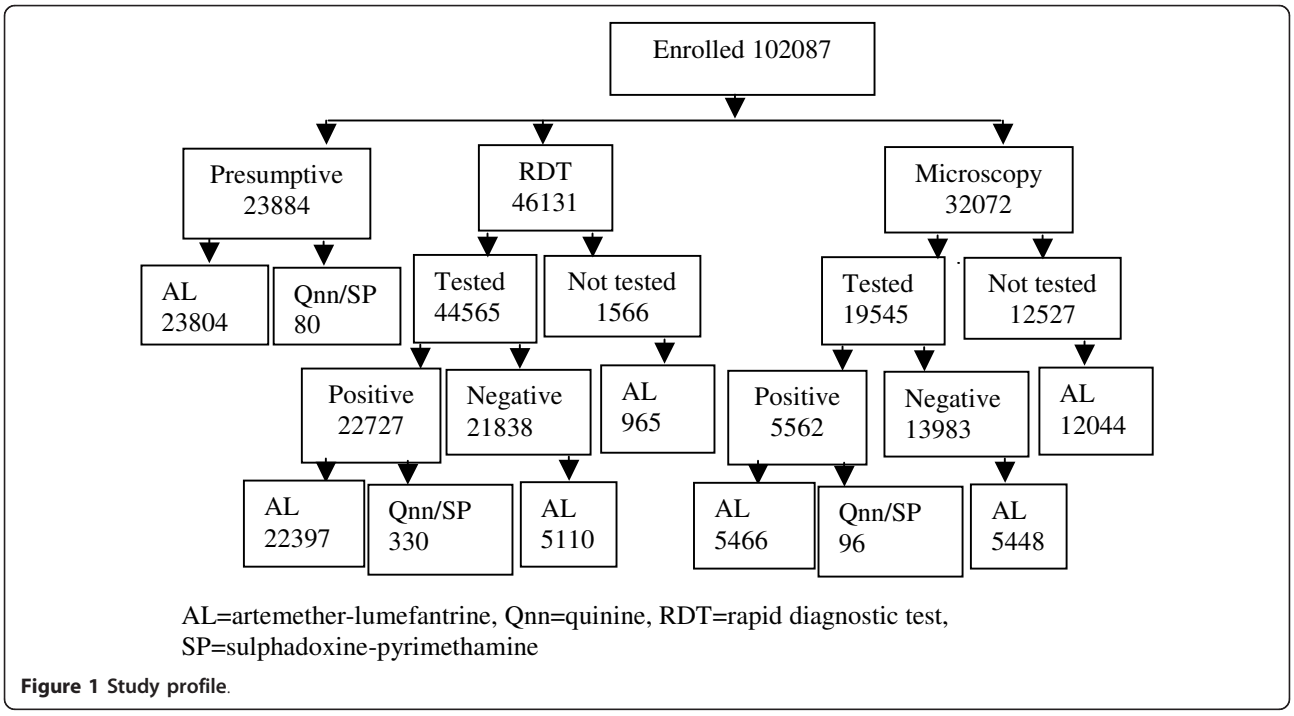


Figure 1 Study profile.

Table 1 Selected characteristics of study sample

Characteristic	Presumptive (%) [Int. range]	RDT (%) [Int. range]	Microscopy (%) [Int. range]
Level at the health delivery system	Sub-county	Sub-county	Sub-county
Number of health centres per arm	10	10	10
Total enrolment	23884	46131	32072
Gender (female)	14239(59.6)	26613(57.8)	19024(59.3)
Children under-five years of age	5265(22.0)	12828(27.8)	8328(26.0)
Median age (in years)			
< 5 years	2[1-3]	1.6[1-3]	1.7[1-3]
≥5 years	21[13-34]	23[14-35]	20[12-35]
Proportion with history of fever			
< 5 years	2695(94.0)	3393(87.5)	4224(88.0)
≥5 years	5568(73.7)	11936(70.3)	9276(68.4)

Int. range = inter-quartile range, RDT = rapid diagnostic test

the first day, few staff especially nursing assistants had a challenge in collecting blood for the RDT using a loop in children under five years. However, they had gained adequate skills by the end of the first week. In the microscopy arm, all staff attended the practical session. Although they were able to prepare a thick blood smear, reading and interpretation of results was the responsibility of the laboratory personnel. Eight of the HCs (four in each district) in the microscopy arm did not have electricity. They used natural light when scanning for malaria parasites. In the remaining two HCs, black-out was frequent and electricity as source of light for malaria microscopy was unreliable.

Duration of outpatient visit

The effective average contact time of clinicians with febrile outpatients (excluding time for investigation) was 11.4 min [95%CI: 11.1-11.7], and was similar in the three arms (Table 2). The mean effective contact time for malaria investigations was significantly shorter when using RDT 7.6 min [95%CI: 7.1-8.0] compared to microscopy 11.0 min [95%CI: 10.6-11.4]. On average, outpatients spent about two and half hours to complete a HC visit. However, the time spent accessing services in the RDT arm 134.4 min [95%CI: 123.5-145.3] was not different from that of presumptive treatment, but significantly shorter than when using microscopy 188.5 min [95%CI: 173.8-203.3].

Malaria test results

Overall, 64, 110 in the two intervention arms were tested (Table 3). The proportions tested in the two transmission settings were not statistically different [RR: 1.10; 95%CI: 0.87-1.40]. However, patients were 1.59 times more likely to have a malaria test done in the RDT arm 44, 565 (96.6%) [95%CI: 93.8-99.4] compared to the microscopy arm 19, 545 (60.9%) [95%CI: 47.9-74.0], [RR: 1.59; 95%CI: 1.31-1.92]. Still patients were more likely to be tested in the RDT arm compared to microscopy arm in children under-five years of age [RR: 1.56; 95%CI: 1.20-2.02], patients five years and above [RR: 1.59; 95%CI: 1.34-1.90], patients in low transmission area [RR: 1.45; 95%CI: 1.41-1.49] and in the high transmission setting [RR: 1.71; 95%CI: 1.06-2.76].

