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Full Length Research Paper

Temporal Dynamics of Malaria Incidence and Vector Diversity in Northern Namibia

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The prevalence of malaria parasite, *Plasmodium falciparum* and the abundance of malaria vector species were studied in selected areas of northern Namibia during the wet and the dry seasons. Female mosquitoes were collected using exit window traps. Blood smears were prepared and body temperatures recorded from suspected malaria patients in order to determine the prevalence of malaria parasites and confirm clinical malaria through microscopic examination. The study revealed that *Anopheles (Cellia) arabiensis* was the most common (84.4%) of the malaria vector species collected. Other vector species included *Anopheles funestus* (5.0%) and non-malaria vector species (10.6%). *Anopheles (Cellia) arabiensis* were more prevalent and significantly more abundant during the wet (85.1%) than the dry (14.9%) season. The opposite was true for *A. funestus* (35.1% in the wet and 64.9% in the dry season). There was no significant difference between the number of patients diagnosed with malaria and those with no malaria during the wet and the dry seasons (χ^2 = 0.55, *df* = 1, P>0.05). Furthermore, 72.1% of patients with fever tested negative for *P. falciparum* when clinical diagnosis was employed. Results reveal that diagnosis of malaria using fever is inaccurate compared to microscopic examination of blood smears. This leads to over-diagnosis of the prevalence of malaria and consequent wastage of malaria drugs on patients who do not have clinical malaria. The use of microscopic examination should therefore be promoted to improve diagnosis of malaria and hence facilitate treatment of patients with malaria.

Key words: *Anopheles*, clinical malaria, fever, malaria, seasonal variation, vectors, Namibia.

INTRODUCTION

Malaria, one of the most important parasitic diseases of man, is estimated to cause between 300 and 500 million cases of clinical malaria illness and about half a million deaths of children each year in Africa alone and an estimated 2.7 million deaths worldwide every year (WHO, 2003). WHO (1997) estimated that of all patients who die in hospitals in Africa, between 17% and 30% die from malaria. In Namibia, malaria is the major cause of outpatient consultations and admissions in health facilities in northern Regions where about one in four inpatient children are hospitalised (Obeid et al., 2001). In Namibia, malaria is the leading cause of illness and death amongst children under the age of five years and the third most important cause of death among adults, after HIV /AIDS and tuberculosis (MoHSS, 2002).

The distribution of malaria is strongly related to rainfall and availability of vector breeding habitats near perennial rivers and stagnant water (Obeid et al., 2001). High prevalence of malaria during rainy season coincides with periods when most farmers are busy with important agricultural activities such as ploughing, planting and growing of their crops (Malaney et al., 2004). Malaria therefore may indirectly contribute to poor crop yields and hence malnutrition. It also robs farmers of their much needed economic livelihoods from farming. Other economic costs of malaria arise from direct costs for treatment and prevention of malaria both at individual, national and global scales. In 2002, about US$ 200 million was earmarked for malaria control worldwide compared with an estimated US$ 60 million in 1998

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(WHO, 2003). In Namibia, the Ministry of Health and Social Services spent about US$781, 726 on chemicals for residual spraying to prevent malaria and on drugs to control it (Kamwi, 2005).

The primary vectors of malaria parasite, *Plasmodium falciparum,* in Africa are mosquitoes of the *Anopheles gambiae* complex (Toure et al., 1994). In southern Africa, these comprise of *Anopheles (Cellia) gambiae, Anopheles (Cellia) arabiensis, Anopheles funestus,*
Anopheles merus, *Anopheles quadriannulatus, Anopheles bwambae*, *Anopheles moucheti* and man-biting populations of *Anopheles nili* (Toure et al., 1994; Kiszewski et al., 2004). Knowledge of species identity and seasonal variation in their abundance and behaviour is very important in that it will facilitate determination of vectors involved in transmission of malaria, targeted control of malaria vectors and identification of vectors that are difficult to eradicate (Hay et al., 2010). For instance, for a vector that is predominantly an outdoor resting species and prefers biting animals such as *Anopheles (Cellia) arabiensis,* indoor residual spraying (IRS) of houses would not be an optimal control strategy (Shililu et al., 2004). In contrast, for a species such as *Anopheles (Celliia) gambiae* which feeds predominantly indoors and at night, use of insecticide-treated (mosquito) nets (ITN) or IRS would be more appropriate control interventions (Lengeler, 2004). For example, a recent historical review of malaria control in southern African countries including Botswana, Mozambique, Namibia, South Africa, Swaziland and Zimbabwe revealed that IRS mainly with Dichlorodiphenyltrichloroethane (DDT) is an effective control measure against malaria transmission (Mabaso et al., 2004) especially when supplemented by other interventions such as the use of ITNs and new drug therapies (artermisinin-based combinations).

