Diploma thesis

Mosquito net usage for malaria prevention among secondary school students in east central Uganda

Submitted by

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Declaration of Originality

The undersigned certifies that, to the best of his knowledge, the following diploma thesis has been written only by the undersigned and without any assistance of third parties. Furthermore, the undersigned confirms that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

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1 Abstract

1.1 English

Background: In 2010, Malaria was responsible for 219 million cases (154 to 289 million) and an estimated 660,000 deaths (610,000 to 971,000) according to WHO data. The highest mortality rate remains in Sub-Saharan Africa with insecticidal mosquito nets being the most important vector control intervention next to indoor residual spraying for endemic regions.

Objectives: To investigate mosquito net usage among secondary school students in east central Uganda, a region with high, year-round malaria transmission. To analyze differences in mosquito net usage according to socioeconomic background, level of knowledge about malaria, health care seeking behavior, sex, and additional factors that might influence mosquito net usage.

Methods: 286 students were invited to fill out a questionnaire. Information about socioeconomic background, level of knowledge about malaria, health care seeking behavior, mosquito net usage, frequency of net use, reasons for the lack of a net, and malaria morbidity in the last month was collected.

Results: 258 students met the inclusion criteria. More than half of the students (57.4%) did not sleep under a mosquito net. Mosquito net usage was highest among students who lived in brick houses (49.5%), went to school by public transport (77.8%), routinely visited the local health center for malaria treatment (55.1%), did not report malaria morbidity in the last month (75.7%), and were female (47.1%). Mosquito net usage was lowest among students who lived in mud-walled houses (11.4%), usually walked to school (37.1%), did not follow a specific management plan when sick of malaria (8.3%), had suffered from malaria in the last month (29.1%), and were male (38.6%).

Conclusion: Mosquito net usage among students attending a secondary school in east central Uganda was low, falls short of the recommended national and global levels of usage, and needs a net distribution campaign to meet targets of universal coverage by 2015.

1.2 German

Hintergrund: Nach Schätzungen der WHO verursachte Malaria 219 Mio. Krankheitsfälle (154 bis 289 Mio.) und 660,000 Todesfälle (610,000 bis 971,000) im Jahr 2010. Die mit Abstand höchste Mortalitätsrate wird in Afrika südlich der Sahara beobachtet. Insektizid-haltige Moskitonetze sowie das Sprühen von Insektiziden an Wände in Wohnräumen bleiben nach wie vor die effektivsten Interventionen zur Vektorkontrolle in endemischen Regionen.
Ziele: In dieser Studie wurde die Verwendung von Moskitonetzen in einem Kollektiv von SchülerInnen einer Mittelschule in zentral-ost Uganda, einer Region mit hoher, ganzjähriger Malariarate, untersucht. Unterschiede in der Verwendung von Moskitonetzen in Hinblick auf den sozioökonomischen Hintergrund, Wissensstand über Malaria, Therapiewahl, Geschlecht und weitere Faktoren wurden analysiert.
Methoden: 286 SchülerInnen wurden eingeladen, an der Umfrage teilzunehmen und einen Fragebogen auszufüllen. Sozioökonomischer Hintergrund, Wissensstand über Malaria, Therapiewahl, Geschlecht, Verwendung von Moskitonetzen, Häufigkeit der Anwendung, Ursachen für eine Nicht-Anwendung, und Malariamorbidität im letzten Monat wurden evaluiert.
Ergebnisse: 258 SchülerInnen erfüllten die Einschlusskriterien. Mehr als die Hälfte der SchülerInnen (57.4%) verwendete kein Moskitonetz. Die Verwendung von Moskitonetzen war bei den SchülerInnen am höchsten, die in festen Ziegelhäusern wohnten (49.5%), öffentliche Verkehrsmittel zur Schule benutzten (77.8%), Malaria im Krankenhaus behandeln ließen (55.1%), im letzten Monat nach eigenen Angaben keine Malaria hatten (75.7%) und weiblich waren (47.1%). Am geringsten war die Verwendung bei den SchülerInnen, die in Lehmhütten wohnten (11.4%), zu Fuß zur Schule gingen (37.1%), keine gezielte Malariatherapie verfolgten (8.3%), im letzten Monat nach eigenen Angaben Malaria hatten (29.1%) und männlich waren (38.6%).
Schlussfolgerungen: Die Verwendung von Moskitonetzen von SchülerInnen einer ugandischen Mittelschule ist gering, weit unter den globalen und nationalen Empfehlungen. Zur Erreichung der WHO-Zielwerte 2015 ist eine Moskitonetz-Kampagne dringend erforderlich.

2 Introduction

Malaria is a mosquito-borne, parasitic disease caused by the protozoon *Plasmodium (P.)* and is transmitted by female *Anopheles (An.)* mosquitoes. Depending on the *P.* species (spp.) malaria is a potentially lethal disease, especially for children under five years of age and pregnant women. The biggest global malaria burden remains in sub-Saharan Africa (WHO 2012a).
The World Health Organization estimates that for the year of 2010 malaria accounted for 219 million cases (uncertainty range between 154 to 289 million) and an estimated 660,000 deaths (610,000 to 971,000) (WHO 2011). Additional data show that malaria was responsible for approximately 7% of post-neonatal child deaths globally and 15% of post-neonatal child deaths in Africa in 2010 (Liu *et al*. 2012).
Malaria is currently endemic in 104 countries and territories (WHO 2012a) and according to the Centers for Disease Control and Prevention (CDC) 3.3 billion people live in areas where malaria transmission can occur (CDC 2012). In 2010, an estimated 91% of deaths occurred in Africa, 6% in Southeast Asia, and 3% in the Mediterranean and approximately 86% of global malaria deaths were among children (CDC 2012). Malaria remains a major public health challenge to date.

2.1 Etiology

Malaria belongs to the protozoan infections with *P*. being its pathogen. To date, 120 *P.* spp. are identified that can infect birds, reptiles and mammals (Hoffman *et al*. 2011), but only five species are known to infect humans. These are
1) *P. falciparum*
2) *P. vivax*
3) *P. ovale*

4) *P. malariae*

5) *P. knowlesi*

P. falciparum has the highest mortality rate among the five species and currently accounts for more deaths in children <5 years of age than any other infectious agent (Hoffman *et al*. 2011). *P. falciparum* is located worldwide in subtropical and tropical regions, it is the main species in Africa and responsible for the majority of cases of severe malaria and deaths related to the disease (WHO 2012a). CDC estimates that *P. falciparum* is responsible for about 1 million deaths annually, most of them in Africa (CDC 2010a).

P. vivax is the main species outside Africa, spreading across Asia, the Middle East, Central and South America, and some parts of Africa, and has very similar morphologic and biologic characteristics as *P. ovale* (CDC 2010a). Together with *P. ovale,* which is found predominantly in West Africa and on islands in the Pacific, it can develop dormant liver stages (hypnozoites), which can lead to relapse after symptom-free periods of months up to several years (Taylor *et al.* 2012). Generally, *P. vivax* and *ovale* infections have a less severe manifestation of disease than *P. falciparum* infections. One difference between the two species is that *P. vivax* cannot invade red blood cells which do not have the Duffy blood group antigen because it needs the surface antigen to attach to and invade the erythrocyte (Hoffman *et al*. 2011).

P. malariae is distributed worldwide but mainly found in small, circumscribed areas and, like *P. vivax* and *ovale* infections, usually leads to uncomplicated malaria. It is the only malaria parasite with a quartan cycle (characteristic symptoms on days 1 and 4) in contrast to *P. falciparum*, *P. vivax* and *P. ovale*, which have tertian cycles (Hoffman *et al*. 2011).

P. knowlesi is a simian malaria parasite that in most cases chooses macaques as its host, but infection of humans rarely occurs in Southeast Asia, especially in Malaysia, in areas where populations live in proximity to macaques (Lee *et al.* 2013, Singh B *et al.* 2004). *P. knowlesi* has the shortest erythrocytic replication cycle of all malaria species and consequently can progress relatively quickly from uncomplicated to complicated malaria and it is potentially lethal (Cox-Singh *et al.* 2010).

2.2 Life Cycle

When a female *An.* mosquito takes blood from an individual that harbors one of the five malaria parasites known to infect humans, the sexual forms of the *Plasmodia*, female and male gametocytes are ingested by the mosquito (Fig. 1). Absorbed with the blood meal, these gametocytes form gametes which fuse to a diploid zygote and develop to an ookinete. The motile ookinete migrates through the mosquito's gastric mucosa and forms an oocyst that undergoes meiotic division and develops haploid sporozoites. The sporozoites are taken away by the circulatory flow in the gastric wall and migrate to the mosquito's salivary glands, which takes about 1 to 3 weeks (Hoffman *et al.* 2011).

When the infected *An.* mosquito bites a human, the sporozoites are injected with the mosquito's saliva into the skin and they migrate via bloodstream to the liver of the host and within half an hour (*P. falciparum*) to an hour (*P. vivax*) they have penetrated parenchymal hepatocytes after passing Kupffer-Cells (Taylor *et al.* 2012). In the hepatocytes they begin asexual reproduction and form thousands of merozoites and a liver meront/schizont develops. The period from inoculation of the sporozoites to the completed formation of liver or exo-erythrocytic schizonts varies among the different *P.* spp and the host remains asymptomatic during this time (Taylor *et al.* 2012). The schizonts/meronts break open once mature and each schizont releases thousands of merozoites into the blood stream. *P. falciparum* has the quickest intrahepatic development and needs a minimum of 5.5 days from injection of sporozoites to rupture of the mature liver schizonts, releasing approximately 10 000 to 40 000 merozoites (Hoffman *et al.* 2011). This timeframe is called the pre-patent period and is defined as time from injection of sporozoites into the skin until asexual merozoites can be detected in the bloodstream by microscopy or other diagnostics (Taylor *et al.* 2012).

