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ORIGINAL ARTICLE

Malaria epidemiology and comparative reliability of diagnostic tools in Bannu; an endemic malaria focus in south of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The present study was aimed at elucidation of malaria epidemiology and comparing performance of several diagnostic procedures in Bannu, a highly endemic district of Khyber Pakhtunkhwa, Pakistan.

Dried blood spots were collected from patients suspected of malaria visiting a hospital and two private laboratories in district Bannu and processed for species-specific PCR (rRNA). Patients were also screened for malaria through microscopy and RDT. A well-structured questionnaire was used to collect patient information to assess risk factors for malaria.

Of 2033 individuals recruited, 21.1% ($N = 429$) were positive for malaria by at least one method. Overall, positivity detected by PCR was 30.5% (95/311) followed by 17.7% by microscopy (359/2033) and 16.4% by RDT (266/1618). *Plasmodium vivax* (16.9%, $N = 343$) was detected as the dominant species followed by *Plasmodium falciparum* (2.3%, $N = 47$) and mixed infections (1.2%, $N = 39$). Microscopy and RDT (Cohen's kappa k = 0.968, $p = <0.0001$, McNemar test $p = 0.069$) displayed significant agreement with each other. Satisfactory health, sleeping indoors, presence of health-care facility in vicinity (at an accessible range from home), living in upper middle class and in concrete houses significantly reduced malaria risk; whereas, low literacy level, presence of domestic animals indoors and malaria diagnosis recommended by clinician increased the disease risk.

Overall, findings from the study provide reasonable basis for use of RDT as a cost-effective screening tool in field and for clinicians who can proceed with timely treatment of malaria patients. Appropriate management of identified risk factors could contribute to reduction of malaria prevalence in Bannu and its peripheries.

KEYWORDS

Epidemiology; diagnosis; Bannu; Khyber Pakhtunkhwa; malaria

Introduction

Malaria in Pakistan remains the fourth largest cause of death among communicable diseases, and along with Afghanistan, Somalia, Sudan and Yemen, it accounts for more than 95% of the total regional malaria burden. The country has a reported National Annual Parasite Incidence (API) averaging at 1.66 per 1000 population [1–3]. *Plasmodium vivax* (accounting for >80% cases) and *P. falciparum* are the only known prevalent species for malaria in Pakistan, although figures from the past few decades indicate that there is a substantial rise in *P. falciparum* infections [4–6]. Federally Administered Tribal Areas (FATA), Baluchistan and Khyber Pakhtunkhwa (KP) provinces reportedly observe the highest APIs in Pakistan. According to the latest stratification, 66 districts and agencies have been categorized in the high endemicity stratum (API >5 per 1000) [1]. In KP, malaria incidence (particularly *P. falciparum* malaria) is unstable and fluctuates with climatic deviations, that adds to underlying risk for outbreaks as reported in the past [7,8]. Many factors have contributed toward increase of malaria cases over the years including warm autumns (that subsequently prolong the transmission period) [7], emergence of chloroquine resistance across the country [8] and a chronic drop in vector control activities.

Designing efficient control and prevention strategies requires taking into account demographic and climatic factors that probably influence malaria transmission in a region [9–12], e.g. rainfall, age and gender [13], human migration [14], type and location of housing, etc [15–17]. Geographical and chronological differences in malaria transmission require well-timed identification of crucial risk factors, so as to implement targeted control measures [18].

Falciparum malaria, having fatal implications, is increasingly reported from southern and northern districts of KP [19]. District Bannu (population: 1.1 million) has an API of approximately 1.6–3.5 per 1000 population, which is substantially above the national average (0.8 per 1,000 population). The district is of great economic significance for being the

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central hub in the South of KP, in addition to serving as a short route to markets in Central Asia [7,20].

Variations in both human and parasite population in Pakistan demand periodic surveillance of burden and distribution of malaria to ensure appropriate and timely case treatment, particularly in situations where diagnosis by microscopy or species-specific rapid diagnostic tests are not accessible [21]. Misdiagnosis and poor differential diagnosis is a common concern in most endemic countries that usually leads to treatment failure [22]. The present study aimed to investigate epidemiology of malaria in Bannu district, Khyber Pakhtunkhwa, Pakistan. Additionally, we set out to assess and compare performance of common diagnostic techniques in this region of Pakistan.

Methods

Study area and sampling

Bannu (32°43′–33°06′ N; 70°22′–57′ E) is one of the 26 districts in the south of Khyber Pakhtunkhwa province of Pakistan with an area of about 1227 sq. km and a population of 1,167,892 (Pakistan Bureau of Statistics, 2017), majority of which is rural. Bannu is divided into two tehsils Domel/Bannu-1 and Bannu-II, comprising 40 union councils [23]. About 80 public health centers including Medical Teaching Institutes (MTI), District Headquarter Hospitals (DHQ), Regional Health Centers (RHC), Basic Health Units (BHU), Civil Dispensaries (CDs) and Mother & Child Health Centers (MCH) along with 15 private centers operate in Bannu District. In these health centers, first-line treatment for unconfirmed malaria is Chloroquine, for *P. vivax* is Chloroquine-Primaquine, for uncomplicated *P. falciparum* malaria is Artesunate/Sulfadoxine-Pyrimethamine (AS+SP), whereas Artesunate, Artemether or quinine is recommended for treating severe *P. falciparum* malaria or cases with treatment failures [24]. Control interventions being conducted here are run and sponsored by 'Integrated vector control/malaria control program Khyber Pakhtunkhwa' (IVC/MCP-KP) and 'Frontier Primary Health Care' (FPHC). These include regular trainings for focal persons in basic microscopy, RDTs use, malaria case management, outbreaks etc. Other essential prevention strategies include indoor residual spraying, mass distribution of bed nets to every household, antenatal care (ANC) bed net distribution (only pregnant women receive a bed net during her visit to hospital for antenatal care) and community education and mobilization campaigns (*Personal communication* with IVC/MCP-KP).

Blood samples were collected from patients suspected of malaria (patients manifesting classical non-specific clinical malaria symptoms in an endemic area including fever, malaise, headache, myalgias, jaundice and sometimes nausea, vomiting and diarrhoea) [25] visiting Malaria Model Laboratory, Malaria Lab (Women & Children Hospital Bannu) and Siddique Laboratory Bannu from March to October 2013.

All participants gave written informed consent to participate in the study. Ethics Committee at University of Peshawar (ref. no.06/EC-16/Pharm, March 2016) approved the study documentation. To acquire demographic and other relevant information for assessing malaria risk factors, a questionnaire was administered to these suspected patients.