Overall, 28, 289 (44.2%) [95%CI: 30.8-57.5] had a positive test result. In the low transmission setting, the proportion of those who tested positive in the RDT arm was 3, 313 (20.5%) [95%CI: 17.4-23.6] and microscopy arm 1, 548 (14.0%) [95%CI: 11.2-16.7]. The proportion of those testing positive in the RDT arm 19, 414 (68.6%) [95%CI: 62.2-74.9] in the high transmission setting was also significantly higher than in the microscopy arm 4,

Table 2 Mean patient time (in minutes) at different points of seeking care at government health centres

Variable	Presumptive Mean[95%CI]	RDT Mean[95%CI]	Microscopy Mean[95%CI]	Overall Mean[95%CI]
Contact with clinician	11.1[10.6-11.6]	11.9[11.3-12.5]	11.3[10.8-11.9]	11.4[11.1-11.7]
Contact for investigation	N/A	7.6[7.1-8.0]	11.0[10.6-11.4]	9.3[8.9-9.6]
Waiting time for test results	N/A	37.5[32.8-42.3]	123.9[105.9-142.0]	62.4[54.6-70.2]
Overall waiting time	135.6[123.0-148.2]	109.2[98.3-120.1]	156.1[141.4-170.9]	133.7[126.0-141.3]
Time spent at HC	143.8[131.2-156.4]	134.4[123.5-145.3]	188.5[173.8-203.3]	155.6[147.8-163.4]

CI = Confidence Interval, HC = health centre, N/A = not applicable, RDT = rapid diagnostic test

Table 3 Proportion of patients tested and malaria results by age and transmission intensity

Diagnostic method	Low transmission			High transmission		
	Enrolled	Tested n(%)	Positive n(%)	Enrolled n(%)	Tested n(%)	Positive n(%)
Presumptive						
< 5 years	2936	-		2329	-	
≥5 years	14035	-		4584	-	
RDT						
< 5 years	2716	2571(94.7)	583(22.7)	10112	10075(99.6)	7932(79.2)
≥5 years	14921	13624(91.3)	2730(20.1)	18382	18295(99.5)	11482(62.8)
Microscopy						
< 5 years	2900	1768(61.0)	269(15.2)	5428	3502(64.5)	2122(60.6)
≥5 years	14608	9313(63.8)	1279(13.7)	9136	4962(54.3)	1892(38.1)

RDT = rapid diagnostic test

014 (47.4%) [95%CI: 35.7-59.1]. Generally, the risk of having a positive test was higher among children under-five years of age 10, 900 (61.1%) compared to patients five years old and above 17, 124 (37.4%) [RR: 1.62; 95% CI: 1.41-1.86]. This relationship was maintained in the low transmission area [RR: 1.12, 95%CI: 1.05-1.21], and increased in the high transmission setting [RR: 1.29; 1.14-1.47].

Treatment of patients who did not receive a parasitological diagnosis for malaria

All patients in the control arm were treated with anti-malarials (Figure 1 and Table 4). Prescription of AL in the control arm was not significantly different between those under five years and the older age group. A total of 12, 527 (39.1%) in microscopy and 1, 566 (3.4%) in RDT arms were not tested [RR: 11.50; 95%CI: 5.16-

Table 4 Patients treated with artemether-lumefantrine stratified by diagnostic method, age and transmission intensity

	Low transmission				High transmission			
	Presumptive n(%) [95%CI]	Microscopy n(%) [95%CI]	RDT n(%) [95%CI]	Total n(%) [95%CI]	Presumptive n(%) [95%CI]	Microscopy n(%) [95%CI]	RDT n(%) [95%CI]	Total n(%) [95%CI]
Not tested								
Age (yrs)								
< 5	2932(99.9) [99.7-100]	1103(97.4) [95.6-99.2]	129(88.8) [80.1-97.9]	4164(98.8) [97.6-100]	2309(99.1) [97.9-100]	1842(95.6) [94.3-97.0]	33(89.2) [78.6-99.7]	4184(97.5) [95.6-99.3]
≥5	13999(99.7) [99.6-99.8]	5163(97.5) [96.5-98.4]	741(57.1) [54.9-59.3]	19903(96.5) [93.1-99.9]	4564(99.6) [99.4-99.7]	3936(94.3) [92.7-95.9]	62(71.3) [61.5-80.9]	8562(96.8) [94.1-99.5]
Total	16931(99.7) [99.6-99.8]	6266(97.5) [96.5-98.5]	870(60.3) [57.9-62.8]	24067(96.9) [93.9-99.8]	6873(99.4) [99.0-99.8]	5778(94.7) [93.5-95.9]	95(76.1) [69.0-84.2]	12746(97.0) [94.6-99.4]
Tested								
Positive								
Age (yrs)								
< 5	N/A	264(98.1) [95.2-100]	576(98.8) [98.4-99.2]	840(98.6) [97.8-99.4]	N/A	2106(99.3) [97.8-100]	7853(99.0) [97.6-100.]	9959(99.1) [98.1-100]
≥5	N/A	1254(98.0) [96.1-99.7]	2675(98.0) [97.4-98.6]	3929(98.0) [97.5-98.5]	N/A	1842(97.4) [97.8-98.8]	11293(98.4) [96.5-100]	13135(98.2) [96.9-99.5]
Total	N/A	1518(98.1) [96.9-99.2]	3251(98.1) [97.6-98.7]	4769(98.1) [97.7-98.5]	N/A	3948(98.4) [96.9-99.7]	19146(98.6) [96.9-100]	23094(98.6) [97.4-99.7]
Negative								
Age (yrs)								
< 5	N/A	374(25.0) [21.6-28.3]	332(16.7) [11.2-22.2]	706(20.3) [16.1-24.4]	N/A	1138(82.5) [80.4-84.5]	1246(59.6) [57.5-61.8]	2384(68.7) [44.0-93.4]
≥5	N/A	1630(20.3) [16.4-24.2]	1413(13.0) [12.4-13.6]	3043(16.1) [11.7-20.5]	N/A	2298(74.9) [73.3-76.4]	2055(30.2) [29.1-31.3]	4353(44.1) [40.2-48.9]
Total	N/A	2004(21.0) [17.4-24.7]	1745(13.6) [12.9-14.2]	3749(16.8) [12.4-21.1]	N/A	3436(77.2) [75.9-78.4]	3301(37.1) [36.1-38.1]	6737(50.5) [43.1-55.4]