A. funestus is one of the major malaria vectors in sub-Saharan Africa (Hargreaves et al., 2000). Although *A. funestus* was very common and abundant near Kavango and Zambezi Rivers in Namibia (De Meillon, 1951), it is believed to have been eradicated by the 75% DDT wetable powder indoor spraying program initiated from 1964 (Hansford, 1975). The extent of eradication of populations of *A. funestus* in northern Namibia has not been empirically documented hence is largely unknown.

In many parts of Namibia, most health facilities report high numbers of malaria cases even at the time of the year when cases are expected to be low (Kamwi, personal communication). Microscopic examination of malaria is not carried out at most health facilities and clinics in Namibia. Most malaria diagnosis is based on measured body temperatures or reported fever (body temperature >37.5°C) by patients. Failure to accurately diagnose malaria leads to administration of malaria treatment to patients that do not have malaria parasites. The need to substantiate the extent of correct diagnosis or misdiagnosis of malaria by investigating the relationship between the prevalence of malaria determined through microscopic examination of blood smears and malaria diagnosed through measured body temperature or reported fever cannot be over-emphasised.

This study was carried out to determine variation in the vector species responsible for malaria transmission in northern Namibia, to investigate seasonal variation in the prevalence of malaria and to compare the number of patients that are diagnosed to have clinical malaria or not, on the basis of fever and microscopic examination of blood smears.

MATERIALS AND METHODS

Study site

The study was conducted between July 1999 and June 2000 at Bukalo and Kalimbeza in Caprivi Region, Andara and Kangongo in Kavango Region and in Mahenene and Outapi in Omusati Region in northern Namibia, on the basis of demographic, epidemiological and climatic conditions (Figure 1). These sites represent various malaria endemic areas in northern Namibia (Service, 1993).

Malaria vector species and seasonal abundance

Female mosquitoes were collected from huts using Muirhead-Thomson design exit window traps (Muirhead-Thomson, 1947) that were placed in each hut. Once captured, mosquitoes were collected by means of an aspirator and stored in press tubes containing isopropanol. They were all counted and recorded.

Preparation of DNA, PCR analysis and species identification

Mosquitoes were identified to species level using the *A. gambiae* complex species-specific polymerase chain reaction (PCR) following the method of Scott et al. (1993) at the Medical Research Council in Durban, South Africa. Briefly, the following were carried out. The DNA was obtained from abdomens of female mosquitoes using a Qiagen DNA extraction kit. The DNA was analysed on a 1% agarose gel stained with ethidium bromide and the concentration was estimated with a λ DNA standard. Subsequently, the DNA samples were diluted to 10 ηg/µl and were used as templates in PCR amplification. The Go-Green *Taq* Master Mix (Promega) which contains all ingredients needed for DNA amplification (dNTPs, *Taq* polymerase, $MgCl_2$, 10× PCR buffer) was used. The following reaction mixture was prepared and used: Go-Green *Taq* Master Mix 12.5 µl, 5 µM dNTPs, 2 µl of UNFOR 1029 universal primer (5′-GTGTGCCCCTTCCTCGATGT-3′) and with combinations of 2 µl of the following primers GA (5'-CTGGTTTGGTCGGCACGTTT-3'), ME(5'-TGACCAACCCACTCCCTTGA-3'), AR (AGGTGTCCTTCTCCATCCTA-3') and QD(CAGACCAAGATGGTTAGTAT-3') (Scot et al., 1993), 4.5 µl sterile double distilled water and DNA template (10 ηg). The mixture was put in the PCR machine under the PCR amplification profile: an initial of 94°C for 4 min followed by 32 cycles at 94°C for 30 s; 30 s at 62°C, and 1 min at 72°C then a final extension step of 10 min at 72°C and holding at 4°C. PCR products (5%) were analysed on a 1% agarose gel stained with 0.5% ethidium bromide. The remainder of IPCR amplicons were purified and sequenced in an automated sequencer using the big-dye chemistry forward and reverse primers for each of the samples. The forward and reverse sequence of each isolate was aligned appropriately to obtain one complete sequence using BioEdit program. The resulting sequences were identical and only one of them was chosen for use