Sample processing and nested-PCR diagnosis

Finger-prick blood spots on filter paper from patients were processed with RDT and microscopy on-site (thick and thin smears) [26]. Briefly thin and thick slides were made on a clean, grease-free microscope slide and allowed to air dry. The films were stained with 10% Giemsa solution, allowed to air dry and then examined by microscopists at the labs/clinics with light microscopy using an oil immersion objective lens. A slide was declared negative only after observing 100 microscopic fields without finding parasites. Thick films were examined first for the detection of malaria parasites. Thick films in which malaria parasites were identified, were subsequently examined for species identification on their thin films as per standards described by WHO [27].

Rapid tests were conducted by using malaria rapid diagnosis (First response® Malaria Ag, pLDH/HRP2 Combo Card test kit, Cat. No 116FRC30). About 5 µl fresh blood was taken using a disposable pipette, dispensed into the RDT sample well and processed following manufacturer's instructions.

Coarse porosity filter paper discs (Fisher Scientific, Loughborough, UK) were used to obtain blood spots from each patient. Filter papers were wrapped individually in properly labeled airtight sealed bags with silica gel and stored at 4°C until further processing [28]. A standardized disc section of the dried blood spot was punched using a Harris Uni-Core hole punch (2.5 mm diameter) and reconstituted in sodium azide (1 g per 1 L PBS/Tween) as described in [28]. DNA was later extracted using a resin-based Chelex® method [29] and then processed by a two-step Nested-PCR targeting conserved rRNA genes for discriminating *Plasmodium* species (using genus and species-specific primers) as described earlier (Table 1) [30]. In nest2, *P. falciparum* produces a 205 bp PCR product while *P. vivax* a 120 bp product.

Data analysis

Due to financial constraints, PCR was performed only on a subset of samples screened by microscopy but not by RDT. For the reason mentioned above, no sample was screened for all the three methods simultaneously, i.e. some samples were screened for Microscopy & RDT (N = 1618) and another set for Microscopy & PCR (N = 311). Therefore, the performance of RDT was compared

Table 1. Primers and reaction conditions used in diagnostic PCR.

PCR reaction	Forward primers(F)/reverse primer(R) (5′-3′)		PCR mixture	Cycling conditions
Nest 1 (genus specific primers)	F: TTAAAATTGTTGCAGTTAAAACG R: CCTGTTGTTGCCTTAAACTTC		20 µl reaction; 5 µl DNA from filter paper, 10.5 µl dH_2O, 1X NH_4SO_4, 2 mM $MgCl_2$, 250 µM dNTPs, 250 nM primer, 1 unit Taq polymerase	95°C, 5 minutes 25 cycles 58°C, 2 minutes 72°C, 2 minutes 94°C, 1 minute 1 cycle 58°C, 1 minute 72°C, 5 minutes 25°C, 10 minutes
Nest 2 (species specific primers)	**rV1V2** **rV1V2** **Rfal2** **Rfal1**	F: ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA R: CGCTTCTAGCTTAATCCACATAACTGATAC F: ACACAATGAACTCAATCATGACTACCCGTC R: TTAAACTGGTTTGGGAAAACCAAATATATT	20 µl reaction; 1 µl nest 1 product, 14.5 µl dH_2O, 1X NH_4SO_4, 2 mM $MgCl_2$, 250 µM dNTPs, 250 nm primer, 1 unit Taq polymerase	95°C, 5 minutes 30 cycles 58°C, 1 minutes 72°C, 1 minutes 94°C, 1 minute 1 cycle 58°C, 1 minute 72°C, 5 minutes 25°C, 10 minutes

against microscopy as the 'gold standard', whereas for microscopy, PCR was taken as 'gold standard' since PCR demonstrates high analytical sensitivity and is especially recommended for detecting low-grade infections (chronic, afebrile or mildly symptomatic, <100 parasites/µL) [31,32] in epidemiological research and surveys particularly in low-transmission areas [33].

Cross-tabulation and tests for comparison of diagnostic techniques were carried out in IBM SPSS Statistics 20. Kappa statistic (frequently used to test interrater reliability) [34–36] sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and McNemar's marginal homogeneity were also calculated for each diagnostic method against a selected true positive standard [37,38].

All malaria positives were matched by gender and age (<5, 5–20, >20 years) to obtain matched case–control pairs in Stata v13 (Statacorp, 2013). Matching leads to a balanced number of cases and controls across levels of the selected matching variables leading to reduced variance in the parameters of interest, which ultimately improves statistical efficiency [39]. Using this paired data, univariable and step-wise multivariable conditional logistic regression was used to investigate socioeconomic and demographic risk factors for malaria in the study population.

Results

Samples were collected from 2033 individuals suspected of malaria visiting 3 major laboratories of Bannu. Of the total, 429 (21.1%) were positive for malaria by at least one diagnostic technique (Table 2). Prevalence among males (21.2%) and females (20.8%) were comparable. Similarly, prevalence among all age groups was almost similar, i.e. ≤5 (20.7%, *N* = 143), 5–25 yrs (22.5%, *N* = 170) and >25 (19.8%, *N* = 116).

In Bannu, *Plasmodium vivax* (14.2%) was the dominant species followed by *P. falciparum* (1.7%) and mixed infections (1.3%). Even though the largest number of cases were reported in Bannu, the positivity rate did not seem to differ between areas from where the patients originated (Table 2). Patients that belonged to neighboring districts possibly had to travel to Bannu due to non-availability of local diagnostic services or health facilities. Here, health centers in Bannu received maximum patients originating from agricultural belts of Bannu, Sarai Naurang, Domel and Lakki Marwat (Figure 1). Within Bannu, patient visits were more frequent from Bannu II which is predominantly urban (Table 3).

Apparently, the prevalence of *P. vivax* cases was lowest in March followed by a steady increase until it peaked in August. On the other hand, *P. falciparum* infections were consistently low throughout the year with an abrupt rise in October where these infections peaked. No cases were reported in August and September by us. Similarly, mixed infections displayed constant low rates that peaked in October (Figure 2).

PCR outperformed the other two diagnostic methods by displaying highest positivity (30.5%) followed by microscopy (17.7%) and RDT (16.4%) (Table 4). With PCR as a true positive test, microscopy presented a poor sensitivity value of 41% and a reasonable specificity of 73%. Sensitivity and specificity of RDT was 79%

Table 2. Region-wise distribution of *Plasmodium* species.

