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Malariological baseline survey and in vitro antimalarial drug resistance in Gulu district, Northern Uganda

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Malariologische Basisuntersuchung und in vitro Resistenzprüfung im Distrikt Gulu, Nord-Uganda

Zusammenfassung. Eine umfassende, repräsentative Basisuntersuchung über Malaria wurde in einer Flüchtlingspopulation im Distrikt Gulu im Norden Ugandas durchgeführt. Die Studie schloss 74 Haushalte und 390 Personen ein. Die erfassten Parameter erstreckten sich auf sozio-ökonomische Information, Umwelt, individuelle physische Daten, Malaria und die Arzneimittelempfindlichkeit von *Plasmodium falciparum*. Die Prävalenz von Infektionen mit *Plasmodium falciparum* betrug 54.4% mit einer mittleren asexuellen Parasitämie von 229/µl Blut, ein Hinweis auf hyperendemischen Status. *P. falciparum* zeigte hochgradige Resistenz gegen Chloroquin und Amodiaquin und bereits reduzierte Sensibilität gegenüber Lumefantrin und Artemisinin, offensichtlich das Resultat freizügigen Einsatzes der Lumefantrin-Artemether Kombination ohne die Basis evidenzgesteuerter Indikation.

Summary. A comprehensive, representative malaria survey has been carried out in a population of internally displaced persons (IDP) in the district of Gulu, Northern Uganda. It included 74 households and 390 persons, and covered socio-economic and environmental information, individual physical data, malaria and the drug sensitivity of *Plasmodium falciparum*. The prevalence of infections with *Plasmodium falciparum* was 54.4% at a geometric mean asexual parasitaemia of 229/µl blood, typical for hyperendemic conditions. *P. falciparum* turned out to be highly resistant to chloroquine and amodiaquine. It showed also reduced sensitivity against lumefantrine and artemisinin, obviously the result of the liberal use of the lumefantrine-artemether combination without evidence-based indication.

Key words: Malaria, prevalence, *Plasmodium falciparum*, drug sensitivity, Uganda.

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Introduction

Nearly two decades of conflict in Northern Uganda have resulted in the displacement of up to 2.3 million persons [1]. In the Acholi region with the districts Gulu, Kitgum, and Pader, 90% of the population relocated in camps as internally displaced persons (IDPs) [2]. Camps accomodating between 400 and 60,000 people are characterised by overcrowded living conditions with increased vulnerability and exposure to risk factors for malaria [2]. Since IDPs suffered long isolation and poor access to medical care the government of Uganda called for the assessment of basic health indicators in the IDP population. In 2005, the Ministry of Health and the World Health Organisation (WHO) conducted a representative survey of all IDP camps in Gulu, Kitgum, and Pader districts. In Gulu district, with an estimated IDP population of 462,580, self-reported cause of death due to malaria/fever accounted for 25.3% in all age groups and for 47.5% in children under 5 years [3]. The high burden of malaria is also reflected by hospital data from the region. A retrospective analysis of discharge records concerning 70,304 in-patients admitted to St. Mary's Lacor Hospital in Gulu municipality between 1992 and 1997 revealed malaria as the most frequent cause of admission and the second leading cause of death in hospital [4]. Disease incidence and treatment outcome of malaria depend, in part, on the endemicity level in the region [5]. Therefore, classification of the malaria endemicity is an essential basis for planning antimalarial measures based upon the epidemiological characteristics. Malariometric surveys in Uganda were started in 1964 in the course of the malaria pre-eradication programme [6]. However, there are no recent data pub-

lished on the intensity of malaria transmission in Northern Uganda.