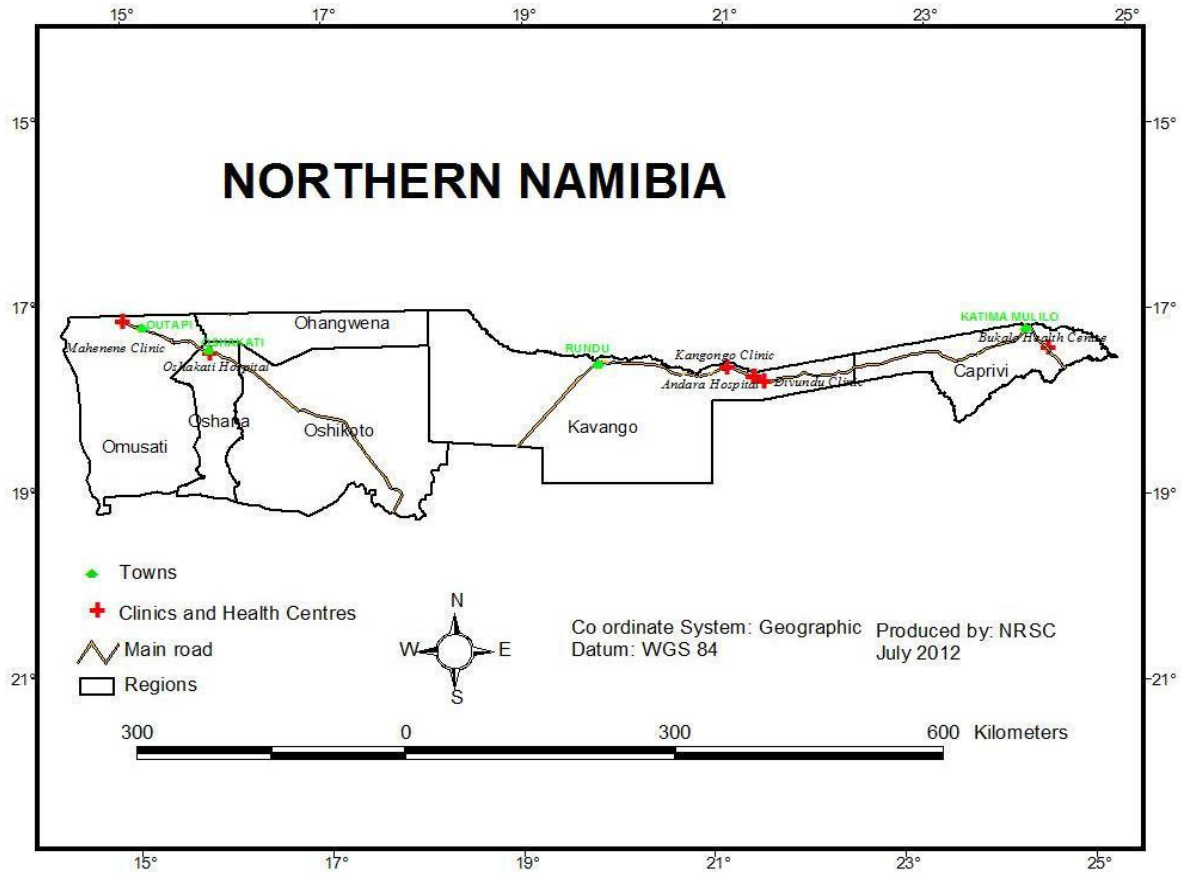

Figure 1. Map showing sampling sites in the northern Namibia. The sites included Outapi Hospital and Mahenene in Omusati Region, Kangongo and Andara in Kavango Region and Bukalo and Kalimbeza in Caprivi Region.

in BLAST searches at the NCBI Genbank in order to identify the species of the malaria vectors. *A. funestus* group were identified using morphological methods following Coetzee (1989). It is noted that more recently, a cocktail PCR assay to identify members of the *A. funestus* group has been developed (Koekemoer et al., 2002).