P. vivax and *ovale* schizonts burst open between 6 and 9 days, but these two species have the potential of forming hibernating liver stages, which do not rupture at the time of acquired infection but remain dormant for a period of a few months up to five years and can cause relapse (Taylor *et al*. 2012, Hoffman *et al*. 2011).

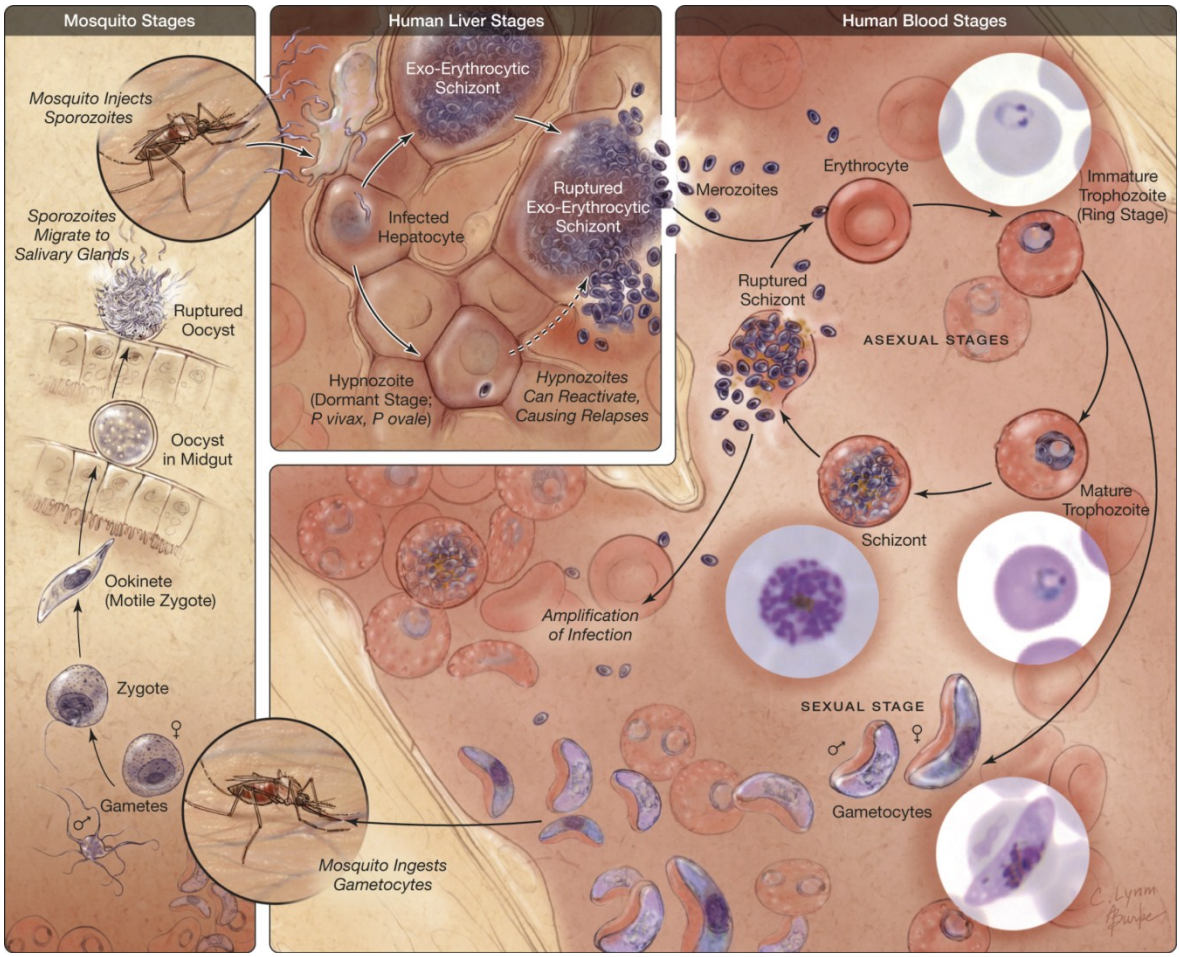


Fig. 1. Life cycle of *P*. spp. (from: Griffith KS *et al*. Treatment of malaria in the United States: a systematic review. JAMA. 2007 May 23;297(20):2264-77. PubMed PMID: 17519416.)

Once the hepatocytes burst apart and set merozoites free into the blood stream, the erythrocytic cycle begins and merozoites stick to red blood cells (RBCs) and invade them. However, merozoites of different malaria species show a certain erythrocyte preference*. P. ovale* only invade reticulocytes, *P. vivax* invades reticulocytes and erythrocytes of up to two weeks old. *P. malariae* prefers to penetrate older erythrocytes and *P. falciparum* has not shown a clear preference but invades all ages (White *et al.* 2011).

After RBC invasion of merozoites, ring stage trophozoites form inside the RBCs which grow into erythrocytic schizonts, feeding on the RBC's hemoglobin as their main substrate. Hemozoin or malaria pigment is the histologic correlate as end-product of the parasitic intra-erythrocytic metabolism (White *et al.* 2011). Once the erythrocytic schizonts mature, the erythrocytes break open and release once again

merozoites that finally produce characteristic malaria symptoms and continue to invade more RBCs.

The intra-erythrocytic life cycle takes about 24 hours for *P. knowlesi*, 48 hours for *P. falciparum*, *P. vivax* and *P. ovale* and 72 hours for *P. malariae* (Taylor *et al.* 2012). Depending on the *P.* spp. a five- to thirtyfold replication of parasites takes place during every new intra-erythrocytic cycle and when the replication reaches a parasite density of more than 50 per µL blood or a total body parasite count of more than 100 million parasites symptoms generally appear and parasitemia can be detected by microscopy or rapid diagnostic tests (Hoffman *et al.* 2011). The period from sporozoite injection to the first malaria symptoms is the incubation period, which is strongly influenced by the host's immunity against the parasite, but is 8.9 days on average (range 7-14 days) (Taylor *et al.* 2012). Non-immune travelers can become symptomatic already at low parasitemias and therefore have a relatively short incubation period, whereas semi-immune individuals from endemic areas might tolerate high parasitemias without developing severe symptoms at all (Nankabirwa *et al.* 2013).

After several asexual life cycles for *P. falciparum* or straight after the liver stage for *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* the sexual forms, gametocytes, develop (White *et al.* 2011). When a female *An.* mosquito takes a blood meal from an infected individual these gametocytes are ingested by the mosquito and the life cycle starts again.

2.3 Epidemiology

The epidemiology of malaria infection and the development of disease are determined primarily by the *Anopheles* species (*An*. spp.), the *P.* spp., climate, and human factors.
A common epidemiologic measure to quantify the malaria transmission intensity is the entomological inoculation rate (EIR). The EIR is the number of bites by infected female *An*. mosquitoes in a specified time frame, usually per year (annual entomological inoculation rate AEIR). An AEIR below 10 is considered low transmission, an AEIR >10 to 49 intermediate transmission and an AEIR >50 high transmission (Taylor *et al.* 2012, Kelly-Hope *et al.* 2009).

2.3.1 Anopheles mosquitoes

About 3,500 mosquito species are currently classified and subdivided into 41 genera. Only the genus *An.* is capable of transmitting human malarial protozoa and among them only female *An.* transmit malaria. Currently, there are about 469 recognized *An.* spp. and more than 50 still unnamed members of spp. complexes that are spread worldwide (Harbach 2013). Of all *An.* spp., 41 species were classified to be dominant malaria vector spp. and vector spp. complexes (Hay *et al*. 2010, Sinka *et al*. 2012, Fig. 2). *An*. with the greatest effectiveness of transmitting malaria parasites, inhabit large parts of Africa (*An. gambiae*), the Amazon (*An. darlingi*), and Southeast Asia (*An. dirus*) (Fig. 2).
The malaria transmission rate is not only influenced by the abundance of potent vectors but also by each species' biting habits. While some female *An.* spp. use human blood as a protein source for their egg production (anthropophilic), others prefer animals (zoophilic). The two main malaria vectors in Sub-Saharan Africa, *An. funestus* and *An. gambiae* show a strong human biting preference and are therefore among the most efficient malaria vectors globally (Hoffman *et al.* 2011). The majority of the *An.* spp. is active either during night (nocturnal) or at dusk or dawn (crepuscular). This is the timeframe with greatest risk of acquiring an

infection and therefore intervention methods such as bed nets specifically try to prevent transmission during that time.
Indoor or outdoor biting habits (endophagic vs. exophagic) and indoor or outdoor resting preferences after blood meals (endophilic vs. exophilic) need to be considered and play an important role in the choice of vector control in a malarious area. While endophagic and endophilic *An*. spp. can be targeted by insecticide treated nets (ITNs) or spraying the inside walls of homes with residual insecticides (indoor residual spraying, IRS), exophagic and exophilic *An*. spp. need a different approach (CDC 2010b).