In 2000 the first line malaria treatment regimen in Uganda changed from chloroquine (CQ) monotherapy to CQ plus sulfadoxine/pyrimethamine (SP). Since 2002, Uganda has implemented the Home Based Management of Fever (HBMF) program with CQ/SP. As part of the HBMF program pre-packaged, age specific, malaria treatment kits are distributed to caregivers of young children with instructions on proper use. A survey conducted in 2005 among IDPs in Gulu district showed a caretakers' adherence to pre-packaged antimalarial treatment of 96.3% [7]. As resistance to CQ/SP continued to rise, Uganda changed its first line treatment policy at the health facility level to combination therapy with artemether and lumefantrine (AL). Furthermore, since 2006 HBMF with AL has been implemented in the northern districts Gulu, Pader, Kitgum, and Kotido [8]. Monitoring the response of *Plasmodium falciparum* to antimalarial drugs is essential in the development and evaluation of malaria drug policies. However, data are lacking on the current drug sensitivity level of *P. falciparum* in the region.

Assessment of *P. falciparum* endemicity and resistance patterns represents the basis for programmatic decisions about public health interventions and antimalarial treatment regimens in the region. The present study had the objective of determining the current *P. falciparum* prevalence and in vitro antimalarial drug sensitivity status in a representative sample of the IDP population in Gulu district.

Materials and methods

A household survey was conducted in Amuru IDP camp situated 30 km from Gulu municipality. Amuru IDP camp has a population of 24,254 people and is divided into 7 clearly demarcated zones (July 2007, Norwegian Refugee Council, personal communication). The time of the study, a 4 weeks' period in September 2007, coincided with the peak of the rainy season. We selected households from the 7 camp zones with a probability proportional to size, using the Expanded Programme on Immunisation (EPI) methodology [9]. Ethical approval was obtained in advance of the study from St. Mary's Lacor Hospital and Vienna Medical University.

Interview and examination

Sampled households were visited for a maximum of 3 times on consecutive days for inclusion into the study. Trained interviewers conducted standardized interviews using the Household Questionnaire of the Malaria Indicator Survey (MIS) from the WHO Roll Back Malaria Monitoring and Evaluation Reference Group [10], assessing personal and demographic details, socioeconomic factors and risk factors for malaria of household members and visitors. All individuals listed in the Household Questionnaires were invited to participate in the study. Standardised examinations were conducted in individuals having provided written informed consent. Measurement of axillary body temperature was performed in degree Celsius. Height and weight were measured in patients wearing light indoor clothes and no shoes. Spleen size was palpated from the right lower to the left upper abdomen and left rib bow and quantified according to Hackett's classification (grade 1–5) [11]. Venous blood samples were drawn for the assessment of haemoglobin, haematocrit, and automated white blood count. Trained microscopists performed thick film examinations and parasite counts for the assessment of *P. falciparum* asexual parasite density per microliter (µl) blood. Underweight was defined as body mass index (BMI) < 18.5 kg/m^2 at age > 19 years and as BMI for age (weight for age) < 3. percentile at age > 5 – ≤ 19 (≤ 5) years [12, 13]. Spleen enlargement was defined as any grade of palpable spleen (Hackett's classification grade 2–5) [11]. Anaemia was defined as haemoglobin measurement below the lower limits of normal values for age and sex [14]. Leukocytosis was defined as leukocyte count above the normal age and sex specific range [14].

In vitro tests

Blood samples with > 1000 asexual *Plasmodium falciparum* parasites per µl blood from persons without antimalarial treatment within the time-frame of therapeutic efficacy of administered drugs were eligible for resistance testing. Overall, in vitro tests were carried out with 30 fresh isolates of *Plasmodium falciparum*, following the protocol of the WHO Standard Microtest Mark II (MAP/87.2) and measuring the inhibition of schizont maturation. Parallel tests were carried out with chloroquine,

Table 1. Characteristics of included households (N = 74)