CI = confidence interval, N/A = not applicable, RDT = rapid diagnostic test

25.65]. Subsequently, 12, 044 (96.1%) and 965 (61.6%) respectively were treated with AL.

Treatment of patients with a positive RDT or microscopy

All patients in both microscopy and RDT arms with positive results received anti-malarial treatment (Figure 1). Overall, parasitological confirmation of malaria was associated with a reduction in the prescription of AL between presumptive and RDT [RR: 0.62; 95%CI: 0.47-0.82], and between presumptive and microscopy [RR: 0.72; 95%CI: 0.60-0.86].

Treatment of patients with negative RDT or microscopy results

Overall, 10, 558 (29.5%) were treated with AL. Patients with RDT negative results were more often prescribed AL in the high transmission setting 3, 301 (37.1%) compared to those in the low transmission area 1, 744 (13.6%) [RR: 2.74; 95%CI: 1.05-7.15]. In the microscopy arm, there was a two to five-fold likelihood of prescribing AL to patients with negative results in the high transmission area 3, 436 (77.2%) than in the low transmission setting 2, 002 (21.0%) [RR: 3.67; 95%CI: 2.43-5.54]. This implies that the likelihood of not accepting negative microscopy results was higher than that for RDT in a setting of high malaria transmission.

Within the low transmission setting, children under-five years of age with negative microscopy results were more often prescribed AL compared to those in the RDT arm [RR: 1.49; 95%CI: 1.14-1.97]. A similar pattern in AL prescription was observed among patients five years and above. In the high transmission setting there was no statistically significant difference in AL prescription among children under-five years when those treated under microscopy were compared to those under RDT. Among patients five years and above in the high transmission setting 2, 055 (30.2%) in the RDT arm and 2, 298 (74.9%) in microscopy arm were prescribed AL [RR: 2.48; 95%CI: 0.83-7.41].

Discussion

This study provides data on the comparative feasibility of microscopy and RDT among outpatients attending government rural primary health care centres located within areas of varying transmission intensities. The study findings indicate that RDT was more feasible than microscopy and prescribers were unlikely to adhere to negative results especially in the microscopy arm.

It is reported here that patients attending HCs with RDT as the method for malaria investigation had a higher probability of getting a parasitological test done regardless of age and transmission setting. Out of 133 clinical and laboratory staffs trained in 30 HCs, 56 were in the RDT arm and they performed the testing of patients during the whole period of study implementation. In the microscopy arm, the staff either had clinical or laboratory roles, although clinicians were able to prepare usable thick smears. Microscopy was the sole responsibility of the laboratory assistants. This meant that the RDT diagnostic services were not interrupted even if the laboratory assistants were away on leave. Performing RDT takes a much shorter time than microscopy. Therefore given a similar time, more patients could be tested in RDT compared to microscopy. RDTs reduced the patient waiting time compared to microscopy and were thus more convenient for health workers and patients. An earlier investigation [21] also reported a high number of patients tested by RDT. However, that study did not have a microscopy arm and therefore constraining a full comparison with the current findings. In Tanzania, a study that introduced routine use of malaria RDTs only resulted into 35% of patients being tested [22].

The risk of a febrile patient not getting a malaria diagnostic service was higher in HCs with microscopy and even far higher among patients under-five years of age. The current malaria treatment policy in Uganda and WHO [1] recommend that malaria case diagnosis be based on parasitological confirmation either by microscopy or RDT. An earlier publication reported a shortage of staff where only 34% of laboratory assistant posts were filled, although only four HCs had functioning laboratories at the time [16]. In order to improve the availability of microscopy services, there is need to functionalize the laboratories and to train and post at least two laboratory assistants at each HC. The low rate of malaria tests done in the microscopy arm adds to previous reports [13-15,23] regarding the microscopy limitations, signifying the difficulties surrounding its feasibility and scale up of the service.

With routine use of parasitological confirmation of malaria, prescription of AL was reduced by 28.1% between presumptive and microscopy; and by 38% between presumptive and RDT. This benefit was also reported in other studies [21,24], but it was offset by continued prescription of AL to patients with negative results. Indeed treatment of this "negative syndrome" with AL is a cause for concern in both intervention arms, and it was significantly higher in the microscopy arm in both transmission settings and age groups. This might imply prescribers were unlikely to adhere to negative microscopy results. Prescribing anti-malarials among patients with negative results has also cited [2-4,9,21,22,25-27]. The behaviour of treating negative patients with AL may reflect the hangover of previous practices of presumptive treatment, doubting accuracy of test methods, patients having been on anti-malarials before, or clinicians not knowing how to treat patients

with negative results due to lack of clear guidelines. The continuous prescription of anti-malarials by clinicians disregarding negative test results is likely to impact on the cost-effectiveness of the diagnostic methods, clinical care of patients as well as increasing the costs of diagnosis and that of the overall treatment. Furthermore if negative patients continue receiving anti-malarials, health workers are more likely not to see the need for parasite-based diagnosis and may not be motivated to implement the policy. Therefore, service providers need support and guidelines on how to manage patients with negative results.