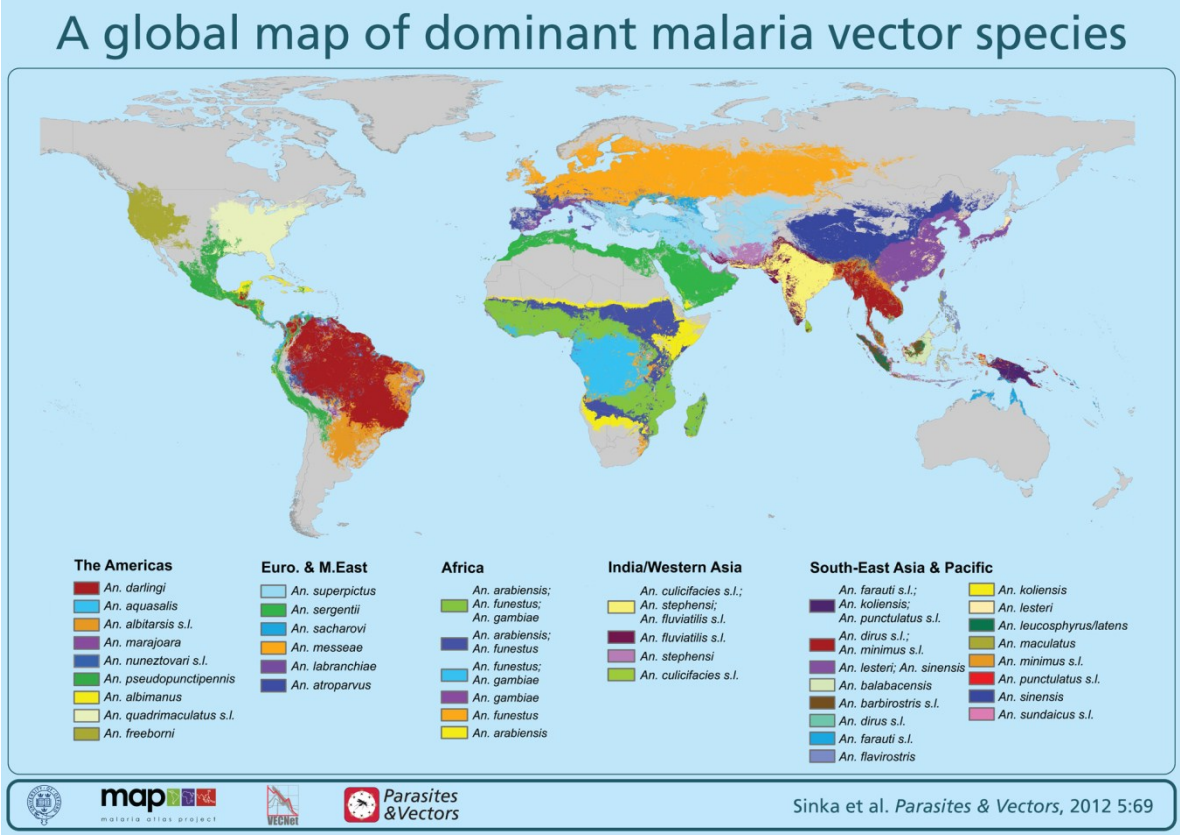


Fig. 2. Worldwide distribution of dominant malaria vector spp. (from Sinka *et al.* Parasites & Vectors 2012, 5:69).

Furthermore the lifespan of a mosquito plays a crucial role in parasite transmission. Whether malaria parasites that are ingested by *An*. are able to complete their life cycle within the mosquito (extrinsic cycle or sporogony) depends on the longevity of the *An*. harboring the parasite. The parasite's

development from ingested gametocytes to sporozoites in the salivary glands, ready to be injected (the time when the mosquito becomes infective) lasts about 10 to 21 days, depending on the ambient temperature and the parasite species (CDC 2010b). Consequently, the mosquito's lifespan needs to surpass the duration of the parasite's extrinsic cycle in order to become infectious to humans. The average survival of *An*. is about 20 to 25 days and therefore gametocytes need to be ingested in the early days of a mosquito's life to produce an infectious *An*. (Hoffman *et al.* 2011).

An. mosquitoes go through four different stages in their life cycle, starting from an egg after oviposition of a female adult into an aquatic habitat. Out of the egg hatches a larva which feeds on bacteria, algae and other microorganisms just below the water surface, and when mature, sheds the exoskeleton and becomes a pupa. The first three stages of the mosquito development, egg to pupa, take approximately 5 days to two weeks depending on *An.* species and ambient temperature (CDC 2010b). Different water sources serve as breeding grounds, such as the eddies of rivers or streams, rain puddles, rice fields, swamps, marshes, lakes, leaf axils of plants, tree holes, and others (CDC 2010b). If the breeding ground dries or alters in a way that makes the mosquito's development impossible such as flooding or draining before the adult mosquito emerges, the development can be interrupted (CDC 2010c). On the contrary, irrigation schemes and dams can create new breeding grounds for mosquitoes (Kusumawathie *et al.* 2006) and therefore influence transmission intensities.

After 5 days to two weeks of aquatic stages, the adult mosquito leaves the water source.

2.3.2 Climate

Ambient temperature and humidity are key determining factors that influence *An.* mosquitos' likelihood of survival and longevity and the parasite's extrinsic development. The minimum temperature for the parasite to complete its cycle within the mosquito is 16°C for *P. vivax* and 21°C for *P. falciparum* (White *et al.* 2011), the mean humidity should be around 60 percent and a high relative humidity increases the mosquito's lifespan (Taylor *et al.* 2012). Below 16°C

sporogony cannot be accomplished and consequently no infection can take place. At 25°C the parasite needs about 9 days up to three weeks depending on the *P.* spp. to complete its development from gametocyte to infective sporozoite (CDC 2010c). Warmer climates catalyze sporogony and therefore increase the likelihood of infection. The aquatic stages of the mosquito life cycle are determined by the water temperature (Taylor *et al.* 2012).

2.3.3 Plasmodium species

One of the five *P.* spp. needs to be prevalent to cause malaria infection. Differences in mortality and morbidity are related to the predominant *P*. spp. in a certain area. *P. falciparum* has by far the highest mortality of all *P*. spp. and almost all malaria related deaths are attributable to *P. falciparum* (White *et al.* 2011). *P. vivax* has a widespread prevalence in densely populated areas of Asia. In both spp., *P. falciparum* and *vivax*, strains have evolved which are resistant to anti-malarial drugs such as to chloroquine, sulfadoxine-pyrimethamine and more recently even to artemisinins (Talisuna *et al.* 2012) and thus present a public health challenge to populations living in areas where resistance occurs (Fairhurst *et al.* 2012).
P. vivax and *P. ovale* infections generally result in less severe forms of malaria, but are capable of forming dormant liver stages (hypnocytes) in hepatocytes over periods of months up to years and need treatment with primaquine in combination with an schizontocidal agent (chloroquine or artemisinin) in order to prevent relapse (Fernando *et al*. 2011).

2.3.4 Human factors

Innate Immunity
Some authors suggested that *P.* infection has put more pressure on the human genome than any other infectious pathogen (Hoffman *et al.* 2011). As a consequence, over the millions of years of repeated *P.* infections, genetic mutations have occurred in order to gain benefits and secure survival in malaria

endemic areas. These mutations generally affect the hemoglobin and erythrocyte structure. Several hemoglobin mutations, so called hemoglobinopathies, are known to reduce the risk of malaria infection, such as the sickle cell trait (HbAS), hemoglobin C (HbC), hemoglobin E (HbE), hemoglogin F (HbF), alpha and beta thalassemias, erythrocyte enzyme deficiencies like glucose 6 phosphate deficiency or structural differences in the cytoskeleton of RBCs, such as ovalocytosis (White *et al.* 2011).

The sickle cell trait is found mainly in regions where *P. falciparum* is endemic, and although the homozygous state, HbSS or sickle cell disease, shows a significant reduction in life expectancy, the heterozygous trait HbAS protects from high density parasitemias, severe malaria related anemia, cerebral malaria and malaria related deaths, especially in children between 2 to 16 months of age (Aidoo *et al.* 2002). It is assumed that the HbAS is a poorer substrate for the parasites and that erythrocytes with the sickle cell trait are subject to earlier sequestration in the spleen, reducing the risk of cytoadherence in the microcirculation of brain, kidneys, and other vital organs that is characteristic of severe *P. falciparum* infection (White *et al.* 2011).

Not only *P. falciparum*, but also *P. vivax* infections assumingly resulted in RBC mutations to reduce the risk of symptomatic malaria. Most Africans show a mutation of the Duffy RBC surface antigen and, when homozygous recessive, *P. vivax* cannot penetrate erythrocytes because the Duffy surface antigen is required for *P. vivax* adhesion and invasion of RBCs (Hoffman *et al*. 2011).

Acquired Immunity

The acquisition of immunity depends on the transmission setting. In areas of high transmission, repeated infections occur more frequently than in areas of low transmission. As a result, the development of partial immunity is generally only observed in high transmission settings, whereas individuals in areas of low transmission may develop symptomatic malaria until adulthood. Newborns in endemic areas are protected by maternal antibodies, transferred via the placenta, for about six months and rarely present with high-density parasitemia (Hoffman *et al*. 2011). Additionally fetal hemoglobin is believed to have a protective effect against *P*. replication and severe disease. Children may develop a partial immunity

against severe malaria, depending on the transmission intensity, usually between age three - in areas of intense transmission - to age 10 or older - in areas of seasonal transmission (Hoffman *et al*. 2011).
Antibodies target primarily the erythrocytic stage parasites and surface antigens expressed on infected red blood cells, such as the *P. falciparum*-erythrocyte-membrane-protein-1 (PfEMP1) (White *et al.* 2011). Consequently, in partially immune individuals the risk of sequestration of infected RBCs in the microcirculation of vital organs is reduced, capillary flow and metabolism maintained and parasitized RBCs are increasingly removed in the spleen (Hoffman *et al*. 2011). This results in a less severe manifestation of disease.