After species identification, the numbers of malaria vector mosquitoes captured from each hut were recorded appropriately. Non-malaria vector mosquitoes that were captured in exit window traps, for example, *Culex quinquefasciatus,* were excluded from further analysis.

Prevalence of malaria parasites and the relationship between fever and clinical malaria

Data for clinical malaria and fever were collected from patients around Bukalo health centre in Caprivi Region, Mahenene health centre and Outapi district hospital in Omusati Region and at Andara health centre and Kangongo health clinic in Kavango Region. Community and personal consent was sought from all patients that participated in the study. Each patient completed a questionnaire to obtain data including their bio-data. The body temperature of each patient was recorded using clinical thermometers to determine whether patients had fever (body temperature > 37.5°C) or not. Thick and thin blood smears were prepared for each patient following standard staining procedure. The prepared blood smears were examined for the presence or the absence of *P. falciparum*. The rationale of using both thin and thick blood smears to detect hemoparasites, as done for *P. falciparum* in the present study, was to maximise detection of the malaria parasites. Thin blood smear is only useful in acute stages when parasitemia is high and parasites can easily be detected in blood. However, in chronic stages when parasitemia is low, one needs a thick blood smear to increase chances of detecting parasites (WHO, 1991). A total of 4,346 thick and thin blood smears were prepared from suspected malaria patients.

A cross-sectional survey was conducted at Bukalo health centre in Caprivi Region and Mahenene health centre in Omusati Region in order to determine the prevalence of malaria parasites and to investigate the relationship between fevers and clinical malaria diagnosed via microscopic examination of blood smears. Blood was drawn from a total 1,390 randomly selected subjects that were recruited for the survey at the health centres. Thin and thick blood smears were prepared and later examined for malaria parasites. The body temperature (to the nearest °C) of each person whose blood sample was drawn, was recorded.

Data analysis

Chi-square (χ^2) tests were employed to determine significant differences in the abundance of *Anopheles (Cellia) arabiensis* during the wet and dry season, number of patients that had malaria during the dry and wet season and prevalence of malaria and the number of patients that had malaria or not determined through microscopic examination of blood smears and fever (body

Table 1. Proportion (%) of vector species in malaria transmission at different sampled sites in northern Namibia at which female mosquitoes were collected from exit window traps set in different huts. 'Other species' refers to mosquitoes other than *Anpheles (Cellia) arabiensis* and *A. funestus* that were not identified to species level and were non-malaria vector species.

Vector sampling site	Total number of mosquitoes collected	Percentage of *Anopheles arabiensis*	Percentage of *Anopheles funestus*	Percentage of other species
Kalimbeza	258	88.7	1.5	9.8
Andara	58	79.3	13.8	6.9
Outapi	62	75.8	6.5	17.7
Kangongo	19	68.4	21.1	10.5
Total	397	84.4	5.0	10.6

Table 2. Seasonal abundance of mosquitoes collected in Kalimbeza and Andara villages in northern Namibia between July 1999 and June 2000. A total of 444 mosquitoes were captured.

Season	Number of *Anopheles arabiensis*	Number of *Anopheles funestus*	Number of other species
Wet	297 (85.1)	13 (35.1)	31 (53.4)
Dry	52 (14.9)	24 (64.9)	27 (46.6)
Total	349 (78.6)	37 (8.3)	58 (13.1)

Figures in parentheses are percentages.

temperature > 37.5°C). Frequencies and percentages were calculated and tabulated for the following data; vector species in malaria transmission, seasonal abundance of vector species and the number of patients diagnosed to have clinical malaria or not on the basis of fever and microscopic examination of blood smears. Data analysis employed SPSS version 10.1 and Microsoft Excel.