Characteristic	
Number of individuals per household, mean (SD)	7.2 (3.7)
Main source of drinking water	
Tube well or borehole, N (%)	57 (77.0)
Dug well-protected well, N (%)	3 (4.1)
Dug well-unprotected well, N (%)	6 (8.1)
Water from spring-protected spring, N (%)	5 (6.8)
Water from spring-unprotected spring, N (%)	2 (2.7)
Piped water/public tap/standpipe, N (%)	1 (1.4)
Kind of toilet facilities used	
Pit Latrine without slab/open pit, N (%)	50 (67.6)
Pit Latrine with slab, N (%)	23 (31.1)
No facility/bush/field, N (%)	1 (1.4)
Ownership of	
Electricity, TV, Refrigerator, N (%)	0 (0)
Radio, N (%)	29 (39.2)
Telephone, N (%)	5 (6.8)
Motorcycle/scooter, car/truck, N (%)	0 (0)[a]
Bicycle, N (%)	24 (32.4)
Main material of the floor	
Earth/sand, N (%)	40 (54.1)
Dung, N (%)	34 (46.0)
Type of fuel mainly used for cooking	
Firewood/straw, N (%)	70 (94.6)
Charcoal, N (%)	4 (5.4)
Indoor spraying against mosquitoes in past 12 months, N (%)	0 (0)[b]
Ownership of at least one mosquito net, N (%)	29 (39.2)
Number of mosquito nets per households, mean (SD)	0.5 (0.7)

[a] 1 missing value, [b] 2 missing values.

Table 2. *Plasmodium falciparum* parasitaemia in study participants at the time of examination by age groups (N = 390)

Parasitaemia	Total	Age groups			p-value
	N = 390	< 5 N = 99	5–15 N = 160	> 15 N = 131	
%	54.4	54.6	62.5	44.3	0.01
n	212	54	100	58	
μl^{-1}, geom. mean*	229.0	228.5	299.3	144.7	0.03

n Number of individuals with parasitaemia; * in individuals with parasitaemia.

amodiaquine, artemisinin, lumefantrine, monodesbutyl-benflumetol, and quinine. For the test, 0.3 ml of heparinized blood was diluted in 2.7 ml RPMI-1640 medium, complete with HEPES and sodium bicarbonate. Aliquots of 50 µl blood-medium-mixture (BMM) were added to the scheduled wells of the test plates (Falcon 3070, BD). After closing the test plates with a lid (Falcon 3071, BD), the plates were inserted in a candle box that was tightly closed after lighting the candle. The candle container was then placed in an incubator set at 37.5°C and held there for 23.5 hours. After removing the supernatant fluid from the test wells, thick films were prepared from the sediments, placing the 8 films of the test series in a set order on the slide. After thorough drying the slides were stained at pH 6.85 with a 3% dilution of a commercial Giemsa stock solution. The test slides were read by classifying ≥200 asexual parasites into trophozoites and schizonts (parasites with ≥3 chromatin bodies) in each film.

Statistics

Descriptive statistics were used to report characteristics of households and household members/ visitors included in the study. Prevalence of *P. falciparum* parasitaemia and parasite density per µl blood were compared between age groups. Demographic and clinical characteristics at the time of examination were compared between study participants with and without *P. falciparum* parasitaemia. The t-test, analysis of variance, and median-test were used for differences in continuous variables and the χ^2-test for those in proportions. Analyses were performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Since the response of *Plasmodium falciparum* to the test drugs is log-concentration normal, the in vitro test analysis followed the method of Litchfield and Wilcoxon [15], using an electronic version [16].

Results

Overall, 74 households agreed to participate in the study and completed the interview following the Household Questionnaire of the MIS. Main characteristics of included households are presented in Table 1. Socio-economic status is approximated by household item ownership, main source of drinking water, main material of the floor, and type of fuel mainly used for cooking. Mosquito net ownership was reported by 39.2% of households and the mean number of mosquito nets per household was 0.5 (SD 0.7). Within the interviewed households a total of 531 individuals were identified. Overall, 390 individuals agreed to participate in the study corresponding to a response rate of 73.4%. *Plasmodium falciparum* parasitaemia was found in 54.4% (95% CI: 49.4–59.3) of study participants, with a geometric mean asexual parasite density per µl blood of 229.0 (Table 2). Significant differences were observed in the *P. falciparum* prevalence and parasite density between age groups with the highest parasite prevalence (62.5%) and density (299.3 per µl blood) in the age group of 5–15 years. Demographic and clinical charac-