In the preparation for the study, RDTs, AL and laboratory supplies were delivered by the study team to the district medical stores. However, AL stock-out occurred in the high transmission setting that resulted into a reduction in the number of outpatient attendance. Subsequently this impacted on the number of patients enrolled in the high transmission setting. RDTs were in-stock throughout the study implementation period. The training of staff for this study took one day. However, other cadres of staff without such skills as HIV testing might require slightly longer period (three to five days). To acquire adequate skills for example in testing malaria with RDT, it is important to make frequent supervisory visits and tapering the number of visits with time. In this study, it was planned to have three diagnostic arms (presumptive, RDT and microscopy). Further research should consider incorporating the arm of RDT plus microscopy.

Conclusion

RDT was more feasible than microscopy and patients with negative results received significantly more AL in the microscopy arm compared to those in the RDT arm. To realise the benefits of parasitological confirmation of malaria, service providers need to adhere to test results and they need guidance regarding management of patients with negative results.

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Authors' contributions

All authors conceived and designed the study; VB and FN collected, analysed, interpreted the data and drafted the manuscript; PM critically revised the manuscript. All authors read and approved the final manuscript

Competing interests

The authors declare that they have no competing interests.

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Antibiotic use among patients with febrile illness in a low malaria endemicity setting in Uganda

Vincent Batwala[1,3*], Pascal Magnussen[2] and Fred Nuwaha[3]

Abstract

Background: Uganda embraced the World Health Organization guidelines that recommend a universal 'test and treat' strategy for malaria, mainly by use of rapid diagnostic test (RDT) and microscopy. However, little is known how increased parasitological diagnosis for malaria influences antibiotic treatment among patients with febrile illness.

Methods: Data collection was carried out within a feasibility trial of presumptive diagnosis of malaria (control) and two diagnostic interventions (microscopy or RDT) in a district of low transmission intensity. Five primary level health centres (HCs) were randomized to each diagnostic arm (diagnostic method in a defined group of patients). All 52,116 outpatients (presumptive 16,971; microscopy 17,508; and RDT 17,638) aged 5 months to ninety five years presenting with fever (by statement or measured) were included. Information from outpatients and laboratory registers was extracted weekly from March 2010 to July 2011. The proportion of patients who were prescribed antibiotics was calculated among those not tested for malaria, those who tested positive and in those who tested negative.

Results: Seven thousand and forty (41.5%) patients in the presumptive arm were prescribed antibiotics. Of the patients not tested for malaria, 1,537 (23.9%) in microscopy arm and 810 (56.2%) in RDT arm were prescribed antibiotics. Among patients who tested positive for malaria, 845 (25.8%) were prescribed antibiotics in the RDT and 273(17.6%) in the microscopy arm. Among patients who tested negative for malaria, 7809 (61.4%) were prescribed antibiotics in the RDT and 3749 (39.3%) in the microscopy arm. Overall the prescription of antibiotics was more common for children less than five years of age 5,388 (63%) compared to those five years and above 16798 (38.6%).

Conclusion: Prescription of antibiotics in patients with febrile illness is high. Testing positive for malaria reduces antibiotic treatment but testing negative for malaria increases use of antibiotics.

Trial Registration: ClinicalTrials.gov: NCT00565071

Keywords: Antibiotic treatment, febrile patients, malaria diagnosis

Background

Recent World Health Organization (WHO) guidelines recommend a universal 'test and treat' strategy for malaria, mainly by use of rapid diagnostic test (RDT) and microscopy in all transmission areas [1]. In rural settings, however, febrile outpatients present with multiple complaints at health facilities and receive antibiotics in addition to anti-malarial treatment. Although WHO recommends rational use of medicines, it is estimated that in developing countries, the proportion of patients treated according to clinical guidelines for common diseases in primary care is less than 40% in the public sector and 30% in the private sector [2].

Antibiotics are routinely prescribed for colds, non-specific upper respiratory tract infections and acute bronchitis [3,4], as concomitant medications to anti-malarials [5]. In almost all cases, these are viral, self-limited conditions in which antibiotic use does not enhance illness resolution and is not recommended [6,7]. For other infections, such as otitis media, antibiotics provide some benefit, but the value of their use as first-line treatment has been debated [8].

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Even for conditions in which antibiotic use might be justified, experts have expressed concern about substantial overuse [4] that impacts on health care costs. Inappropriate use of these drugs promotes antimicrobial resistance [9], adverse drug reactions and erodes patient confidence in health services [2]. In absence of urgent and corrective actions, the world is heading towards a post-antibiotic era in which many common infections will no longer have a cure and, once again, kill unabated [10,11].

Although the Uganda national malaria guidelines now recommend confirmation of parasitaemia before initiation of treatment, data on how results of use of malaria diagnostics influence antibiotic treatment among febrile outpatients is lacking. The current study assessed antibiotic prescribing rates among febrile outpatients attending rural health centres (HCs) where feasibility of rolling out parasitological diagnosis for malaria was being tested. The primary outcome measures were the proportions of patients with febrile illness that were prescribed antibiotics when: not tested for malaria, they test positive and when they test negative.

Methods

Study design

Data collection on antibiotic treatment was carried out within a cluster randomized feasibility trial of presumptive malaria diagnosis (control) and two diagnostic interventions (microscopy and RDT). Fifteen out of twenty sub-county level government HCs in a district of low malaria endemicity in Uganda were randomly selected for the trial. HCs were the primary sampling units, and were allocated to the three diagnostic arms using simple randomization. Finally, there were five HCs per arm (diagnostic method in a defined group of patients).