RESULTS

Vector species in malaria transmission areas

A total of 397 female mosquitoes were collected from exit window traps (Kalimbeza, n=258, Kangongo, n=19, Andara, n=58 and Outapi, n=62). *A. arabiensis* was the most common malaria vector species at all the four sampled sites (Table 1). When data of mosquitoes from all sampled sites were combined, *Anopheles (Cellia) arabiensis* comprised of 84.4% of the number of vectors collected while *A. funestus* and other species of mosquitoes made up 5.0 and 10.6%, respectively.

The seasonal abundance of vector species

In order to investigate seasonal abundance of malaria vector species, a total of 444 mosquitoes were captured in Kalimbeza and Andara villages. Of the total, 349 were *Anopheles (Cellia) arabiensis* (78.6%), 37 were *A. funestus* (8.3%) and 58 (13.1%) belonged to other non-malaria vector species (Table 2). The present study revealed that there was a significant difference (χ^2 = 67.298, $p<0.05$, *df* = 2) in the abundance of vector species collected from Kalimbeza and Andara villages during the wet and the dry season (Table 2). Clearly, there were more *Anopheles (Cellia) arabiensis* during the wet (85.1%) than the dry (14.9%) season. The opposite was true for *A. funestus* species (64.9% in the dry season and 35.1% in the wet season). The abundance of other mosquito species did not differ significantly between the dry season (46.6%) and the wet (53.4%) season (Table 2).

Variation in prevalence of malaria

A total of 4,346 blood samples were collected during the malaria cross-sectional survey that was carried out in Bukalo health centre in Caprivi Region, Mahenene health centre and Outapi district hospital in Omusati Region and at Andara health centre and Kangongo health clinic in Kavango Region (Table 3). Chi-square (χ^2) test revealed that there was no significant difference (χ^2 = 0.555, df =1, p = 0.456) between the number of patients that had malaria (positive for *P. falciparum*) and those that did not have malaria (negative for *P. falciparum*) during the wet and the dry seasons (Table 3).

The relationship between fever and clinical malaria

The results of this study showed that 72.1% of patients that had fever (those that presented with body temperatures >37.5°C) tested negative for *P. falciparum*

Table 3. Number of study subjects who tested positive or negative for *P. falciparum* based on blood smears, during the malaria cross-sectional survey conducted Bukalo health centre in Caprivi Region, Mahenene health centre and Outapi district hospital in Omusati Region and at Andara health centre and Kangongo health clinic in Kavango Region.

Season	Total number tested	Number positive for *P. falciparum*	Number negative for *P. falciparum*
Wet	2,190	104 (4.7)	2,086 (95.3)
Dry	2,156	113 (5.2)	2,043 (94.8)
Total	4,346	217 (5.0)	4,129 (95.0)

Figures in parentheses are percentages.

Table 4. Number of patients that were diagnosed to have malaria or not*, on the basis of fever (Body temperature >37.5°C) and microscopic examination of blood smear at Bukalo and Mahenene Health Centres in northern Namibia.

Method of diagnosis of malaria	Number diagnosed to have malaria	Number diagnosed not to have malaria	Total number tested
Fever	1358 (97.7)	32 (2.3)	1390
Microscopic examination of blood smear	388 (27.9)	1002 (72.1)	1390

Figures in parentheses are percentages.*Note that body temperatures and blood smears were taken for all the 1390 patients. Comparison is made here to reveal how the use of fever (most commonly used method in many health centres and clinics in Namibia due to lack of microscopic examination facilities and qualified technicians) leads to misdiagnosis of clinical malaria.

when blood smears were examined microscopically (Table 4). There was a significant difference between having fever (body temperature > 37.5°C) and testing positive for *P. falciparum* (χ^2 = 0.138, *df* = 1, P<0.05) indicating that the majority of patients that presented with fever actually did not have clinical malaria based on microscopic examination of blood smears.