2.4 Pathogenesis

The presentation of clinical malaria symptoms is related to blood-stage parasites. Exo-erythrocytic stages, sporozoites or gametocytes do not seem to play a role in the presentation of symptoms. The onset of symptoms is linked with the destruction of red blood cells, the release of parasites into the blood stream and the host's immune reaction that is triggered by these events (White *et al.* 2011). Several changes in parasitized erythrocytes take place before the clinical symptoms actually develop. These include the parasite's metabolism of hemoglobin into hemozoin, or malaria pigment. At the same time parasitic proteins are expressed on the surface of infected RBCs which result in an irregular shape and a stiffening of the cell membrane and an increased exposure of antigens (White *et al.* 2011). The most important of these surface antigens on *P. falciparum* parasitized RBCs is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which attaches to receptors in the host's capillaries (Hoffman *et al.* 2011). Each *P.* spp. binds over a specific erythrocyte membrane protein to different vascular receptors such as the intercellular adhesion molecule 1 (ICAM-1) in the brain, chondroitin sulfate B in the placenta and CD36 receptors in most other organs (White *et al.* 2011). This process is responsible for the majority of malaria

symptoms because parasitized erythrocytes stick to receptors in the microcirculation and eventually obstruct venules and capillaries (cytoadherence) (Taylor *et al.* 2012). The parasitic proteins expressed on the surface of infected red cells not only enable the adherence to vascular proteins, but also to surface proteins on non-infected RBCs (rosette formation) or to other infected RBC (agglutination) (White *et al.* 2011). Cytoadherence, rosette formation and agglutination play an important role and predominate in *P. falciparum* infection and eventually lead to sequestration of erythrocytes in the microcirculation impairing blood flow and metabolism. When developing in the brain, sequestration can lead to cerebral malaria with capillary obstruction, reduced perfusion, endothelial cell activation and micro-thrombi formation, and ultimately anoxic damage (Taylor *et al.* 2012). The sequestration of infected RBCs in vital organs counteracts the host's protective mechanism of parasitized erythrocyte processing in the spleen. As a consequence, peripheral blood films taken from *P. falciparum* infected patients generally show only the younger ring forms of the *P.* whereas the older parasites remain largely undetected in the peripheral blood (White *et al.* 2011). Therefore, the level of parasitemia can be underestimated, because the peripheral blood may not be representative of the actual parasite count. The overall life-span of RBCs in infected individuals is reduced due to a decreased deformability of the erythrocyte membrane (infected and uninfected) when flowing through obstructed microcirculation and thus being exposed to early rupture (Hoffman *et al.* 2011). Another mechanism contributing to the severity of *P. falciparum* infections and distinguishing *P. falciparum* from *P. vivax* and *ovale* is caused by the ability of *P. falciparum* to invade all ages of RBCs while the other *P*. spp. show age preferences (White *et al.* 2011).

2.5 Clinical manifestations

A malaria infection is usually associated with unspecific prodromal signs that include fever, headache, fatigue, muscle pain, cough, dyspnea, abdominal discomfort, nausea, vomiting, hypotension, and a general lack of well-being (White *et al*. 2011). The most common initial clinical symptom is unspecific fever related to schizont rupture, parasite release and an activation of macrophages and pro-inflammatory cytokines (White *et al*. 2011).

Another characteristic but rather unspecific finding in malaria patients is anemia due to rupture of parasitized erythrocytes, an accelerated destruction of uninfected erythrocytes and an insufficient erythropoiesis in the bone marrow despite elevated serum erythropoietin levels (Hoffman *et al.* 2011).

Hypoglycemia (blood glucose <40mg/dL) with CNS symptoms is an important biochemical aberration particularly in children with severe *P. falciparum* malaria (Taylor *et al.* 2012). Hypoglycemia is attributed to increased glucose metabolism of the parasites and to insufficient hepatic gluconeogenesis. The level of hypoglycemia is an important prognostic factor especially if it occurs before antimalarial treatment is started, because certain antimalarials such as quinine can stimulate the pancreatic insulin secretion and glucose levels can drop (Taylor *et al.* 2012).

Metabolic acidosis is another clinical sign suggesting severe malaria (capillary blood pH <7.25, plasma bicarbonate <15 mmol/L or plasma lactate levels >5 mmol/L) (White *et al.* 2011). Acidosis is accompanied by a slower respiratory rate and can lead to acidotic breathing, which shows higher mortality rates.

Pulmonary edema due to increased pulmonary capillary permeability is another clinical feature of severe malaria especially in adult patients and commonly resulting in respiratory distress. It is observed less frequently in kids (Hoffman *et al.* 2011).

Renal impairment with an elevation of creatinine and blood urea nitrogen, proteinuria and hemoglobinuria may be observed as well in severe malaria and acute renal failure can develop which is more common in adults than children (Taylor *et al.* 2012). In cases of severe hemoglobinuria the term blackwater fever is used to describe the staining of the urine in a black color (Hoffman *et al.* 2011).

Additionally, cerebral malaria is a severe complication of *P. falciparum* infection and occurs more frequently in children than in adults. It is caused by a sequestration of infected red blood cells in the cerebral capillaries with resulting obstruction of the microcirculatory flow, hypoperfusion and anoxia. Besides, an impairment of the blood-brain barrier is witnessed and activation of endothelial cells and platelets lead to micro-thrombi formations (Taylor *et al*. 2012). Additionally, systemic inflammatory processes might be involved in the pathogenesis of cerebral malaria with cytokine release and destruction of parasitized red cells by the host's immune cells (Idro *et al.* 2005). Cerebral malaria can lead to coma and is related to an increased mortality rate despite treatment (Hoffman *et al.* 2011).
Shock is another feature of severe malaria and is diagnosed with a systolic blood pressure of <80 mmHg in adults and <50 mmHg in children, core/skin temperature difference of more than 10°C and prolonged capillary refill >2 seconds (White *et al*. 2011)
Jaundice, associated with a rupture of red blood cells, is observed more frequently in children than in adults and resolves when the patient recovers (Hoffman *et al.* 2011).

2.6 Diagnostics

Because malaria infection can mimic a broad spectrum of infections with a different etiology and vice versa, laboratory testing is crucial to correctly diagnose and treat the disease. The WHO recommends a prompt parasitological confirmation by either light microscopy or rapid diagnostic tests (RDT) of each suspected malaria case before treatment is started (WHO 2012a). To minimize drug interactions, side effects and development of drug resistance it is crucial to avoid the use of antimalarial therapy in parasite negative individuals (WHO 2012a). In patients with negative microscopy or RDT test results an alternative diagnosis needs to be established and treated accordingly. Presumptive treatment

based on clinical presentation alone should only be an option if parasitological diagnosis cannot be achieved in less than two hours from the time of the patient's presentation (WHO 2012a). Generally, the two options most widely used for parasitological confirmation are light microscopy and RDT.

Light microscopy of thin and thick blood films usually stained with Giemsa has a high sensitivity and specificity if performed by an expert microscopist and allows a differentiation between various *P*. spp. (White *et al.* 2011). Thick blood films are used for parasite detection, whereas thin films serve to identify the *P.* spp.. Microscopy is associated with relatively high acquisition costs, requires medical personnel training and continuing quality assurance (Taylor *et al.* 2012). It depends on electricity, which can be scarce in remote settings in the developing world although solar power can solve that problem (Wongsrichanalai *et al.* 2007). In endemic areas and hospitals with a high turn-over, operational costs are low (Taylor *et al.* 2012).

Rapid diagnostic tests include monoclonal antibodies and detect malarial antigens from finger- or heel-prick, are easy to handle and interpret and do not need extensive training. The two most commonly targeted antigens are histidine-rich protein 2 (HRP2), which is specific for *P. falciparum*, and parasite lactate dehydrogenase (pLDH), which can either detect the presence of any *P.* spp. (pan-malaria), *P. vivax*, or *P. falciparum* (Wongsrichanalai *et al*. 2007). They have a good sensitivity and specificity (Abba *et al*. 2011), do not require electricity and provide a result in 5 to 20 minutes but have the disadvantage of not being able to quantify the level of parasitemia but instead only the presence or absence of *P.* antigens (WHO 2012b). Therefore it is difficult to provide a prognosis based on a rapid antigen test. Additionally, RDTs are limited for follow-up visits, because *P*. antigens can remain elevated for several weeks even if the patient had been treated effectively (Wongsrichanalai *et al.* 2007). The WHO regularly performs testings of RDTs in cooperation with the CDC in order to ensure product quality and accuracy (WHO 2012b).

2.7 Treatment

The CDC currently recommends the following guidelines for the treatment of malaria (CDC 2011). All treatment guidelines provided are based on the current guidelines for treatment of malaria in the United States and are taken from CDC Global Health - Division of Parasitic Diseases and Malaria available online (CDC 2011). All doses provided are for adults.

For uncomplicated malaria caused by *P. falciparum* or an unidentified *P.* spp. from a suspected chloroquine-resistant region the following drugs are currently endorsed:
1) Atovaquone-proguanil AP (Malarone™): 250 mg atovaquone/ 100 mg proguanil
4 adult tablets po daily for 3 days
2) Artemether-lumefantrine AL (Coartem™): 20 mg artemether/ 120 mg lumefantrine
4 tablets po as initial dose, second dose 4 tablets po 8 hours later, followed by 4 tablets twice daily for 2 days - 3 day treatment and 6 oral doses in total.
3) Mefloquine (Lariam™): 684 mg base (=750 mg salt) po as initial dose, followed by 456 mg base (=500 mg salt) po given 6-12 hours after the initial dose;
the total dose is 1,250 mg salt
(CAVE: mefloquine is contraindicated for use in persons who acquired infection in Southeast Asia due to drug resistance.)
4) Quinine sulfate plus one of the following: doxycycline, tetracycline, or clindamycin. Quinine sulfate: 542 mg base (=650 mg salt) po three times daily for 3 days (for 7 days if infection was acquired in Southeast Asia)
Doxycycline: 100 mg po twice daily for 7 days
Tetracycline: 250 mg po four times daily for 7 days
Clindamycin: 20 mg base/kg/day po divided three times daily for 7 days
(CAVE: doxycycline and tetracycline are contraindicated in children younger than 8 years of age but treatment options one, AP, and two, AL, are recommended for this age group.)
According to the CDC all four options are equally recommended except option

three (mefloquine), which has shown severe neuropsychiatric adverse events at treatment doses and is therefore only suggested if all other options cannot be used.