District total no. of patients screened	No. of positive cases *N* (%)	*P. vivax N* (%)	*P. falciparum N* (%)	Mixed infections *N* (%)
Bannu *N* = 1566	349 (22.3%)	288 (18.4%)	34 (2.17%)	27 (1.7%)
FATA *N* = 320	52 (16.3%)	40 (12.5%)	10 (3.1%)	2 (0.6%)
Karak *N* = 19	4 (21.1%)	2 (1.5%)	0	2 (10.5%)
Lakki Marwat *N* = 128	24 (18.7%)	13 (10.2%)	3 (2.3%)	8 (6.3%)
Total *N* = 2033	429 (21.1%)	343 (16.9%)	47 (2.3%)	39 (1.9%)

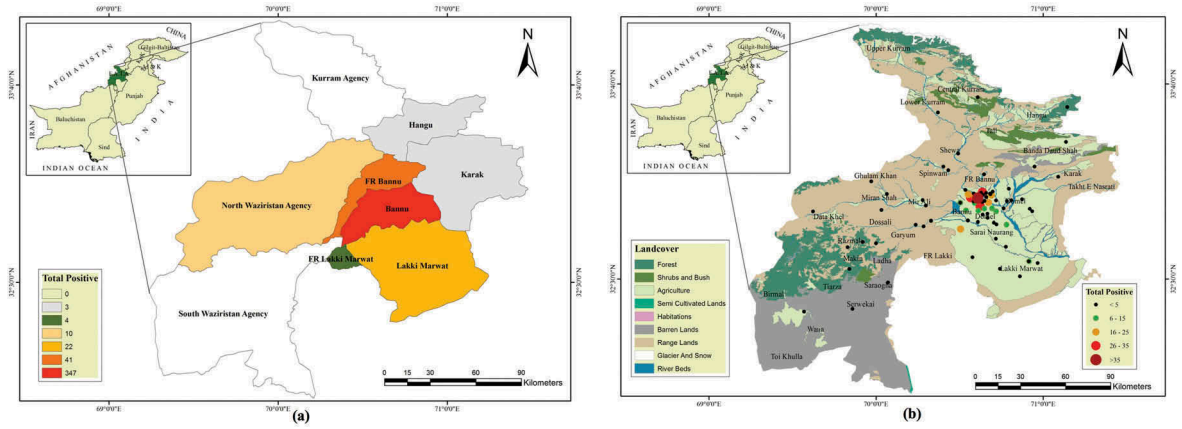

Figure 1. Origin of malaria patients in District Bannu and its vicinities a) Abundance of malaria patients b) Abundance on land cover map.

Table 3. Tehsil-wise distribution of *Plasmodium* species in district Bannu.

Tehsil total no. of patients screened	No. positive cases *N* (%)	*P. vivax N* (%)	*P. falciparum N* (%)	Mixed infections *N* (%)
Bannu 1 *N* = 468	91 (19.4)	74 (15.8)	9 (1.9)	8 (1.7)
Bannu 2 *N* = 1098	258 (23.5)	214 (19.5)	25 (2.3)	19 (1.7)
Total *N* = 1566	349 (22.3)	288 (18.4)	34 (2.2)	27 (1.7)

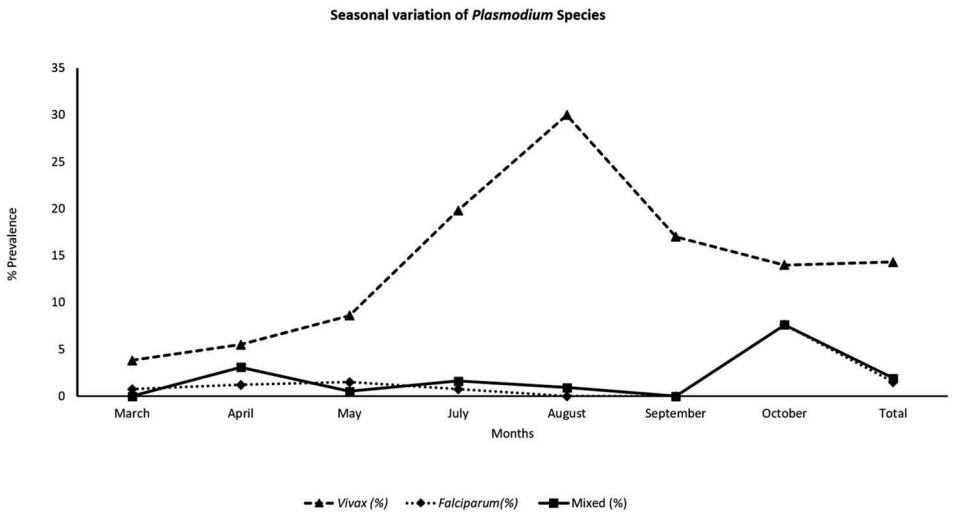

Figure 2. Seasonal abundance of *Plasmodium* infection.

Table 4. *Plasmodium* species detected using three diagnostic techniques.

	No. of malarial infections *N* (% positivity)			
Diagnostic Method no. of patients screened for each method	*P. vivax*	*P. falciparum*	Mixed	No. of total positive *N* (% positivity)
Microscopy *N* = 2033	290 (14.3)	30 (1.5)	39 (1.9)	359 (17.7)
RDT *N* = 1618	197 (12.2)	30 (1.9)	39 (2.4)	266 (16.4)
PCR *N* = 311	79 (25.4)	16 (5.1)	0 (0)[a]	95 (30.5)

[a]Subset of samples screened for PCR had no positive mixed infection by microscopy.

and 99%, respectively, using microscopy as a reference test. Significantly strong agreement between microscopy and RDT was observed (Cohen's kappa k = 0.968, p = <0.001, McNemar test p = 0.595). On the other hand, there was a moderate agreement between PCR and microscopic procedures (Cohen's kappa k = 0.14, p = 0.026. McNemar test p = 0.069) (Table 5).

Paired cases and controls were utilized to evaluate risk factors for malaria (Supplementary Table 1). Assessment of risk factors for malaria revealed satisfactory health (OR = 0.24, 95% CI 0.16–0.35), sleeping inside the room (OR = 0.62, 95% CI 0.46–0.84), presence of health-care facility in vicinity (OR = 0.65, 95% CI 0.47–0.88), an upper middle class (OR = 0.68

Table 5. Comparative performance of diagnostic methods.

Diagnostic Method No. of patients screened for each method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	McNemar marginal homogeneity test (*p*-value)	Cohen's Kappa value (*p*-value)
Microscopy [a] *N* = 311	41	73	41	73	0.069	0.14 (0.026)
RDT [b] *N* = 1618	79	99	98	99	0.593	0.968 (<0.001)

PCR: polymerase chain reaction; RDT: rapid diagnostic test; PPV: positive predictive value; NPV: negative predictive value.
[a]Using PCR as a true positive test.
[b]Using microscopy as a true positive test.