DISCUSSION

Vector species in malaria transmission areas

Female mosquitoes that were collected in the present study consisted of two main vector species, namely *Anopheles (Cellia) arabiensis* (84.4%) and *A. funestus* group (5.0%) (Table 1). Species included in the *A. funestus* group were *A. funestus*, *Anopheles vaneedeni* and *Anopheles rivulorum*. Some mosquitoes that were trapped, including *Culex quinquefasciatus,* were non-malaria vector species; these made up 10.6% of the total number captured. The high numbers of *Anopheles (Cellia) arabiensis* captured from exit window traps is not surprising. *Anopheles (cellia) arabiensis* is widespread in most African countries and it prefers drier savannah areas (Mnzava and Kilama, 1986). *Anopheles (Cellia) arabiensis* is the major malaria vector in Namibia (Hansford, 1975; Service, 1993). Although *A. funestus* is considered as the most important malaria vector after *Anopheles (Cellia) gambiae* and *Anopheles (cellia) arabiensis* (Gillies and Coetzee, 1987), comparatively few *A. funestus* were collected from exit window traps in Kalimbeza village (Table 1) in this study. This could be due to the effects of indoor house spraying of DDT against malaria vectors. The Ministry of Health and Social Services in Namibia has undertaken indoor house spraying in most malaria-prone areas in Namibia since the 1960s (Ministry of Health and Social Services (2002); Hansford, 1975). The efficacy of insecticides against malaria vectors varies among species. Sharp and le Sueur (1996) reported that *Anopheles (Cellia) gambiae* and *A. funestus* were eliminated in many affected provinces of South Africa in the 1950s with the exception of *Anopheles (cellia) arabiensis*. These eliminated species are probably highly susceptible to indoor house spraying with residual insecticides. It is also possible that some of the *A. funestus* collected at Kalimbeza in the present study came from nearby Zambia where insecticide spraying is not carried out. Kamwi (2005) reported higher abundance of *A. funestus* in samples collected in nearby Calueque, in southern Angola where spraying of residual insecticides against malaria vectors is not undertaken, than in Mahenene in Northern Namibia. Differences in the behaviour of malaria vector species could have contributed to the differential abundance of vectors in northern Namibia. Some vectors such as *A. funestus* rest indoors after a blood meal hence are highly likely to be susceptible to residual insecticides that are sprayed in-doors (Lengeler, 2004).

The abundance of mosquito vectors in Kalimbeza and Andara villages showed seasonal variation (Table 2). Significantly more *Anopheles (Cellia) arabiensis* were captured in the wet season (85.1%) than in the dry

season (14.9%) while more *A. funestus* were collected in the dry (64.9%) than in the wet (35.1%) season (Table 2). Seasonal changes such as temperature, rainfall and humidity affect anopheline populations and hence the incidence of malaria (Service, 1993). Although mosquitoes breed throughout the year, populations are low during the dry season because of the paucity of suitable habitats for breeding. Anopheline populations however increase dramatically from the onset of rains. The abundance of *Anopheles (cellia) arabiensis* is rainfall dependent (White, 1974). This may explain why more *Anopheles (cellia) arabiensis* were captured during the wet season. The low numbers of *A. funestus* in the wet season may be due to increased frequency of in-door spraying during the rainy season to control mosquitoes when they breed most. *A. funestus* may be more susceptible to insecticides than *Anopheles (Cellia) arabiensis*. Many anopheline species have developed DDT (Brown, 1986) and pyrethroid (N'Guessan et al., 2007) resistance. For instance, *Anopheles arabiensis* has developed Malathion resistance while Permethrin resistance of *Anopheles (cellia) arabiensis* and *Anopheles (cellia) gambiae* has been reported in laboratory studies (WHO, 1980).

Seasonal variation in prevalence of malaria

Although microscopic examination of blood smears from patients revealed that about 95.0% tested negative for malaria in the study area, the statistical outcome that there was no significant difference ($\chi^2 = 0.555$, *df* = 1, p=0.456) in the prevalence of *P. falciparum* in patients examined during the wet and dry season (Table 3) suggests that patients were equally likely to have malaria during the dry and the wet seasons. This is rather surprising as high prevalence of malaria is expected during the wet season when malaria vectors such as *Anopheles (Cellia) arabiensis* are most abundant (Table 2). Significant seasonal variation in the prevalence of malaria has been reported in West Sumba district, in Indonesia (Syafruddin et al., 2009). Gil et al. (2003) reported peak densities of malaria vectors that were coincident with incidences of malaria cases in two district endemic areas in Brazilian Amazonia.