For uncomplicated malaria caused by *P. falciparum* or an unidentified *P.* spp. from chloroquine-sensitive regions (Central America west of the Panama Canal, Haiti, the Middle East) the following drugs are currently recommended:
1) Chloroquine phosphate (Aralen™): 600 mg base (=1,000 mg salt) po followed by 300 mg base (=500 mg salt) po at 6, 24, and 48 hours
Total dose: 1,500 mg base (=2,500 mg salt)
2) Hydroxychloroquine (Plaquenil™): 620 mg base (=800 mg salt) po followed by 310 mg base (=400 mg salt) po at 6, 24, and 48 hours; Total dose: 1,550 mg base (=2,000 mg salt).

For uncomplicated malaria caused by *P. malariae* or *P. knowlesi* chloroquine phosphate or hydroxychloroquine (both treatments as above) are recommended without any geographic limitations.

For uncomplicated malaria caused by *P. vivax* or *P. ovale* from chloroquine-sensitive regions, chloroquine phosphate or hydroxychloroquine (both treatments as above) are recommended, however primaquine phosphate 30 mg base po daily for 14 days needs to be given in addition to chloroquine or hydroxychloroquine to eradicate hypnozoites (dormant liver stage parasites that may form in *P. vivax* and *P. ovale* infections) to prevent relapse. (CAVE: primaquine can cause hemolytic anemia in G6PD-deficiency and G6PD screening is obligatory before start of treatment.)

For uncomplicated malaria caused by *P. vivax* from chloroquine-resistant regions (Indonesia, Papua New Guinea) the following three treatment options are recommended:
1) Atovaquone-proguanil AP plus Primaquine phosphate (both treatments as above).
2) Mefloquine plus Primaquine phosphate (both treatments as above).
3) Quinine sulfate plus Doxycycline or Tetracycline; plus Primaquine phosphate

(all treatments as above).
(CAVE: Some cases of chloroquine-resistant *P.vivax* malaria have been documented outside Indonesia and Papua New Guinea in Myanmar, Central America, South America, and India, among others and if chloroquine treatment does not prove effective in *P. vivax* infections, management needs to be changed to a chloroquine-resistant *P. vivax* treatment.)

The majority of cases of severe malaria are caused by *P. falciparum* and require aggressive parenteral treatment. The following treatment is currently recommended:
1) Quinidine gluconate plus Doxycycline, Tetracycline, or Clindamycin:
Quinidine gluconate: 6.25 mg base/kg (=10 mg salt/kg) loading dose IV over 1-2 hours, then 0.0125 mg base/kg/min (=0.02 mg salt/kg/min) continuous infusion for at least 24 hours. Once oral medication can be taken and parasite density is below 1%, a complete treatment with oral quinine is recommended. Quinidine/quinine is given three days if the infection is acquired in Africa and for 7 days if it is acquired in Southeast Asia.
Doxycycline and clindamycin treatments are given as above or if oral medication is not feasible, treatment is given IV initially and then switched to oral medication. Artesunate is a newer alternative for quinidine.

2.8 Prevention

The most important and cost-effective tool for malaria prevention in endemic countries remains the mosquito net. Mosquito nets, especially insecticide treated nets (ITNs) and since several years long-lasting insecticide treated nets (LLINs), are a well-established and evidence-based vector control method for populations at risk of acquiring malaria (Lengeler 2004, Diallo *et al.* 2004, Schellenberg *et al.* 2001). The two population groups with the highest risk for malaria morbidity and mortality, pregnant women and children under five years of age, benefit most by

sleeping under a mosquito net (WHO 2012a). An overall increase in child survival of 27% has been demonstrated (Schellenberg *et al.* 2001) and long-term follow-ups after implementation of insecticide treated curtains (ITC) for malaria prevention show an estimated reduction in child mortality of 19-24% (Diallo *et al.* 2004). The efficacy of using mosquito nets to reduce malaria morbidity and mortality in endemic countries is undoubted, the public health impact significant, and distribution of insecticide treated nets to all populations at risk has become the main pillar in malaria control for endemic countries next to indoor residual spraying (WHO 2012a). Not only is sleeping under a mosquito net highly effective to prevent disease, it is also a practical and cost-effective means to achieve protection (Lengeler 2004). Achieving universal mosquito net coverage and use among all populations at risk was made a consensus goal of the Roll Back Malaria partnership, the World Health Organization and the US President's Malaria Initiative to achieve a 75% reduction of malaria cases by 2015 signed by the World Health Assembly in Abuja, Nigeria (Roll Back Malaria 2003, The US President's Malaria Initiative 2006).

2.9 Malaria in Uganda

This survey was conducted in east central Uganda, a country with mostly stable, year-round malaria transmission. Uganda is a small and landlocked East African country located north of Lake Victoria and borders Tanzania to the south, Kenya to the east, South Sudan to the north, the Democratic Republic of Congo to the west, and Rwanda to the south-west. It has an area of 241,038 square kilometers and a population of 34,758,809, 48.9% of which is below age 14 (median age 15.5 years) (CIA 2013). Life expectancy at birth is 53.98 years (CIA 2013).
In 2013 Uganda had the world's third highest birth rate with an estimated average of 44.5 births per 1,000 people, the world's fourth highest total fertility rate of 6.06 children born per woman and the fourth highest population growth rate globally of

3.32% (CIA 2013). Estimated maternal and infant mortality rates are extremely high with 310 deaths/100,000 live births (Austria: 4 deaths/100,000 live births) and 62.47 deaths/1,000 live births (Austria: 4.21 deaths/1,000 live births), respectively (CIA 2013). A total of 91.3% of Ugandan households do not have access to electricity (Uganda Bureau of Statistics 2010).

Uganda's tropical climate with two rainy seasons (generally from March to May and September to December) favors high and year-round malaria transmission in most of the country. About 95% of Uganda is considered highly endemic for malaria (Yeka *et al*. 2012) and some of the highest transmission intensities throughout Africa have been recorded in Uganda (infective bites per person per year or annual entomological inoculation rate AEIR >1500) (Okello *et al.* 2006). Transmission intensities vary greatly in different regions throughout the country but Uganda's Demographic Health survey (UDHS) estimates that 70% of Uganda experiences very high transmission (AEIR >100), 20% medium to high (AEIR 10-100), and 10% low transmission (AEIR <10), especially in the highland areas in the West (Ruwenzori range) and East (Mount Elgon) (Uganda Bureau of Statistics 2010). 88% of Uganda's population is considered to live in areas of moderate to very high year-round transmission intensities (Ministry of Health 2008).

Malaria is the leading cause of morbidity and mortality in Uganda, with about 30-50% of outpatient visits, 15-20% of hospital admissions and 9-14% of hospital deaths (Uganda Bureau of Statistics 2010). Disease burden is greatest in children <5 years of age and the Ministry of Health estimates that 25-30% of deaths among children <5 years admitted to health facilities are caused by malaria, accounting for an estimated 70,000-100,000 deaths among children under 5 annually (Ministry of Health 2008). Malarial parasitemia was prevalent in about 42% of Ugandan children aged 0-59 months (microscopy-verified) in 2010 (Uganda Bureau of Statistics 2010).
According to the 2008 WHO World Malaria Report Uganda had the sixth highest number of malaria cases and third highest number of malaria deaths worldwide (WHO 2008). In 2012 Uganda was ranked fifth in malaria cases and ninth in malaria deaths of all countries being affected by malaria globally (WHO 2012a). However, these data need to be observed with caution since the estimated

number of unreported cases and deaths is considered to be very high and sufficient data are limited. Recent surveys even suggest that there is no evidence that the impact of malaria in Uganda has declined in recent years and it may even be rising (Yeka *et al*. 2012). This is confirmed by new WHO data which shows that hospital admissions have been increasing in Uganda from 2000 to 2011 (WHO 2012a).

With exception of *P. knowlesi*, all other four human *P.* spp. have been documented in Uganda, however an estimated 90-98% of cases are caused by *P. falciparum,* followed by about 2% *P. vivax*, 2% *P. malaria* and less than 1% *P. ovale* (Uganda Bureau of Statistics 2010). The parasites are mainly transmitted by *An. gambiae s.l.* and *An. funestus*, two potent malaria vectors that bite and rest inside the house (endophagic and endophilic) and therefore make mosquito nets and indoor residual spraying the preferred and most widely used vector control interventions in Uganda (Yeka *et al.* 2012).

Mosquito nets and newer long-lasting insecticide treated nets (LLINs) are the major vector control method in Uganda and households owning at least one mosquito net (any net) have increased from 34% in 2006 to 59% in 2009 and to 74% in 2011 (Uganda Bureau of Statistics 2010, 2012). However, on average only 3 in 10 Ugandan households (28%) have at least one mosquito net (ITN) for every two people living in a house (Uganda Bureau of Statistics 2012). This falls way short of the target of 80% coverage by 2010 for people at risk and raises the question whether Uganda can meet the target of 100% vector control coverage and a 75% reduction of cases from 2000 levels by 2015 (Roll Back Malaria 2008).