0.46–0.99) and living in concrete house type (OR = 0.54, 95% CI 0.29–1.01) as statistically significant factors for reducing the disease risk. Other exposure variables that were identified to increase risk of malaria exposure included primary literacy level (OR = 1.97, 95% CI 1.18–3.30), presence of domestic animals within houses (OR = 1.36, 95% CI 1.03–1.78) and malaria diagnosis recommended by clinician (OR = 1.84, 95% CI 1.39–2.43) (Supplementary Table 1). Stepwise multivariate analysis for risk factors, as assessed for all diagnostic methods and microscopy identified primary literacy level (OR = 1.98, 95% CI 0.16–0.36), a diagnosis recommended by clinician (OR = 1.47, 95% CI 1.09–2.00), weak and satisfactory health status (All positives OR(weak) = 0.56, 95% CI 0.37–0.84 and OR (satisfactory) = 0.18, 95% CI 0.12–0.26; Microscopy OR (satisfactory) = 0.24, 95% CI 0.16–0.36), sleeping indoors (All positives OR = 0.72, 95% CI 0.53–0.96; Microscopy OR = 0.69, 95%CI 0.50–0.95), body aches (OR = 0.66, 95% CI 0.47–0.93) and presence of healthcare facility in vicinity (OR = 0.61, 95% CI 0.44–0.85) as factors crucial for malaria transmission and control (Table 6).

Discussion

The study was one of the first to provide a comprehensive understanding of malaria epidemiology in Bannu district and to assess the comparative efficiency of different diagnostic methods in this endemic focus.

Overall, 21.1% of the cases were positive by at least one method. Bannu was considered as one of the most malarious areas of the province [40]. Internal displacement of FATA population in 2012 (about 700,000 people from neighboring North Waziristan) was suspected to have added to the malaria burden in Bannu since FATA regions are known to exhibit highest rates of disease incidence in the country [21,41,42]. Corroborating previous studies, *P. vivax* infections were more prevalent compared to *P. falciparum* and mixed infections [43–45]. Dominance of one malaria species over the other is primarily determined by the parasite's biology and by an area's climatic and seasonal variations [46]. Larger influx of *P. vivax* is possible because true relapses do not occur in *P. falciparum*, whereas in *P. vivax*, relapses are common due to prolonged survival of *Vivax* hypnozoites in liver cells [47–49].

In our study, *P. vivax* cases peaked in the summer month (August) while *P. falciparum* and mixed infections in winter (October). Similar distribution trends are known to exist in certain areas of neighboring Afghanistan that share similar climatic settings [20,50,51]. Generally, in Pakistan, *P. falciparum* transmission starts in the summer monsoon (July) when the temperature and humidity are optimum and it prevails until the end of the year when the temperature falls below the critical value (December). *P. vivax* usually observes two transmission peaks, one is its early transmission period during the wet months of spring (probably facilitated by true relapses) and the other is with the *P. falciparum* (monsoon) [7,20,40,52].

Table 6. Risk factors for malaria based on multivariate conditional logistic regression of variables from case–control pairs.

Diagnostic method	Microscopy *N* = 718	Positive by any method *N* = 858
Variables	Odds ratio (95% CI)	Odds ratio (95% CI)
General health (compared with poor health)		
Weak	0.87 (0.57–1.32)	0.56* (0.37–0.84)
Satisfactory	0.24* (0.16–0.36)	0.18* (0.12–0.26)
Literacy (compared to illiterate)		
Primary	–	1.98* (1.13–3.47)
Secondary & above		1.25 (0.89–1.76)
Indoor sleeping habit (compared to outdoors)	0.69* (0.50–0.95)	0.72* (0.53–0.96)
Presence of health care facility in living area	0.61* (0.44–0.85)	–
Having body aches	–	0.66 (0.47–0.93)
Malaria diagnosis recommended by clinician (compared self-diagnosis without recommendation by clinician)	–	1.47* (1.09–2.00)

CI: confidence interval.
*Factors with *p*-value <0.05.

Unlike the findings of our study, age has earlier been associated with malaria acquisition [53,54] where children ≤5 years are shown to carry a comparatively higher risk [55–57]. The acquired immunity is both exposure- and age-dependent, and the older children are likely to have developed some degree of immunity because of repeated infections [58,59]. There also exists a possibility of extended exposure due to inattentiveness of parents/guardians toward protective/treatment measures [56]. Further, we documented no significant association with gender. Although, several earlier studies in Khyber Pakhtunkhwa and elsewhere prove males to be at a higher risk of malaria compared to females [43,45,60–68] suggesting increased exposure to mosquito bites since males are more likely to work outdoors and are not traditionally well covered as females (usually veiled) [52]. Cultural and social norms might have stemmed low influx of female patients to health-care facilities.

In the present study, microscopy displayed poor sensitivity when compared to rRNA PCR. Studies suggest that microscopy, when compared to molecular diagnostic methods like PCR, often exhibit considerable discrepancies (usually showing lower sensitivity to mixed infections), especially in submicroscopic infections [69–72]. Parasite densities measured by microscopy correlate with parasite gene copy number in quantitative PCRs and studies indicate that in submicroscopic infections lower copy numbers are expected in low transmission settings than those in the high transmission. These deviations eventually impact diagnostic outcomes [73,74]. Moreover, mixed infections pose challenges to microscopists since similarities exist between developmental stages of different *Plasmodium* species. Microscopy and RDT are prone to miss infections with density less than 100 parasites/μL, while the detection limit of PCR is generally <5 parasites/μL [75,76]. Other factors like lack of expertise and lack of standard good quality blood films also often contribute to such incongruities [71,77]. On the other hand, PCR will rarely miss microscopy positives due to poor DNA quality, poor quality standards in techniques or missed priming due to mutations [78]. Although studies demonstrate superior performance of PCR traditional diagnostics means in field [79–81], PCR cannot be used as a routine diagnostic tool due to its technologically advanced lab requirements and expenditures [82–84].

WHO recommends RDTs and microscopy as primary methods for diagnosis of clinically suspected malaria in all epidemiological settings, including low transmission areas like Khyber Pakhtunkhwa [85]. In our study, among 1618 samples screened through RDT, 266 (16.4) were detected positive for *Plasmodium* infection. RDT presented satisfactory sensitivity (79%) when compared to microscopy. Overall findings from the study provide reasonable basis for use of RDT as a screening tool in field and for clinicians to proceed with timely treatment of malaria patients. Studies also support the superior performance of RDT compared to microscopy in routine clinical settings and especially in remote locales where medical units face deficit in resources like contained labs, electricity, trained microscopists, etc. [71,86]. RDTs can serve as a cost-effective method for clinical diagnosis, mainly due to improved treatment and health outcomes for non-malaria febrile disease [87]. Incorrect diagnoses are a common observation in labs like the ones selected in our study, largely due to huge numbers of patients availing for free diagnosis. Furthermore, we observed that many of the patients visited the labs for diagnosis without referral by clinicians.