Table 3. Demographic and clinical characteristics of study participants at the time of examination by *Plasmodium falciparum* parasitaemia

Characteristic	Total N = 390	Parasitaemia N = 178	No parasitaemia N = 212	p-value
Age, mean (SD), y	15.1 (14.7)	13.5 (13.3)	16.9 (16.0)	0.02
Age , median (IQR), y	10.2 (16.0)	10.2 (11.0)	11.7 (21.0)	0.31
Age < 5, %	25.4	25.5	25.3	1.00
Women, %	50.3	49.1	51.7	0.61
Women 15–49, %	17.4	12.7	23.0	0.01
Body temperature (BT), °C				
BT, mean (SD)	36.1 (0.7)	36.1 (0.6)	36.1 (0.7)	0.70[a]
BT ≥ 37.5, %	2.1	2.4	1.7	0.73[a]
Underweight, %	9.2	9.0	9.6	0.86
Spleen enlargement, %	28.5	31.1	25.3	0.21[b]
Anaemia, %	23.6	25.5	21.3	0.38[c]
Leukocytosis, %	6.7	6.6	6.7	1.00[c]

Underweight: age > 19: BMI < 18.5 kgm^2, age > 5 – ≤19: BMI for age < 3. percentile for age and sex, age ≤ 5: weight for age < 3. percentile for age and sex; Spleen enlargement: Hackett's classification grade 2–5; Anaemia: haemoglobin below the lower limits of normal values for age and sex; Leukocytosis: leukocyte count above the normal range for age and sex; [a]1 missing value; [b]4 missing values; [c]30 missing values.

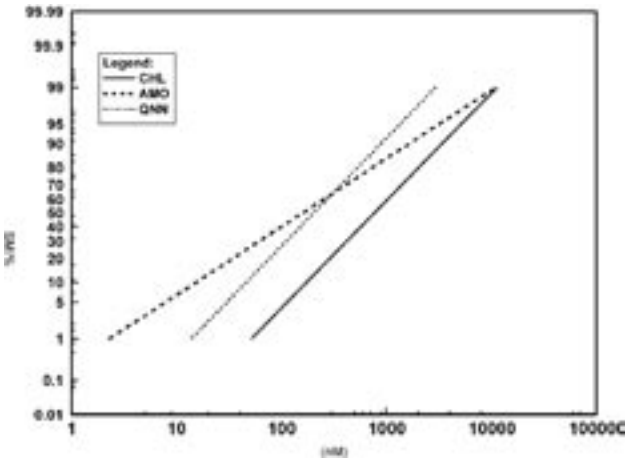

Fig. 1. Log-probit regressions of the response of *Plasmodium falciparum* to chloroquine (*CHL*), amodiaquine (*AMO*) and quinine (*QNN*). Gulu District, Uganda, 2007

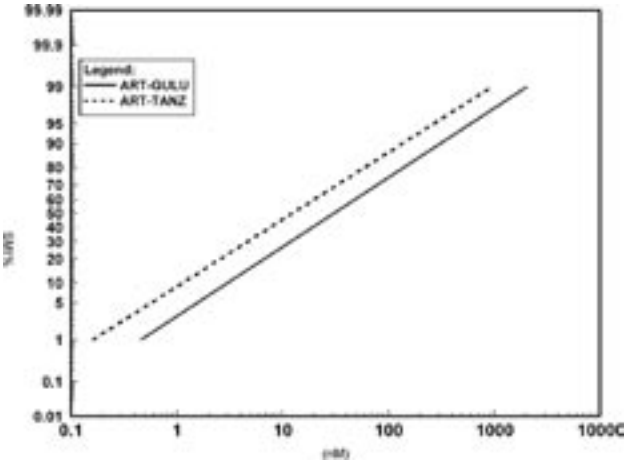

Fig. 3. Log-probit regressions of the response of *Plasmodium falciparum* to artemisinin in Gulu District (*GULU*) 2007 and in Tanzania (*TANZ*) 1994, baseline