Inspection of blood smears through microscopy revealed that the majority of positive *P. falciparum* during the dry season were in fact gametocytes. This demonstrated that gametocytes can remain in human blood of some individuals throughout the year. This may be an evolutionary survival strategy by the malaria parasites. Natural selection may have favoured those parasites that remain as gametocytes even during the dry season. This would be an adaptive strategy that facilitates survival and pick-up of malaria parasites by mosquitoes at the beginning of the transmission season when rains come (Bruce-Chwatt, 1988).

The relationship between fevers and clinical malaria

In Namibia, malaria diagnosis in most clinics and health centres is based on clinical symptoms of reported fever (body temperatures >37.5°C). Patients that present with fever are presumed to have malaria (Chandramohan et al., 2002). Often they receive malaria treatment without confirmation of the presence of the malaria parasite *P. falciparum.* Although more accurate and reliable diagnosis of clinical malaria is most commonly done through microscopic examination of blood smears from patients suspected to have malaria, such examinations however are not conducted at most clinics and health centres in Namibia due to a variety of factors. Microscopic examination of blood smears are not done because either there are no laboratory facilities to carry out examinations of blood smears or there are very few or no trained personnel at most health centres to carry out these examinations. The other problem is that many patients especially in rural areas of Namibia, travel long distances to the clinic or health centres and since they have to return home the same day, it is sometimes not possible to complete microscopic examination of blood smears that same day due to inadequate laboratory staff. Doctors and health personnel therefore resort to the use of fever as an indicator of malaria. The extent to which this is accurate to diagnose malaria is largely unknown.

In the present study, comparison of diagnosis of malaria using fever and results of microscopic examination of blood smears for malaria parasite was investigated at Bukalo health centre in Caprivi Region and Mahenene health centre in Omusati Region. Results revealed that the majority of patients that were diagnosed to have malaria using high fever (Body temperature >37.5°C) as a method of diagnosis, tested negative for *P. falciparum* after their blood smears were examined microscopically (Table 4). This implies that high fever in patients does not necessarily indicate presence of malaria. Therefore the use of fever leads to mis-diagnosis of patients and wastage of malaria drugs as well as financial resources. The economic importance of the results of the present study is that the current trend by the Ministry of Health and Social Services to prescribe the more expensive combination therapies (Artemisinin-based Combination Therapies, ACTs) for treatment of malaria due to chloroquine and sulfadoxine pyremethamine (SP) failure will escalate the cost of treating malaria if mis-diagnosis is continued. It is suggested that the Ministry of Health and Social Services should invest in laboratory facilities and training of technicians to enable accurate diagnosis of malaria. The WHO currently restricts treatment based on clinical symptoms to children under the age of five years only in the absence of microscopy and/or rapid diagnostic tests (RDTs) due to current treatment policies with expensive ACTs (International Artemisinin Study Group, 2004).

In conclusion, the study has revealed the following:

Anopheles (cellia) arabiensis is the most important malaria vector in the areas studied in northern Namibia. Both *Anopheles (cellia) arabiensis* and *A. funestus* showed seasonal variation in abundance. *Anopheles (Cellia) arabiensis* was significantly more abundant during the wet season (85.1%) than during the dry season (14.9%), while the opposite was true for *A. funestus* (35.1% during the wet season and 64.9% in the dry season). Despite the high abundance of *Anopheles (Cellia) arabiensis* during the wet season, there was no significant difference in the prevalence of malaria (*P. falciparum*) between the wet and the dry seasons indicating that individuals carry malaria parasites in their blood even during the dry season. Microscopic examination of blood smears is more reliable to detect clinical malaria in patients than to rely on the use of fever (patients presenting high body temperatures >37.5°C) as an indicator of malaria in patients.

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