Setting

The trial was carried out in Bushenyi district in south-western Uganda. The district headquarter is located at about 320 km from the capital city Kampala. The district is mainly rural with a total land area of 3,949 sq. km. It is endowed with diverse natural resources that include arable land, forests, large lake water bodies (Lakes Edward, George and Kazinga Channel), Queen Elizabeth National Park and minerals. The main economic activities are semi-intensive agriculture (growing crops and rearing animals), fishing and trade. The district is multi-ethnic with varying customs and norms. The main inhabitants are Banyankore and Bakiga. The total population is estimated at 731,392, and with 20 public HCs at sub-county level. The population distribution and density varies with physical geography. It is concentrated in the low-lying plateau zones of Sheema, Igara and Ruhinda; and sparse in the hilly-rough and rugged terrain of Buhweju and Bunyaruguru. The climate is relatively wet. The mean annual temperature range is 12.5°-30°C. Most of the district receives 1500-2000 mm of rainfall annually. Although Bushenyi experiences low and unstable malaria transmission, people of all ages are at risk. It is epidemic-prone, with occasional malaria outbreaks occurring shortly after the rains. The annual entomological inoculation rate is not known, but it was reported to be < 10 infective bites per person per year in the neighbouring district of Kanungu [12]. The trial commenced before Bushenyi was partitioned. However, partitioning did not affect the status of the trial HCs and the delivery of health services by the end of data collection. Additional description of the study setting has been published previously [13].

Study procedures

Training of staff

A total of 74 clinical and laboratory staff received a one-day refresher training on-site. The training and subsequent study procedures were a scale-up of activities performed during the assessment of the accuracy of these malaria diagnostic methods [14]. All staff members were trained in theory by re-orienting them to the malaria treatment policy. Staff in the control arm were only re-oriented to the current malaria treatment guidelines but testing of patients was not performed. In the microscopy arm, members were, in addition, trained in 1) finger prick for collection of blood, 2) thick/thin blood smear preparation, 3) staining smears, and 4) blood smear reading. In the RDT arm, the staff were in addition, trained in 1) finger prick for collection of blood and 2) preparation and reading of *Paracheck*®. The staff in HCs with microscopy or RDT were instructed to treat patients for malaria according to test results. Treatment with antibiotics followed national guidelines. HC outpatient registers were modified to record additional variables such as the presenting complaints, drugs dispensed and to indicate those prescribed but out-of stock. The trained staff were charged with training those that were off-duty on the day of training. However, additional clarification was provided during supervision. Supervision by the study team was carried out weekly during the first two months and monthly thereafter. The district laboratory focal persons provided the routine quality control procedures in both microscopy and RDT arms.

Description of the diagnostic arms

Presumptive (control) arm

Patients presenting with fever (by statement or measured) were enrolled to receive service without parasitological confirmation of malaria. Patients were treated on the basis of signs and symptoms only.

Microscopy arm

All patients presenting with fever (by statement or measured) were enrolled. The laboratory assistants prepared thick and thin blood smears by finger-prick using sterile blood lancets on separate frosted slides. Standard staining was performed using the Field's stain method. Laboratory assistants were only familiar with this staining technique. Blood films were read at magnification X1,000. Each film was graded as positive (asexual malaria parasites seen) or negative (no malaria parasites seen) based on inspection of 200 fields. Microscopy test results were recorded in the laboratory registers.

Rapid diagnostic test arm

Patients presenting with fever underwent rapid testing with the "*Paracheck*®" device (Orchid Biomedical Systems, Goa, India). *Paracheck Pf*® is based on the detection of histidine rich protein-2 (Pf HRP-2) produced by *Plasmodium falciparum* trophozoites and young gametocytes. The specimens were drawn by trained nurses or laboratory assistants using a simple finger-prick. The test preparation and interpretation were done following manufacturer's instructions and standard operating procedures. The test was considered positive when the antigen line was visible in the test window and negative when only the control band was visible. RDT results were recorded in the outpatient registers.

Data collection

All outpatients presenting at the study HCs with fever (by statement or measured) from March 2010 to July 2011 were enrolled. Data collection was carried out weekly by the research assistants by extracting information from the laboratory and outpatient registers.

Statistical analysis

The collected data were manually checked and cleaned. Data were double entered by two trained database assistants in a customized entry template with in-built consistency checks in EpiData 3.1 software (The EpiData Association, Odense, Denmark). The two data sets were validated to check for entry errors. Before analysis in Stata version 10 (Stata Corp LP, College Station, Texas, USA), the data were declared a cluster design using the "svyset command" with HCs as primary sampling units. Further, the Poisson regression model was fitted while accounting for clustering. Probability values (*p*-values) were set at 0.05 and confidence intervals (CI) were calculated at the 95% level. Socio-demographic and symptom data were presented using descriptive statistics: distribution by age, number and percent of patients with positive and negative results, and those receiving drugs.

Ethical approval

The study was approved by Makerere University School of Public Health Higher Degrees Research and Ethics Committee; and the Uganda National Council for Science and Technology (Ref: HS 209). The study was registered with the Clinicaltrials.gov (NCT00565071).

Results

Description of the study population

The study was carried out in 15 sub-county level government HCs located in an area of low malaria transmission intensity. Overall, 52,116 outpatients presenting with fever were enrolled: in the presumptive arm 16,971; microscopy arm 17,508; and RDT arm 17,637 (Figure 1). There were 8,552 children under five years (16.4%) with a median age of two [inter-quartile range one to three years]. The median age for those five years and above was 21 [inter-quartile range 13-34 years]. The overall age range was five months to ninety five years. The presenting symptoms of patients are presented in Table 1.