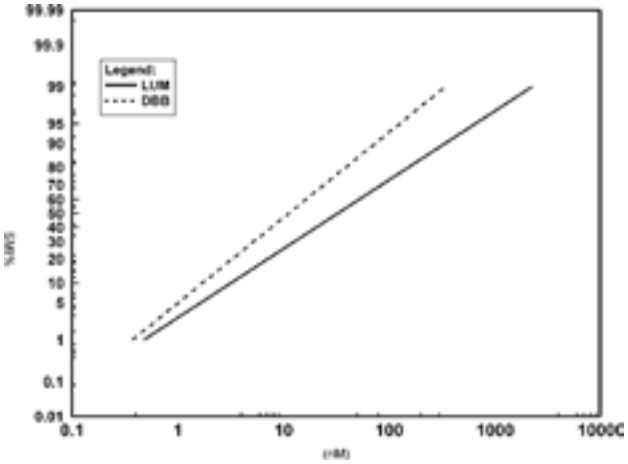

Fig. 2. Log-probit regressions of the response of *Plasmodium falciparum* to lumefantrine (*LUM*) and monodesbutyl-benflumetol (*DBB*). Gulu District, Uganda, 2007

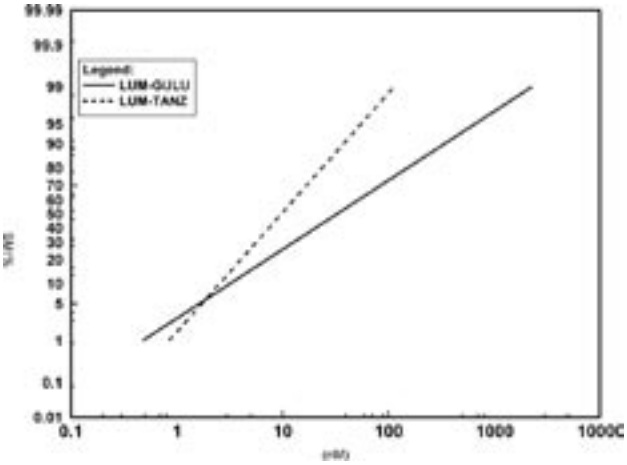

Fig. 4. Log-probit regressions of the response of *Plasmodium falciparum* to lumefantrine in Gulu District (*GULU*) 2007 and in Tanzania (*TANZ*) 1994, baseline

teristics of study participants at the time of examination stratified by *P. falciparum* parasitaemia are presented in Table 3. Significant differences were found in mean age and proportion of women aged 15–49 when compared between study participants with and without parasitaemia.

The results of in vitro tests are illustrated in Figs. 1–5. It is quite evident that resistance to chloroquine and amodiaquine has reached a very high degree, making treatment with these drugs obsolescent. With an EC90 level of 338.79 nM for lumefantrine the sensitivity of local *P. falciparum* is significantly below the baseline of 46.98 nM for northern Tanzania (Fig. 3) [17], obviously the result of heavy drug pressure. Similarly, EC90 and EC99 values for artemisinin of 311.50 nM and 2059.49 nM are far above the levels seen in Thailand although artemisinin derivatives are used there since almost 2 decades, albeit under conditions of very strict control [18] and also well above the baseline sensitivity observed in Tanzania (Fig. 4) [19]. Surprisingly, the sensitivity to quinine seems to have suffered least as the crucial ECs are still within the clinically feasible range.