2.10 Aim of the study

The goal of this study was to investigate mosquito net usage among secondary school students in east central Uganda. Uganda is a country with extremely high, perennial malaria transmission intensities, major malaria related morbidity and mortality, and a weak public health system. Furthermore, there are no comprehensive data available about the impact of malaria control interventions. Mosquito net usage among students aged 14 to 22 was investigated and correlated to socioeconomic background, level of knowledge about malaria, health care seeking behaviour, sex, and other factors that might influence mosquito net usage.

3 Methods

Two hundred and eighty-six Ugandan students attending Matuumu Secondary School (P.O. Box 4877, Buwenge, Kamuli district, Uganda) were invited to participate. The survey was an integrative part of their everyday school life and students were randomly selected to fill out a questionnaire consisting of a brief introduction explaining the purpose of the survey, followed by personal data and 14 questions (questionnaire see Adnex 1). Personal data included name, age, sex, and village or town they lived in. To assess the students' socioeconomic level, information about housing conditions, family size, and transportation to school was collected. To evaluate the students' educational level of the disease, malaria transmission, perceived symptoms, and awareness of prevention methods were investigated.

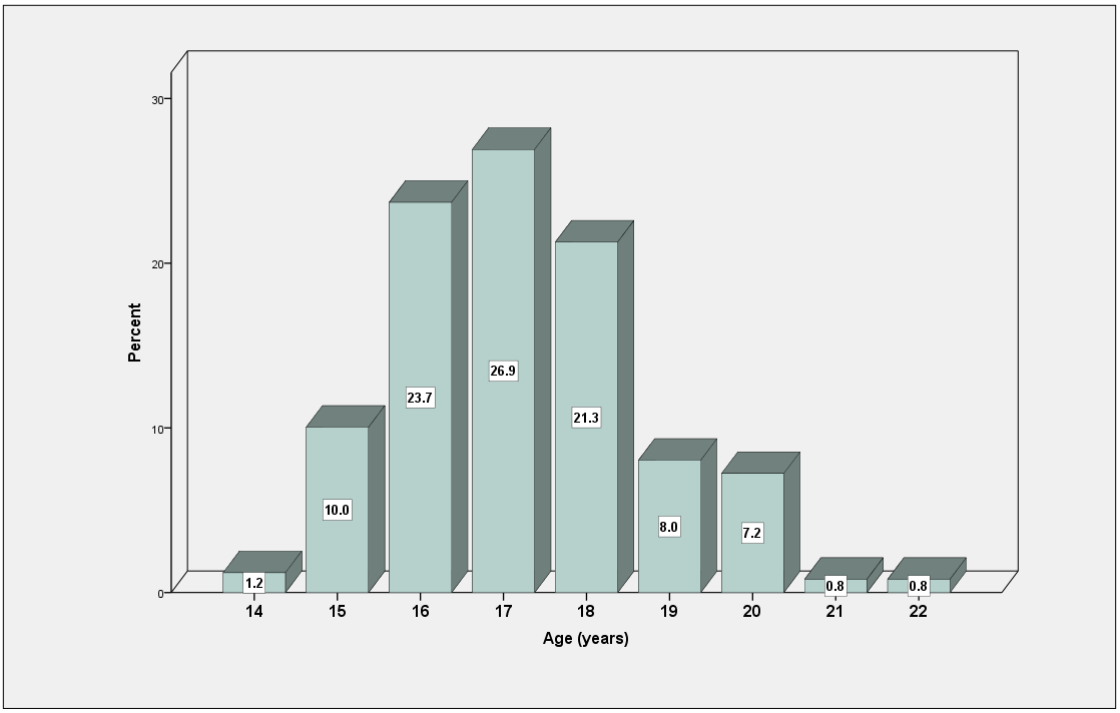
The students were asked whether they sleep under a mosquito net, and if they do so, the frequency of using the net. Furthermore, reasons for the lack of a mosquito net were explored. Finally, the students' management when suffering from malaria, their source of knowledge about the disease, and whether they had been sick of malaria in the 30 days prior to the survey were included in the questionnaire.

Data were collected meeting standards for data protection. All completed questionnaires and those with only one answer missing were included for evaluation. If any contradictory answers were given, the questionnaire was excluded from further analysis. Questionnaires fulfilling inclusion criteria were coded and entered into SPSS, version 20 (IBM Corp., Armonk, NY), for statistical analysis.

4 Results

Of 286 students, 258 (90.2%) met the inclusion criteria. 52.2 percent were male and 47.8% female. The mean age of participants was 17.2 years (age range, 14 to 22 years; Fig. 3).

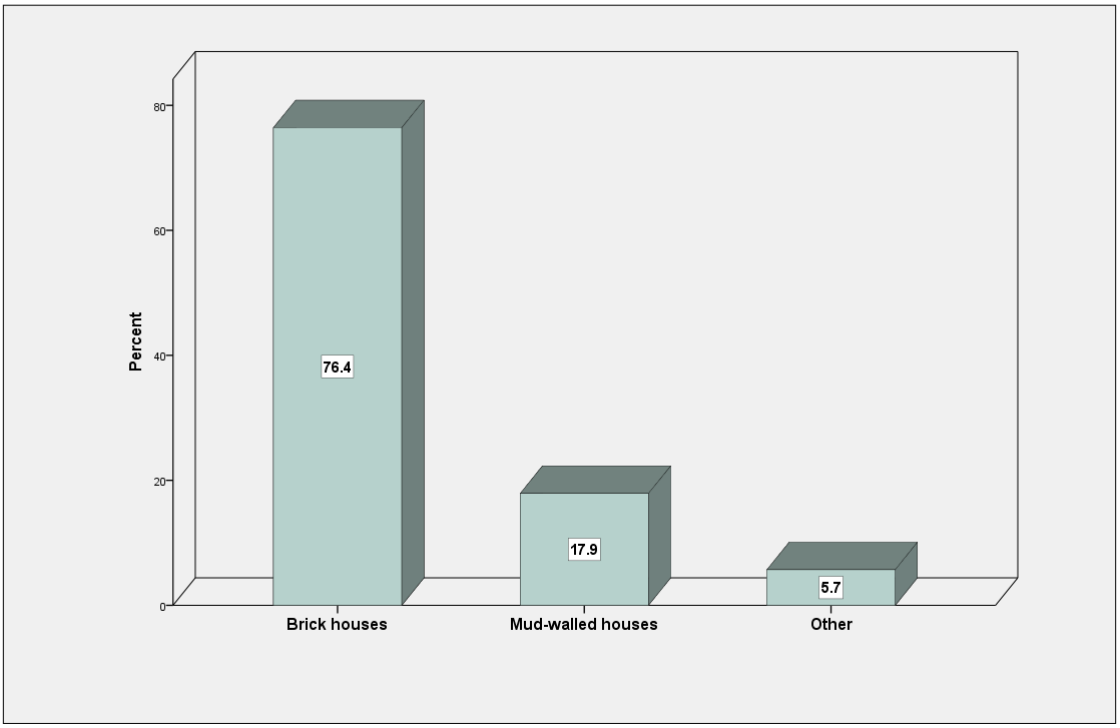
Fig. 3. Age distribution of students included in the study.



4.1 Socioeconomic background

The first part of the survey focused on the socioeconomic background of the students. 76.4% of students reported to live in a brick house, 17.9% in mud-walled houses, and 5.7% in other housing conditions, with all of these students residing in tents (Fig. 4).

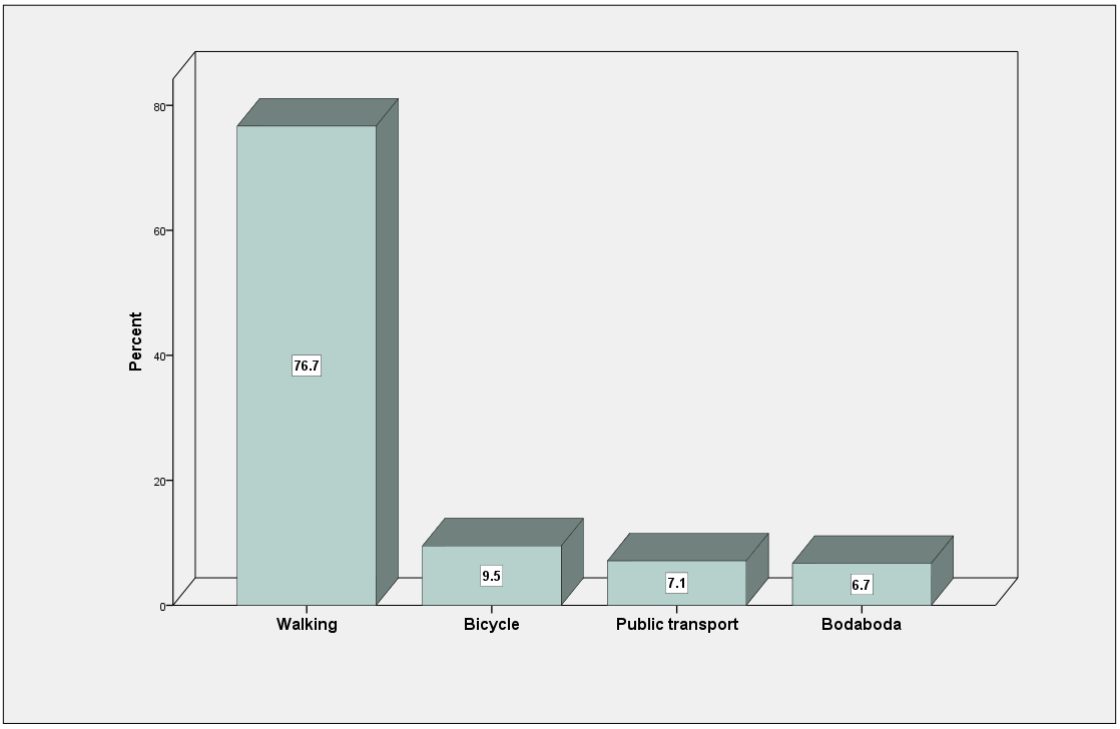
Fig. 4. Housing conditions of students included in the study.