Malaria risk was also investigated through multilevel analysis of individual and household level factors. Keeping livestock indoors was seen as a risk factor for acquiring malaria as described previously [65,88]. Cattle may attract mosquitoes, either by serving as bait or by creating mosquito breeding and resting sites near livestock pens [56,89–91]. Cattle provide an easy source of blood to mosquitoes, which results in increased vector populations, a significant proportion of which is attracted to feed on individuals sleeping outdoors near animals [92]. Risk of mosquito bites could be reduced due to zooprophylaxis [93] especially in situations where vector species predominantly display zoophagic foraging tendencies. However, the benefit of keeping livestock close to human dwellings has been refuted by many authors [94,95]. In Pakistan, *Anopheles culicifacies* and *A. stephensi* are confirmed as malaria vectors [96] and are chiefly Pakistan's zoophilic [97]. *A. culicifacies* is the primary vector in rural areas while *A. stephensi* is considered to be an important vector for malaria transmission in urban areas [98,99]. Livestock are thought to be largely responsible for generating the high mosquito densities in the region.A strong positive correlation between cattle: man ratio and malaria incidence was reported in northern KP province of Pakistan [88].

Frequent outdoor activities also increased probability of infection as mosquitoes are generally active between dusk and dawn [100]. Risk of malaria transmission always exists where mosquito host-seeking behavior coincides with place and times of human presence [101]. In the study, herein, most of the individuals had evening activities while sleeping in open grounds was a common practice.

Sleeping habits especially clustering in sleeping rooms was established as a crucial risk factor. Clustering at household level appears to increase malaria risk probably due to increased release of human chemo-attractants [102,103]. The study area experiences long hours of power outage in summers that aggravates the biting rate.

Concrete houses were observed to reduce risk of malaria since they can decrease mosquito contact by limiting their entry and reducing their resting places [104]. Higher malaria incidence rate is commonly reported from poorly constructed houses (incomplete,

mud, or palm walls and palm thatched roofs) compared to well-constructed houses (bricks/stones) since poor housing likely leads to an increase in human–vector contact [15,17,95,104–106]. On the contrary, several others suggest no such association between malaria incidence and housing quality [16,56,107–110].

Living in an upper middle class was protective against malaria as these households have the amenities to adopt better preventive measures compared to the poor and underprivileged [105,111–116]. Socioeconomic factors may directly or indirectly affect malaria transmission [57,117,118] and failure to consider effects of socioeconomic elements might jeopardize the success of control programs [119].

In Bannu, acquiring only primary education by patients or guardian of patients seemed to increase the risk of malaria. There is a dire need to disseminate information for preventive and control strategies in the study area as majority of the patients denied using any precautionary measures. Commonly, individuals availing government health services belong to lower socioeconomic backgrounds generally with low education levels. These have been associated with poor knowledge regarding utilization of preventative methods and malaria treatment among them [91]. Achieving higher education is known to be protective against malaria [120].

Apparent health status of the individual was indicative of malaria possibly because many patients were referred by clinicians for malaria tests due to their anemic status expected in malaria infections. If left untreated (or inadequately treated), malaria may result in several weeks or months of poor health following repeated attacks of fever and anemia [107,121–123].

Overtreatment of malaria is not only an economic concern, but it has been proposed that restricting antimalarial use to laboratory-confirmed cases will also delay the emergence and spread of resistance to artemisinin derivatives and their partner drugs [124]. The labeling of all febrile patients as having malaria can have severe consequences as the underlying disease would not be properly identified and treated [125]. In our study, we observed that although many of the patients were recommended by clinicians for diagnosis, there were several patients that visited the facility on their own behalf. Many had a history of malaria with self-medication while others were prescribed antimalarials without diagnosis. Many of the private practitioners and labs also prescribed antimalarial drugs without confirmatory malaria tests.

Conclusion

Level of malaria endemicity, the urgency of diagnosis, experience of clinician, the effectiveness of healthcare workers and budget resources are all some factors affecting the choice of malaria-diagnostic method. In this study, RDT was identified as a suitable diagnostic test for its decent sensitivity and specificity; as well as its inter-procedure agreement for detecting parasite compared to microscopy. Population of Bannu, especially the rural lot lacks suitable health infrastructure, which is principal for disease management. Ultimately, targeting effective malaria control programs require essential understanding of epidemiology of malaria in a region.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] WHO (World Health Organization). World malaria report; 2017. Available from: https://www.who.int/malaria/publications/world-malaria-report-2017/en/
[2] Dawn. Malaria resurgence. Daily Dawn. 2016 Sep 20.
[3] Pakistan Medical & Research Council. Malaria indicator survey in 38 high risk districts of Pakistan-2013-14; 2014. Available from: http://dmc.gov.pk/documents/pdfs/3Final MIS Report-From Printe-30 April 2015 (2).pdf.
[4] WHO (World Health Organization). Flooding and communicable diseases fact sheet: risk assessment and preventive measures. WHO Communicable Diseases Working Group on Emergencies; 2011a. Available from: https://www.who.int/diseasecontrol_emergencies/publications/who_cds_2005.35/en/
[5] DMC (Directorate of Malaria Control). Malaria case management desk guide for clinicians and health care providers. Islamabad; 2017. Available from: http://dmc.gov.pk/documents/pdfs/Case_Management_Guidelines.pdf
[6] Ghanchi NK, Martensson A, Ursing J, et al. Genetic diversity among Plasmodium falciparum field isolates in Pakistan measured with PCR genotyping of the merozoite surface protein 1 and 2. Malar J. 2010;9:1.
[7] Bouma MJ, Dye C, Van Der Kaay HJ. Falciparum malaria and climate change in the northwest frontier province of Pakistan. Am J Trop Med Hyg. 1996;55:131–137.
[8] Shah I, Rowland M, Mehmood P, et al. Chloroquine resistance in Pakistan and the upsurge of falciparum malaria in Pakistani and Afghan refugee populations. Ann Trop Med Parasitol. 1997;91:591–602.
[9] Snow RW, Omumbo JA, Lowe B, et al. Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. Lancet. 1997;349:1650–1654.
[10] Lindsay SW, Campbell H, Adiamah JH, et al. Malaria in a peri-urban area of The Gambia. Ann Trop Med Parasitol. 1990;84:553–562.
[11] Drakeley C, Schellenberg D, Kihonda J, et al. An estimation of the entomological inoculation rate for