Discussion

The report reflects the outcome of a malariologic baseline survey and antimalarial drug sensitivity study conducted in Amuru IDP camp in Gulu district, Northern Uganda. Core indicators obtained from the Household Questionnaire delineate a population with limited socio-economic resources and inadequate preventive measures for malaria. The prevalence of *Plasmodium falciparum* parasitaemia in our study population was high. In vitro tests on 30 isolates of *P. falciparum* showed inadequate pharmacologic response to chloroquine

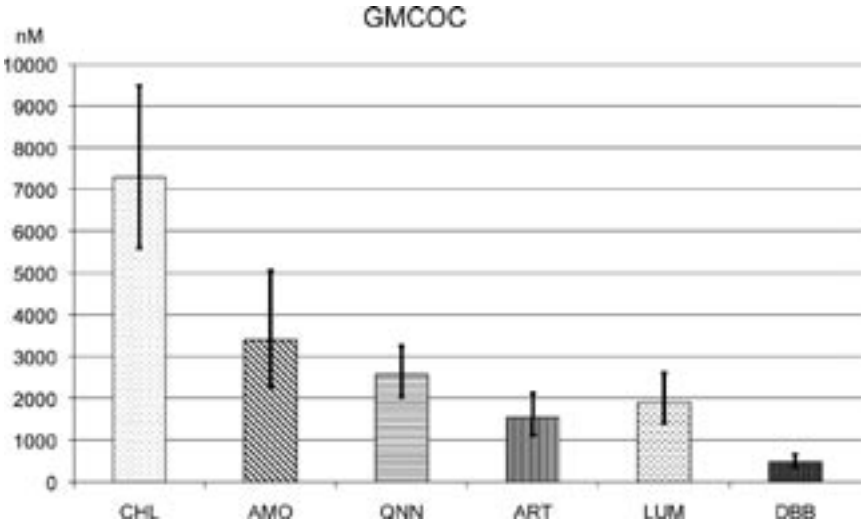

Fig. 5. Geometric mean cut-off concentrations (*GMCOC*) for complete inhibition of schizont maturation in *Plasmodium falciparum* in Gulu District, Uganda, 2007. *AMO* amodiaquine; *CHL* chloroquine; *ART* artemisinin; *LUM* lumefantrine; *DBB* monodesbutyl-benflumetol; *QNN* quinine

and amodiaquine, and decreased sensitivity to artemisinin and lumefantrine.

The results of our study are consistent with findings of previous studies from the same region. Demographic characteristics of households in our sample are in line with regional data from the entire Gulu district [3]. With regard to the prevalence of *P. falciparum* parasitaemia the results of our study are concordant with malariologic surveys undertaken in Northern Uganda in 1966. Onori reported for the Acholi region an overall malaria parasite prevalence of 72.4% and a *P. falciparum* prevalence of 59.7% [6]. Thus, our findings suggest that the endemicity of *P. falciparum* in the region persisted at a high level over the last decades. With regard to the reduced level of in vitro sensitivity to artemisinin and lumefantrin our results might in part be explained by the use of artemisinin-based combination therapy (ACT) in the community without microscopically confirmed diagnosis of malaria.

Our study has a number of limitations. First, small number of households included in the study may limit the validity of indicators obtained at the household level. Second, the results of our study do not reflect seasonal variations of transmission intensity. Third, palpatory assessment of spleen size is subject to observer-dependent variability. However, trained physicians palpated and quantified spleen size using standardised methods in all study participants.

To conclude, the present study provides baseline information for decisions on public health interventions and antimalarial treatment regimens for the IDP population in the study region. The observed high endemicity and reduced sensitivity of *P. falciparum* warrant a continued monitoring of resistance pattern in the region. In the absence of the introduction and strict observance of evidence-based therapy of falciparum malaria it appears to be likely that the efficacy of the currently used combination of artemether and lumefantrine will soon be lost in the study area (and beyond), and with it probably the efficacy of any form of ACT.

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