The mean number of people living with a student in the same house was 8.3 people (range 1 to 35). Nearly half of the students (47.0%) lived with five to eight people.

Besides housing conditions and number of people the students are living with, transportation to school was used as a third parameter to get a better understanding of the students' socioeconomic setting. More than three quarters of students (76.7%) walked to school every day, 9.5% got there by bicycle, 7.1% used public transport, and 6.7% were brought to school on a moped taxi ("bodaboda"; Fig. 5).

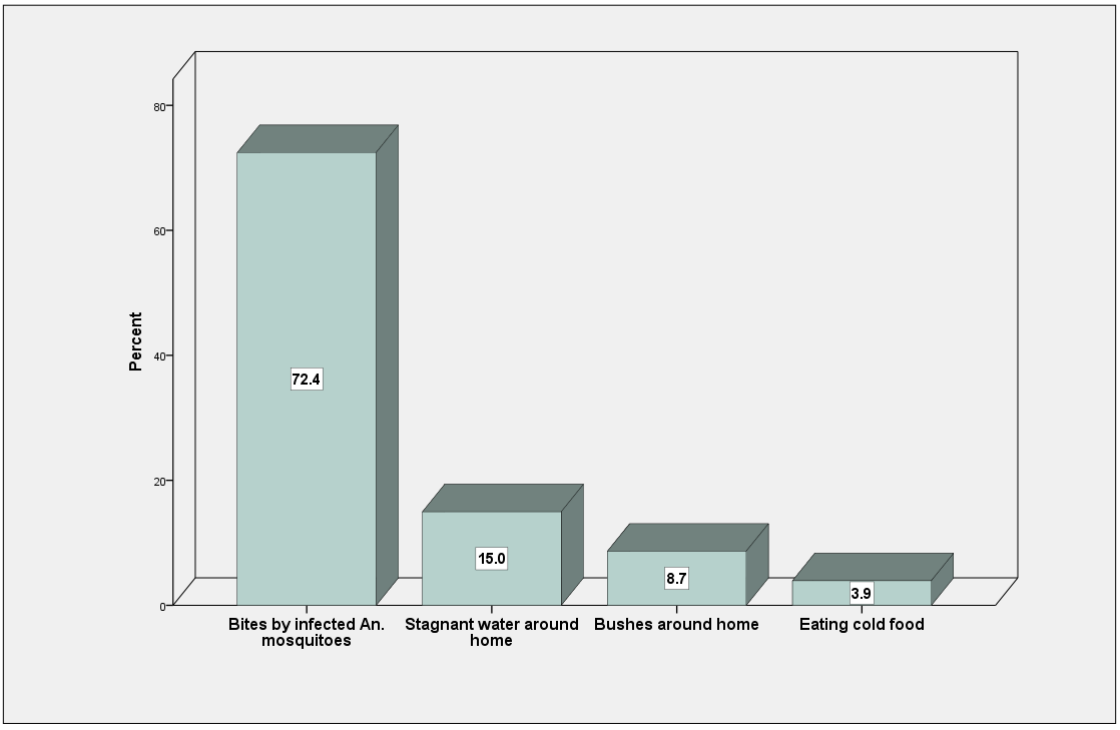
Fig. 5. Means of transportation to school of students included in the study.



4.2 Level of knowledge

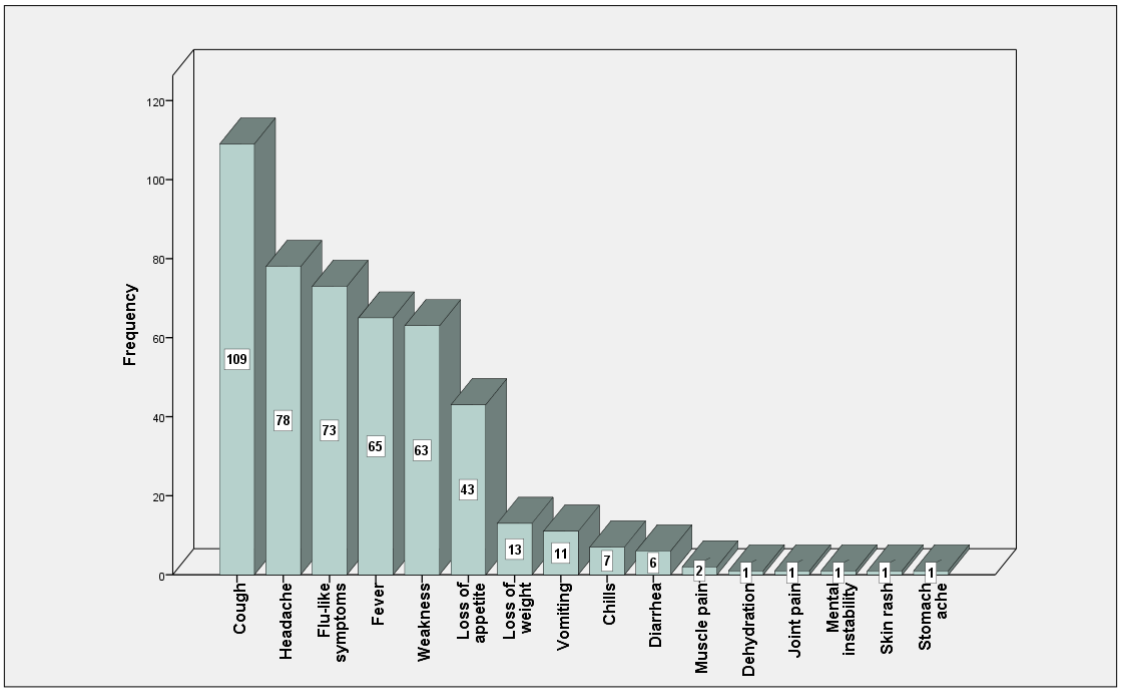
The students' level of knowledge concerning malaria was investigated by collecting information on how malaria is acquired, what the symptoms of infection look like, and how malaria can be prevented. 72.4% of students answered correctly that malaria is transmitted through bites of infected *An.* mosquitoes (Fig. 6). 15.0% believed that malaria is acquired through stagnant water around the house. 8.7% thought that bushes around their home cause the disease, and 3.9% shared the belief that eating cold food results in malaria.

Fig. 6. Perceived cause of malaria according to the students included in the study.



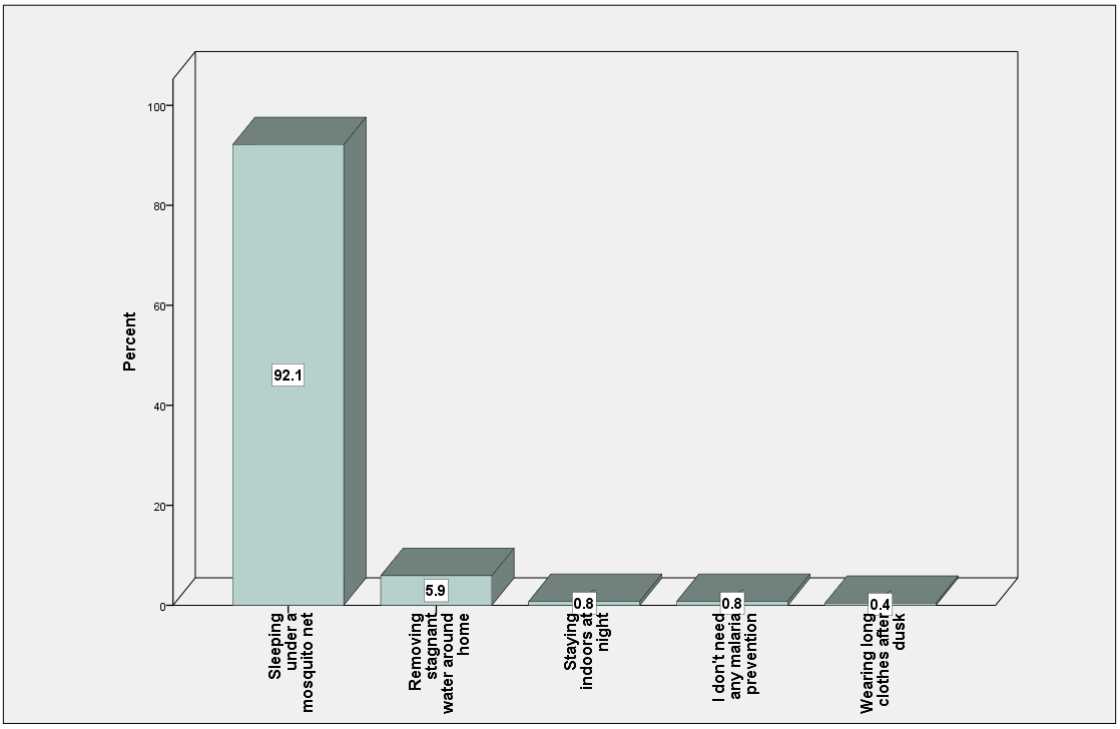
The students were asked to write down up to five symptoms they thought being related to malaria. The frequency of each symptom stated was recorded. Cough was the symptom reported most frequently (109x), followed by headache (78x), flu-like symptoms (73x), fever (65x), weakness (63x), loss of appetite (43x), loss of weight (13x), vomiting (11x), chills (7x), diarrhea (6x), muscle pain (2x), dehydration, joint pain, mental instability, skin rash, and stomach ache (1x each; Fig. 7).

Fig. 7. Malaria symptoms according to the students included in the study.