Ifakara: a semi-urban area in a region of intense malaria transmission in Tanzania. Trop Med Int Health. 2003;8:767–774.
[12] Reyburn H, Mbatia R, Drakeley C, et al. Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria. Jama. 2005;293:1461–1470.
[13] Mendis C, Gamage-Mendis AC, De Zoysa AP, et al. Characteristics of malaria transmission in Kataragama, Sri Lanka: a focus for immuno-epidemiological studies. Am J Trop Med Hyg. 1990;42:298–308.
[14] Klinkenberg E 2001 Malaria risk mapping in Sri Lanka: results from the Uda Walawe area. Proceedings of a health workshop in Embilipitiya, Sri Lanka, March 29.
[15] Gamage-Mendis AC, Carter R, Mendis C, et al. Clustering of malaria infections within an endemic population: risk of malaria associated with the type of housing construction. Am J Trop Med Hyg. 1991;45:77–85.
[16] Van Der Hoek W, Konradsen F, Dijkstra DS, et al. Risk factors for malaria: a microepidemiological study in a village in Sri Lanka. Trans R Soc Trop Med Hyg. 1998;92:265–269.
[17] Gunawardena DM, Wickremasinghe AR, Muthuwatta L, et al. Malaria risk factors in an endemic region of Sri Lanka, and the impact and cost implications of risk factor-based interventions. Am J Trop Med Hyg. 1998;58:533–542.
[18] Klinkenberg E, Van Der Hoek W, Amerasinghe FP. A malaria risk analysis in an irrigated area in Sri Lanka. Acta Trop. 2004;89:215–225.
[19] Raziq F, Khan M. A survey of malarial parasites in Abbottabad. Pak Med J Res. 1995;34:33–34.
[20] MOH (Ministry of National Health). Pakistan national strategic plan for malaria control 2010-2015. Islamabad (Pakistan): MOH; 2010.
[21] Khattak AA, Venkatesan M, Nadeem MF, et al. Prevalence and distribution of human Plasmodium infection in Pakistan. Malar J. 2013;12:297.
[22] Derua YA, Ishengoma DR, Rwegoshora RT, et al. Users' and health service providers' perception on quality of laboratory malaria diagnosis in Tanzania. Malar J. 2011;10:78.
[23] Government of Khyber Pukhtunkhwa. Bannu district demographics [Online]. Peshawar, Pakistan; 2017 [cited 2017 Dec]. Available from: http://kp.gov.pk/page/bannu_district_at_a_glance
[24] WHO (World Health Organization). Country profiles; 2012. Available from: http://www.who.int/malaria/publications/countryprofiles/profile_pak_en.pdf).
[25] Bartoloni A, Zammarchi L. Clinical aspects of uncomplicated and severe Malalria. Mediterr J Hematol Infect Dis. 2012;4:e2012026.
[26] WHO (World Health Organization). Malaria rapid diagnostic test performance. Geneva, Switzerland; 2009. Available from:https://www.who.int/tdr/publications/tdr-research-publications/rdt3_summary.pdf
[27] WHO (World Health Organization). Basic malaria microscopy – 2nd edition. Part 1: learner's guide; 2010. Available from: https://apps.who.int/iris/bitstream/handle/10665/44208/9789241547826_eng.pdf;jsessionid=1D1CD4A1745003713207C226477C6A68?sequence=1
[28] Corran PH, Cook J, Lynch C, et al. Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. Malar J. 2008;7:195.
[29] Plowe CV, Djimde A, Bouare M, et al. Pyrimethamine and proguanil resistance-conferring mutations in Plasmodium falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. Am J Trop Med Hyg. 1995;52:565–568.
[30] Snounou G, Viriyakosol S, Jarra W, et al. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitol. 1993;58:283–292.
[31] Chen I, Clarke SE, Gosling R, et al. "Asymptomatic" Malaria: a chronic and debilitating infection that should be treated. PLoS Med. 2016;13:e1001942.
[32] WHO (World Health Organization). Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 5 (2013). Geneva, Switzerland; 2014a. Available from: https://apps.who.int/iris/bitstream/10665/204118/1/9789241510035_eng.pdf
[33] WHO (World Health Organization) Policy brief on malaria diagnostics in low-transmission settings; 2014b. Available from: https://www.who.int/malaria/publications/atoz/malaria-diagnosis-low-transmission-settings-sep2014.pdf?ua=1
[34] Marston L. Introductory Statistics for Health and Nursing Using SPSS. Thousand Oaks, California: Sage Publications, Ltd; 2010.
[35] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.
[36] Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20:37–46.
[37] Mcnemar Q. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika. 1947;12:153–157.
[38] Parikh R, Mathai A, Parikh S, et al. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol. 2008;56:45–50.
[39] Rose S, Van Der Laan MJ. Why match? Investigating matched case-control study designs with causal effect estimation. Int J Biostat. 2009;5:1–24.
[40] Asif SA. Departmental audit of malaria control programme 2001-2005 north west frontier province (NWFP). J Ayub Med Coll Abbottabad. 2008;20:98–102.
[41] FATA Disaster Managment Authority. 2014. FDMA [Online]. [cited 2017]. Available from: http://www.fdma.gov.pk/
[42] Munir MA, Qureshi H, Safdar N. Malaria Indicator Survey in 38 High Risk Districts of Pakistan- 2013-14. Islamabad (Pakistan): Medical Research Council; 2014.
[43] Razzaq A, Khan N, Khan H. Frequency of slide positivity in clinically suspected malaria cases. Gomal J Med Sci. 2014;12:118–120.
[44] Khan NU. Frequency and seasonal variation of Plasmodium Species in Southern Districts of Khyber Pakhtunkhwa. Pak Armed Forces Med J. 2014;64:518–523.
[45] Khan F, Awan ZUR, Bettani MR. Plasmodium Infection among the Hospitalized Pregnant Women in District Bannu Khyber Pakhtunkhwa, Pakistan. Int J Adv Med Sci. 2016;1:20–22.
[46] Checchi F, Cox J, Balkan S, et al. Malaria epidemics and interventions, Kenya, Burundi, southern Sudan, and Ethiopia, 1999-2004. Emerg Infect Dis. 2006;12:1477–1485.
[47] Robert LS, Janovy JJ, Schmidt GD, et al. Roberts' Foundation of parasitology. London: Mc Graw Hill; 2009.
[48] Garnham P. Relapses and latency in malaria. Protozoology. 1967;2:55–64.