Types of antibiotics prescribed

Oral co-trimoxazole was prescribed to 11,862 patients (51.0%) and amoxicillin capsules to 5,986 (25.8%). These two antibiotics were the most commonly prescribed. Metronidazole was prescribed to 3,950 patients (17.0%), but in combination with other antibiotics. Doxycycline, erythromycin and ciprofloxacin were also prescribed, but in smaller quantities. Other drugs such as analgesics and anti-helminthics were prescribed to 49,574 patients (95.2%) and 10,330 (19.8%) respectively.

Antibiotic use in patients who did not receive a parasitological test for malaria

In the presumptive arm 7,040 patients (41.5%) were prescribed antibiotics. In the microscopy arm 6,427 (37%) did not receive a parasitological diagnosis and of these 1,537 (23.9%) were prescribed antibiotics. In the RDT arm, 1442 (8%) did not receive a parasitological diagnosis and of these 810 (56.2%) were prescribed antibiotics (Figure 1). Prescription of antibiotics was more common for children less than five years of age as compared to those who were older (Table 2). The pattern of antibiotic prescription varied widely across health units: presumptive arm (range 40.7% to 42.2%), microscopy arm (range 22.9% to 25.0%) and in RDT arm (range 53.6% to 58.7%). Overall 9387 (38%) of the patients who did not receive a parasitological diagnosis were prescribed antibiotics.

Antibiotic treatment in patients with a positive RDT or microscopy

Of the 3,313 patients who tested positive for malaria in the RDT arm, 854 (25.8%) were prescribed antibiotics. In the microscopy arm of the 1,548 with positive results,

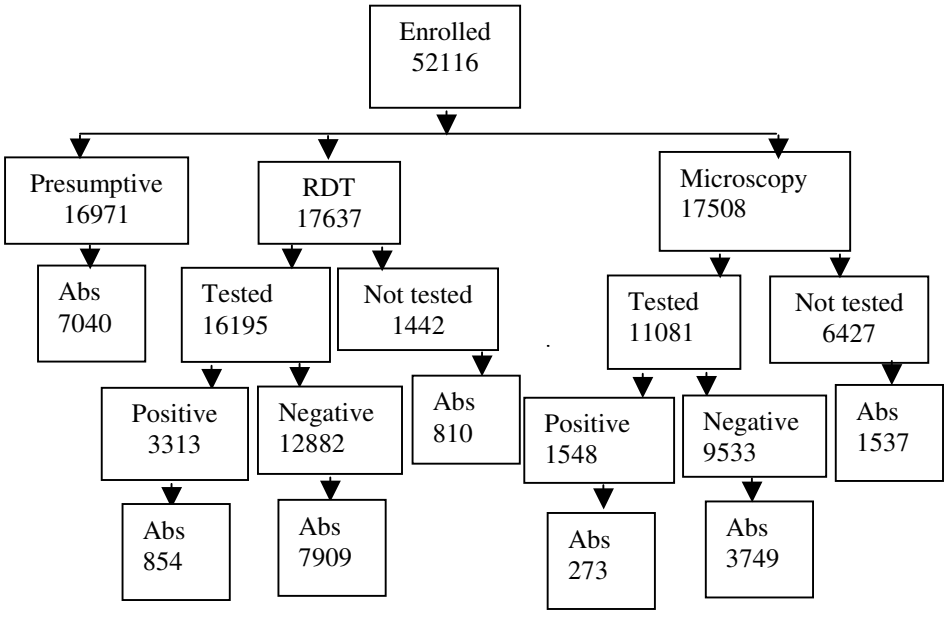


Figure 1 Study profile.

273 (17.6%) were prescribed antibiotics (Table 3). Patients under five years were more often prescribed antibiotics than in those five years and above in both RDT and microscopy arms. Prescription of antibiotics also varied widely between health units (ranging from 15.7% to 27.3%).

Antibiotic use in patients with negative RDT or microscopy

Overall, 11,658 patients (52.1%) with negative results were prescribed antibiotics (Table 4). Patients with negative RDT 7,909 (61.4%) received antibiotics prescription more often than those with negative

Table 1 Selected characteristics of study sample

Characteristic	Presumptive (%) [Int. range]	RDT (%) [Int. range]	Microscopy (%) [Int. range]
Level at the health delivery system	Sub-county	Sub-county	Sub-county
Number of health centres per arm	5	5	5
Total enrolment	16971	17637	17508
Gender (female)	10007(59.0)	10045(57.0)	10440(59.6)
Median age (in years)			
< 5 years	2 [1-3]	2 [1-3]	1.1 [1-3]
≥ 5 years	21 [13-34]	22 [14-35]	20 [13-34]
Non-specific URTI			
< 5 years	1554(52.9)	1626(59.9)	1350(46.6)
≥ 5 years	3635(25.9)	5432(36.4)	2643(18.1)
Otitis media			
< 5 years	6(0.2)	16(0.6)	20(0.7)
≥ 5 years	24(0.2)	8(0.1)	24(0.2)
Sore throat			
< 5 years	24(0.8)	8(0.3)	24(0.8)
≥ 5 years	20(0.1)	204(1.2)	136(0.9)

Int. range = inter-quartile range, RDT = rapid diagnostic test, URTI = upper respiratory tract infection

Table 2 Prescription of antibiotics in patients who did not receive parasitological diagnosis stratified by diagnostic method, age and health centre