[49] Garnham PC. The liver in malaria with special reference to the exoerythrocytic phase. Ann Trop Med Parasitol. 1987;81:531–537.
[50] WHO (World Health Organization). World malaria report; 2011b. Available from: https://www.who.int/malaria/world_malaria_report_2011/en/
[51] Zakeri S, Mehrizi AA, Mamaghani S, et al. Population structure analysis of Plasmodium vivax in areas of iran with different malaria endemicity. Am J Trop Med Hyg. 2006;74:394–400.
[52] Khan HU, Khattak AM, Khan MH, et al. A study of prevalence of malaria in adult population of D. I. Khan, Pakistan. Biomedica. 2006;22:99–104.
[53] Dondorp AM, Lee SJ, Faiz MA, et al. The relationship between age and the manifestations of and mortality associated with severe malaria. Clin Infect Dis. 2008;47:151–157.
[54] Ferede G, Worku A, Getaneh A, et al. Prevalence of malaria from blood smears examination: a seven-year retrospective study from metema hospital, northwest ethiopia. Malar Res Treat. 2013;2013:704730.
[55] Bodker R, Akida J, Shayo D, et al. Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. J Med Entomol. 2003;40:706–717.
[56] Peterson I, Borrell LN, El-Sadr W, et al. Individual and household level factors associated with malaria incidence in a highland region of Ethiopia: a multilevel analysis. Am J Trop Med Hyg. 2009;80:103–111.
[57] Brooker S, Clarke S, Njagi JK, et al. Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. Trop Med Int Health. 2004;9:757–766.
[58] Bodker R, Msangeni HA, Kisinza W, et al. Relationship between the intensity of exposure to malaria parasites and infection in the Usambara Mountains, Tanzania. Am J Trop Med Hyg. 2006;74:716–723.
[59] Woyessa A, Deressa W, Ali A, et al. Malaria risk factors in Butajira area, south-central Ethiopia: a multilevel analysis. Malar J. 2013;12:273.
[60] Sahar S, Akhtar T, Bilal H, et al. Prevalence of Plasmodium falciparum, Malarial Parasite in Muzaffargarh District, Punjab. Pakistan: a Two year Study. Pak J Sci. 2010;64:64–66.
[61] Awan Z, Jan A. Rice fields in relation to malaria in District Bannu. Pak J Zool. 2008a;28:11–21.
[62] Daud M, Ullah N, Khan N, et al. Prevalence of malaria cases in general population of Mithakhel district Karak Pakistan. Rev Prog. 2014;2:1–4.
[63] Kondrachine A. Situation analysis of malaria in the province of Punjab 1–21st September 2008. Islamabad, Pakistan: Country Office. World Health Organization; 2008.
[64] Rahman S, Jalil F, Khan H, et al. Prevalence of Malaria in district Shangla, Khyber Pakhtunkhwa, Pakistan. Jezs. 2017;5:678–682.
[65] Khan IK, Shah AH, Rahman ZU. Epidemiology of Malaria in Urban and Rural Areas of Bannu District Khyber Pakhtunkhwa, Pakistan. Int J Mod Biol Med. 2013;4:30–39.
[66] Awan ZUR, Jan AH. Rice field in relations to the malaria in district Bannu NWFP. Proc Pak Congr Zool. 2008b;28:11–21.
[67] Irshad M, Ali R, Akbar S, et al. Occurrence of malaria in Khwazakhela District Swat Pakistan. PSCI publ. 2013;2:26–28.
[68] Rehman A, Ahmed K, Ustonomiya Y, et al. The prevalence of malaria in an endemic area of Bangladesh. J Trop Med Hyg. 1994;22:13–19.
[69] Mayor A, Moro L, Aguilar R, et al. How hidden can malaria be in pregnant women? Diagnosis by microscopy, placental histology, polymerase chain reaction and detection of histidine-rich protein 2 in plasma. Clinl Infect Dis. 2012;54:1561–1568 %@ 1537–6591.
[70] Arango EM, Samuel R, Agudelo OM, et al. Molecular detection of malaria at delivery reveals a high frequency of submicroscopic infections and associated placental damage in pregnant women from northwest Colombia. Am J Trop Med Hyg. 2013;89:178–183.
[71] Ohrt C, Purnomo, Sutamihardja MA, et al. Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials. J Infect Dis. 2002;186:540–546.
[72] Kaisar M, Supali T, Wiria A, et al. Epidemiology of Plasmodium infection in Flores Island, Indonesia using Realtime PCR. Malar J. 2013;12:169.
[73] Lo E, Zhou G, Oo W, et al. Low parasitemia in submicroscopic infections significantly impacts malaria diagnostic sensitivity in the highlands of Western Kenya. PLoS One. 2015;10:e0121763.
[74] Okell LC, Bousema T, Griffin JT, et al. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun. 2012;3:1237.
[75] Cordray MS, Richards-Kortum RR. Emerging nucleic acid–based tests for point-of-care detection of malaria. Am J Trop Med Hyg. 2012;87:223–230.
[76] Vasoo S, Pritt BS. Molecular diagnostics and parasitic disease. Clin Lab Med. 2013;33:461–503.
[77] Rantala A-M, Taylor SM, Trottman PA, et al. Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malar J. 2010;9:269.
[78] Coleman RE, Sattabongkot J, Promstaporm S, et al. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a Plasmodium falciparum/vivax endemic area in Thailand. Malar J. 2006;5:121.
[79] Rodulfo H, De Donato M, Mora R, et al. Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in endemic areas of Venezuela. Braz J Med Biol Res. 2007;40:535–543.
[80] Zakeri S, Kakar Q, Ghasemi F, et al. Detection of mixed Plasmodium falciparum & P. vivax infections by nested-PCR in Pakistan, Iran & Afghanistan. Indian J Med Res. 2010;132:31–35.
[81] Haghdoost AA, Mazhari S, Bahadini K. Comparing the results of light microscopy with the results of PCR method in the diagnosis of Plasmodium vivax. J Vector Borne Dis. 2006;43:53–57.
[82] Ngasala B, Mubi M, Warsame M, et al. Impact of training in clinical and microscopy diagnosis of childhood malaria on antimalarial drug prescription and health outcome at primary health care level in Tanzania: a randomized controlled trial. Malar J. 2008;7:199.
[83] De Oliveira AM, Skarbinski J, Ouma PO, et al. Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya. Am J Trop Med Hyg. 2009;80:470–474.
[84] Hopkins H, Bebell L, Kambale W, et al. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. J Infect Dis. 2008;197:510–518.
[85] WHO (World Health Organization) Policy brief on malaria diagnostics in low-transmission settings;

2014c. Available from: https://www.who.int/malaria/publications/atoz/malaria-diagnosis-low-transmission-settings-sep2014.pdf?ua=1.
[86] Erdman LK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. Travel Med Infect Dis. 2008;6:82–99.
[87] Shillcutt S, Morel C, Goodman C, et al. Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy. Bull World Health Organ. 2008;86:101–110.
[88] Bouma M, Rowland M. Failure of passive zooprophylaxis: cattle ownership in Pakistan is associated with a higher prevalence of malaria. Trans R Soc Trop Med Hyg. 1995;89:351–353.
[89] Mahande A, Mosha F, Mahande J, et al. Feeding and resting behaviour of malaria vector, Anopheles arabiensis with reference to zooprophylaxis. Malar J. 2007;6:100.
[90] Siri JG, Wilson ML, Murray S, et al. Significance of travel to rural areas as a risk factor for malarial anemia in an urban setting. Am J Trop Med Hyg. 2010;82:391–397.
[91] Tuyishimire J. Spatial modelling of malaria risk factors in Ruhuha sector. Rawanda; 2013.
[92] Wahid S 2013 Assessment of exposure, infection and risk for malaria in refugee camps in Khyber Pakhtunkhwa (KP), Pakistan. Ph.D, London School of Hygiene and Tropical Medicine University of London.
[93] Kaburi JC, Githuto JN, Muthami L, et al. Effects of long-lasting insecticidal nets and zooprophylaxis on mosquito feeding behaviour and density in Mwea, central Kenya. J Vector Borne Dis. 2009;46:184–190.
[94] Bogh C, Clarke SE, Pinder M, et al. Effect of passive zooprophylaxis on malaria transmission in The Gambia. J Med Entomol. 2001;38:822–828.
[95] Ghebreyesus TA, Haile M, Witten KH, et al. Household risk factors for malaria among children in the Ethiopian highlands. Trans R Soc Trop Med Hyg. 2000;94:17–21.
[96] Jahan N, Sarwar M. Malaria prevalence in district Okara, Punjab, Pakistan. Biologia (Pakistan). 2013;59:191–195.
[97] Naz S, Maqbool A, Ahmad M-U-D, et al. Efficacy of ivermectin for control of zoophilic malaria vectors in Pakistan. Pak J Zool. 2013;45:1585–1591.
[98] Carmichael GT. Anopheline control through water management. Am J Trop Med Hyg. 1972;21:782–786.
[99] Reisen WK, Boreham PF. Estimates of malaria vectorial capacity for anopheles culicifacies and anopheles stephensi in rural Punjab province Pakistan. J Med Entomol. 1982;19:98–103.
[100] Dale P, Sipe N, Anto S, et al. Malaria in Indonesia: a summary of recent research into its environmental relationships. Southeast Asian J Trop Med Public Health. 2005;36:1–13.
[101] Ndoen E, Wild C, Dale P, et al. Dusk to dawn activity patterns of anopheline mosquitoes in West Timor and Java, Indonesia. Southeast Asian J Trop Med Public Health. 2011;42:550–561.
[102] Torres-Estrada JL, Rodriguez MH. Physico-chemical signals involved in host localization and in the induction of mosquito bites. Salud Publica Mex. 2003;45:497–505.
[103] Danis-Lozano R, Rodriguez MH, Betanzos-Reyes AF, et al. Individual risk factors for Plasmodium vivax infection in the residual malaria transmission focus of Oaxaca, Mexico. Salud Publica Mex. 2007;49:199–209.
[104] Ayele DG, Zewotir TT, Mwambi HG. Prevalence and risk factors of malaria in Ethiopia. Malar J. 2012;11:195.
[105] Koram KA, Bennett S, Adiamah JH, et al. Socio-economic risk factors for malaria in a peri-urban area of The Gambia. Trans R Soc Trop Med Hyg. 1995;89:146–150.
[106] Keiser J, Utzinger J, Caldas De Castro M, et al. Urbanization in sub-saharan Africa and implication for malaria control. Am J Trop Med Hyg. 2004;71:118–127.
[107] Kimbi HK, Nana Y, Sumbele IN, et al. Environmental factors and preventive methods against malaria parasite prevalence in rural bomaka and Urban Molyko, Southwest Cameroon. J Bacteriol Parasitol. 2013;4:162.
[108] Lenz R. Jakarta kampung morbidity variations: some policy implications. Soc Sci Med. 1988;26:641–649.
[109] Snow RW, Peshu N, Forster D, et al. Environmental and entomological risk factors for the development of clinical malaria among children on the Kenyan coast. Trans R Soc Trop Med Hyg. 1998;92:381–385.
[110] Wolff CG, Schroeder DG, Young MW. Effect of improved housing on illness in children under 5 years old in northern Malawi: cross sectional study. Bmj. 2001;322:1209–1212.
[111] Sintasath DM, Ghebremeskel T, Lynch M, et al. Malaria prevalence and associated risk factors in Eritrea. Am J Trop Med Hyg. 2005;72:682–687.
[112] El Samani FZ, Willett WC, Ware JH. Nutritional and socio-demographic risk indicators of malaria in children under five: a cross-sectional study in a Sudanese rural community. J Trop Med Hyg. 1987;90:69–78.
[113] Macintyre K, Keating J, Sosler S, et al. Examining the determinants of mosquito-avoidance practices in two Kenyan cities. Malar J. 2002;1:14.
[114] Ong'echa JM, Keller CC, Were T, et al. Parasitemia, anemia, and malarial anemia in infants and young children in a rural holoendemic Plasmodium falciparum transmission area. Am J Trop Med Hyg. 2006;74:376–385.
[115] Guthmann JP, Hall AJ, Jaffar S, et al. Environmental risk factors for clinical malaria: a case-control study in the Grau region of Peru. Trans R Soc Trop Med Hyg. 2001;95:577–583.
[116] Banguero H. Socioeconomic factors associated with malaria in Colombia. Soc Sci Med. 1984;19:1099–1104.
[117] Mcmichael AJ, Woodruff RE, Hales S. Climate change and human health: present and future risks. Lancet. 2006;367:859–869.
[118] Temel T. Malaria from the gap: need for cross-sector co-operation in Azerbaijan. Acta Trop. 2004;89:249–259.
[119] Al-Taiar A, Assabri A, Al-Habori M, et al. Socioeconomic and environmental factors important for acquiring non-severe malaria in children in Yemen: a case-control study. Trans R Soc Trop Med Hyg. 2009;103:72–78.
[120] Dike N, Onwujekwe O, Ojukwu J, et al. Influence of education and knowledge on perceptions and practices to control malaria in Southeast Nigeria. Soc Sci Med. 2006;63:103–106.
[121] Tarimo SD. Appraisal on the prevalence of malaria and anaemia in pregnancy and factors influencing uptake of intermittent preventive therapy with sulfadoxine-pyrimethamine in Kibaha district, Tanzania. East Afr J Public Health. 2007;4:80–83.

[122] Ekvall H, Arese P, Turrini F, et al. Acute haemolysis in childhood falciparum malaria. Trans R Soc Trop Med Hyg. 2001;95:611–617.
[123] Sumbele I, Nkuo-Akenji T, Samje M, et al. Haematological changes and recovery associated with treated and untreated P. falciparum infection in children in the Mount Cameroon Region. J Clin Med Res. 2010;2:143–151.
[124] Malisa AL, Pearce RJ, Abdulla S, et al. Drug coverage in treatment of malaria and the consequences for resistance evolution–evidence from the use of sulphadoxine/pyrimethamine. Malar J. 2010;9:190.
[125] Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. Bmj. 2004;329:1212.