Diagnostic method	< 5 years n(%)[95%CI]	≥ 5 years n(%)[95%CI]	Total n(%)[95%CI]
Presumptive			
Karungu	372(69.9) [66.0-73.8]	1327(39.0) [37.4-40.7]	1699(43.2) [41.6-44.7]
Kashenshero	*	1136(34.7) [33.1-36.3]	1136(34.7) [33.0-36.3]
Katunguru	376(59.5) [55.7-63.3]	1418(45.9) [44.1-47.6]	1794(48.2) [46.6-49.8]
Kyangyenyi	1000(56.6) [54.2-58.9]	238 (25.6) [22.8-28.4]	1238(45.9) [44.0-47.8]
Mutara	*	1173(35.2) [33.6-36.8]	1173(35.2) [33.5-36.8]
Total	1748(59.5) [57.8-61.3]	5292(37.7) [36.9-38.5]	7040(41.5) [40.7-42.2]
RDT			
Burere	20(76.9) [59.6-94.3]	148(56.1) [50.9-62.1]	168(57.9) [52.2-63.7]
Bushenyi	27(79.4) [65.1-93.7]	131(51.8) [45.6-58.0]	158(55.1) [49.3-60.8]
Katerera	20(76.9) [59.6-94.3]	123(51.0) [44.7-57.4]	143(53.6) [47.5-59.6]
Kyamuhumga	26(72.2) [56.9-87.6]	184(54.8) [49.4-60.1]	210(56.5) [51.4-61.5]
Kyeizoba	15(65.2) [44.2-86.3]	116(57.1) [50.3-64.0]	131(58.0) [51.5-64.4]
Total	108(74.5) [67.3-81.7]	702(54.1) [51.4-56.8]	810(56.2) [53.6-58.7]
Microscopy			
Bugongi	81(43.1) [35.9-50.2]	146(13.7) [11.7-15.8]	227(18.1) [16.0-20.3]
Kabira	123(47.3) [41.2-53.4]	208(18.3) [16.0-20.5]	331(23.7) [21.4-25.9]
Kabushaho	130(54.9) [48.5-61.2]	234(21.0) [18.6-23.4]	364(26.9) [24.6-29.3]
Kichwamba	123(54.7) [48.1-61.2]	216(23.8) [21.1-26.6]	339(30.0) [27.3-32.6]
Kigarama	107(48.2) [41.6-54.8]	169(15.8) [13.6-17.9]	276(21.3) [19.1-23.6]
Total	564(49.8) [46.9-52.7]	973(18.4) [17.3-19.4]	1537(23.9) [22.9-25.0]

CI = confidence interval, RDT = rapid diagnostic test, *children under-five records missing

microscopy results 3,749 (39.3%) [RR: 1.56; 95%CI: 1.41-1.73, $p < 0.001$]. Again children under five years of age with negative results in both RDT and microscopy arms were more often prescribed antibiotics than the older age group. Further, antibiotic prescription varied widely across health units (ranging from 38.4% to 62.4%).

Discussion

This article reports on a large assessment of the effect of malaria diagnostics on the probability of receiving antibiotics in a Ugandan population living in an area of unstable malaria transmission. The findings indicate that the rate of antibiotic treatment was high; there was a

Table 3 Prescription of antibiotics in patients with positive RDT or microscopy by age and health centre

Diagnostic method	< 5 years n(%)[95%CI]	≥ 5 years n(%)[95%CI]	Total n(%)[95%CI]
RDT			
Burere	50(42.7) [33.6-51.8]	104(19.4) [16.0-22.8]	154(23.6) [20.3-26.8]
Bushenyi	29(33.3) [23.2-43.4]	82(17.9) [14.4-21.5]	111(20.4) [17.0-23.8]
Katerera	47(45.2) [35.5-54.9]	115(21.3) [17.9-24.8]	162(25.2) [21.8-28.6]
Kyamuhumga	84(54.2) [46.3-62.1]	162(25.8) [22.3-29.2]	246(31.4) [28.1-34.6]
Kyeizoba	43(35.8) [27.1-44.5]	138(24.3) [20.7-27.8]	181(26.3) [23.0-29.6]
Total	253(43.4) [39.4-47.4]	601(22.0) [20.5-23.6]	854(25.8) [24.3-27.3]
Microscopy			
Bugongi	17(34.7) [20.9-48.5]	36(14.2) [9.9-18.6]	53(17.6) [13.2-21.9]
Kabira	23(37.7) [25.2-50.2]	38(12.8) [8.9-16.6]	61(17.0) [13.1-20.9]
Kabushaho	16(38.1) [22.8-53.4]	30(12.1) [8.0-16.2]	46(15.9) [11.6-20.1]
Kichwamba	26(41.9) [29.3-54.6]	39(13.8) [9.8-17.9]	65(18.9) [14.7-23.1]
Kigarama	23(41.8) [28.4-55.3]	25(12.6) [8.0-17.3]	48(19.0) [14.1-23.8]
Total	105(39.0) [33.2-44.9]	168(13.1) [11.3-15.0]	273(17.6) [15.7-19.5]

CI = confidence interval, RDT = rapid diagnostic test

Table 4 Prescription of antibiotics in patients with negative RDT or microscopy by age and health centre

Diagnostic method	< 5 years n(%)[95%CI]	All age ≥ 5 years n(%)[95%CI]	Total n(%)[95%CI]
RDT			
Burere	299(80.0) [75.6-83.8]	1258(58.5) [56.4-60.6]	1557(61.7) [59.8-63.6]
Bushenyi	180(81.1) [75.9-86.3]	767(54.0) [51.4-56.6]	947(57.7) [55.3-60.1]
Katerera	285(82.6) [78.6-86.6]	1190(63.0) [60.8-65.2]	1475(66.1) [64.1-68.0]
Kyamuhumga	485(81.1) [78.0-84.3]	1826(60.8) [59.0-62.5]	2311(64.2) [62.6-65.7]
Kyeizoba	353(79.0) [75.2-82.8]	1266(52.8) [50.8-54.7]	1619(56.9) [55.1-58.7]
Total	1602(80.6) [78.9-82.4]	6307(58.1) [57.1-60.0]	7909(61.6) [60.7-62.4]
Microscopy			
Bugongi	202(64.9) [59.6-70.3]	470(29.8) [27.5-32.0]	672(35.5) [33.4-37.7]
Kabira	213(65.5) [60.3-70.7]	543(32.7) [30.4-34.9]	756(38.0) [35.9-40.2]
Kabushaho	207(70.7) [65.4-75.9]	570(36.8) [34.4-39.2]	777(42.2) [39.9-44.5]
Kichwamba	189(70.3) [64.8-75.8]	572(39.4) [36.9-41.9]	761(44.2) [41.9-46.6]
Kigarama	195(64.9) [59.6-70.4]	590(33.0) [30.9-35.2]	785(37.6) [35.5-39.7]
Total	1006(67.2) [64.8-69.5]	2745(34.2) [33.2-35.2]	3751(39.4) [38.4-40.4]

CI = confidence interval, RDT = rapid diagnostic test

reduction in antibiotic prescription among patients with positive malaria test results; there was an increase in antibiotic treatment in those testing negative; the rate of antibiotic prescription was higher in children under five years of age; and antibiotic prescription varied widely across HCs suggesting that prescribers' behaviour is a big factor in use of antibiotics.

The level of antibiotic treatment reported here is higher than that demonstrated in Kabale [15], a district in the same region of Uganda with similar malaria endemicity, but comparable to that reported in Zanzibar [16]. In this study it is unlikely that symptoms alone justify this rate of antibiotic prescription as less than 5% of the patients would probably need antibiotics based on the clinical presentation. Other reasons such as expectations of the patient [17], service provider (prescriber) behaviour [18] and the social interaction between the patient and prescriber [17,18] have been cited. It was reported that for a satisfactory outcome of the consultation process, the clinician provides technically correct care, but this corresponds with the patient's expectations in order to legitimize the illness [18]. Also an earlier study [19] demonstrated that patient preference can stimulate inappropriate antibiotic prescribing. Further, other studies provided information about the other reasons for unnecessary antibiotic use [20-22]. Of particular importance are the inadequate staffing and the varying levels of professional training of staff manning outpatient clinics reported in a previous publication [12]. Understaffing is impacted by the heavy patient load, creating a need to finish the queue at the earliest possible time. Some reports also indicated that overuse of medicines is a consequence of diagnostic uncertainty by service providers, inappropriate unethical promotion of medicines by pharmaceutical companies, overworked health staff with limited time to spend with patients, and unrestricted availability of medicines [2,21,23]. These results complement these observations as diagnostic uncertainty and prescribers' behaviour were important determinants of antibiotic use in this study.

Patients who were in the RDT arm were more likely than those in the microscopy arm to be prescribed antibiotics. This difference is unlikely to be attributed to use of RDT. Generally in the microscopy arm, antibiotic prescription was low among: those not tested, who tested positive and those who tested negative. The trend of antibiotic use, however, was similar in the two diagnostic arms (decreasing among patients who test positive and increasing among negative patients). A more likely explanation for this difference in rates of antibiotic use among the diagnostic arms is prescribers' behaviour [i.e. higher likelihood of service providers more likely to treat with antibiotics in the RDT arm].

The rate of antibiotic prescription generally decreased among patients with positive results. This might indicate clinicians' acceptance of malaria-positive results as the only likely cause of illness at that point and therefore restrained from prescribing concurrent medications. However, the proportion of parasitaemic patients prescribed antibiotics reported here is higher than that observed in other settings [24]. Enormous resources have been invested in improving the targeting of antimalarials but the concern of concurrent or otherwise antibiotic treatment has not received equal attention. Although antibiotic prescription to febrile outpatients appears complex because of frequent presentation with multiple complaints, antibiotic treatment in parasitaemic patients may be an indicator of the likely inability to utilize the clinical guidelines. Antibiotic prescribing for cough or non-specific upper respiratory tract infections

was reported to be neither cost-effective nor cough-effective [25]. Therefore, there is need to enhance the treatment decisions at the lower level of care since staff manning these units have varying levels of professional training.

In this study, the chance of prescribing antibiotics increased if a febrile patient tested negative for malaria. These data are similar to what has been reported elsewhere [24,26-28]. Thus it appears that there is a compensatory antibiotic prescription in patients with negative results. This scenario of antibiotic prescribing has the potential to erode the financial savings that could accrue from widespread implementation of the universal 'test and treat' strategy for malaria. Besides the universal test and treat strategy does not provide adequate guidance on treating patients who test negative for malaria. Therefore, there is need to develop and implement guidelines regarding antibiotic treatment in febrile patients who test negative for malaria.

In some settings, interventions that promote rational antibiotic use have been shown to be effective. These emphasize careful diagnosis especially of upper respiratory syndromes, deferral of antibiotic use, and a watch-and-wait approach (along with symptom relief) for which antibiotics are not immediately indicated [29], and a targeted educational intervention [30]. Unless the use of antibiotics is curtailed, there is a prospect of higher costs, increased morbidity, and higher rates of death from common bacterial infections [2].

Conclusions

Prescription of antibiotics in patients with febrile illness is high. Testing for malaria reduces antibiotic treatment in patients with positive results but increases in those testing negative. Antibiotic use also depends on age and prescriber behaviour. It is essential that malaria diagnostics are rolled-out in all primary level health units and guidelines for antibiotic treatment especially among children developed and distributed. In addition, continuing professional education for prescribers should be enforced.

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Authors' contributions

All authors conceived and designed the study; VB and FN collected, analysed, interpreted the data and drafted the manuscript; PM critically revised the manuscript. All authors read and approved the final manuscript

Conflicts of interests

The authors declare that they have no competing interests.

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