The third question evaluating the students' level of knowledge about malaria focused on preventive methods of the disease. Most of the students (92.1%) reported that sleeping under a mosquito net is effective to prevent malaria, while 5.9% stated that removing stagnant water around the house prevents infection (Fig. 8). 0.8% reported that staying indoors at night is protective and the same percentage stated to not need any malaria prevention at all (0.8%). One student (0.4%) reported that wearing long clothes after dusk is effective to prevent the disease.

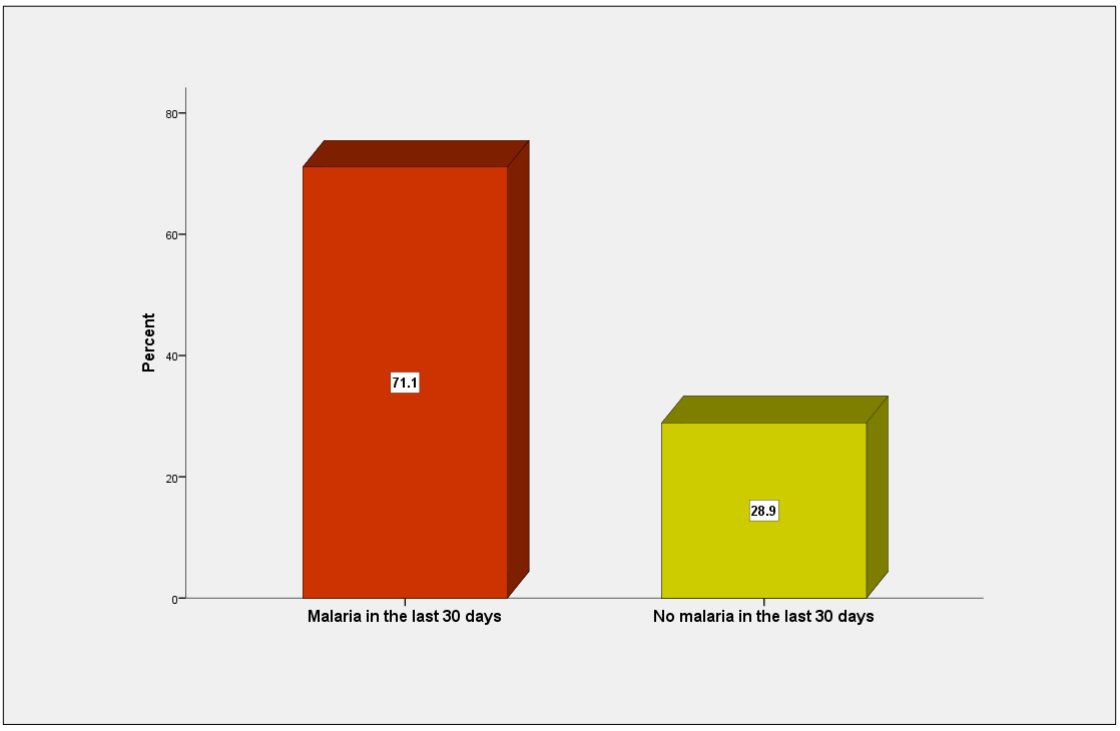
Fig. 8. Means of malaria prevention according to the students included in the study.



4.3 Self-reported malaria morbidity, disease management, and main source of information

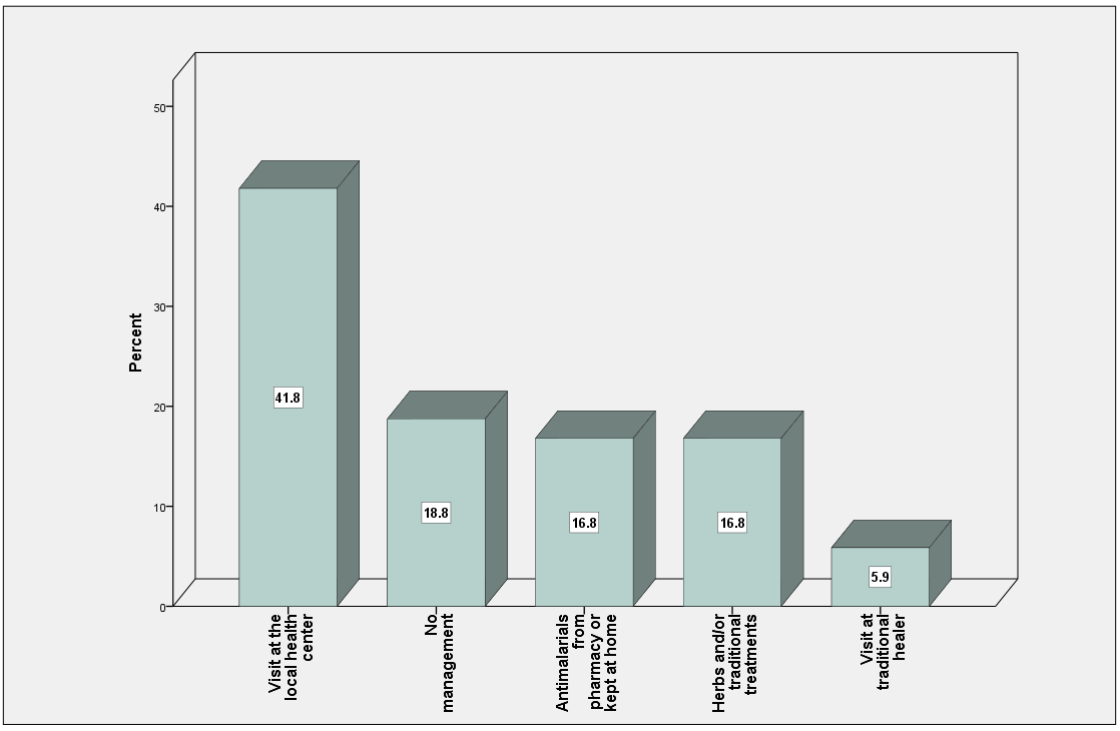
The students were asked if they had been sick of malaria in the previous 30 days. 71.1% reported that they had suffered from malaria in the previous 30 days, whereas 28.9 percent of students stated that they had not (Fig. 9).

Fig. 9. Self-reported malaria morbidity in the previous 30 days according to the students included in the study.



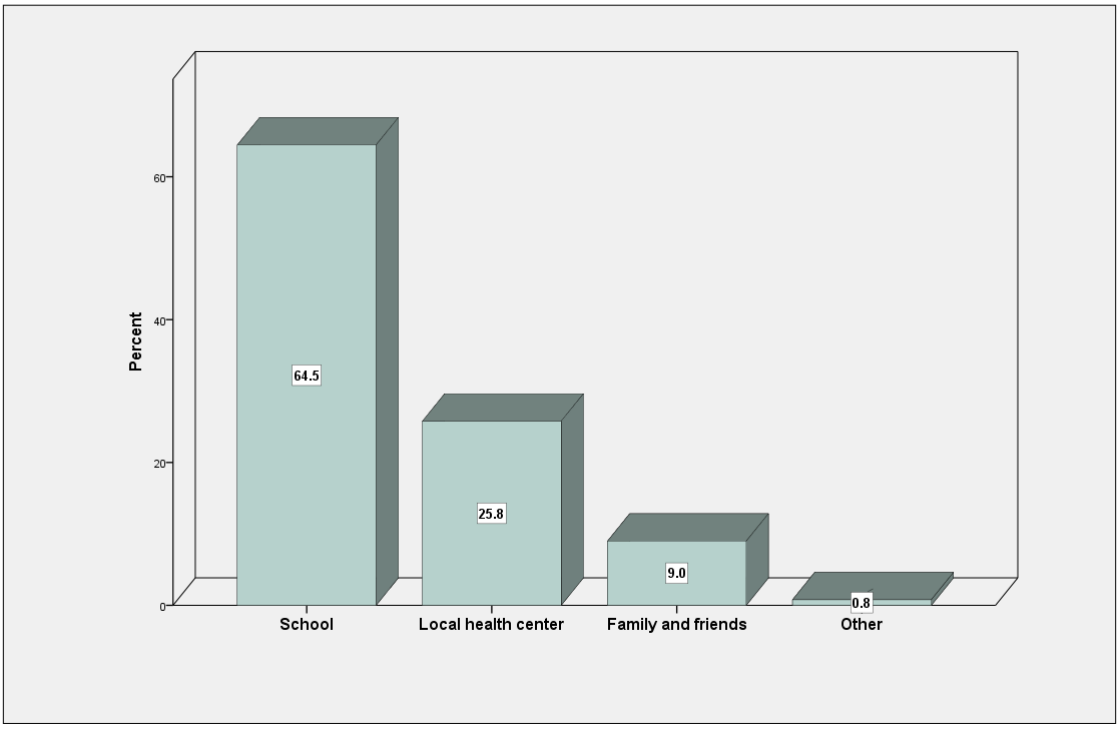
Furthermore, the students' management when suffering from malaria was addressed. 41.8% of the students reported to visit the local health center when experiencing malaria symptoms (Fig. 10). 16.8% mentioned to take antimalarial drugs purchased in a pharmacy or stored at home and an additional 16.8% stated to use herbs and/or other traditional treatments. 5.9% of students indicated that they usually visit a traditional healer for malaria management and 18.8% commonly did not follow a specific management plan.

Fig. 10. Management of malaria infection according to the students included in the study.



Finally, the students' main source of information concerning malaria was examined. 64.5% of the participants indicated that school is their primary source of information provider about the disease, followed by the local health center with 25.8% of students claiming it as their most important source of information (Fig. 11). 9.0% of students reported that family and friends play a crucial role in providing information about the disease. Only one student mentioned radio and another student newspaper as sources of information about malaria (Other source 0.8%).

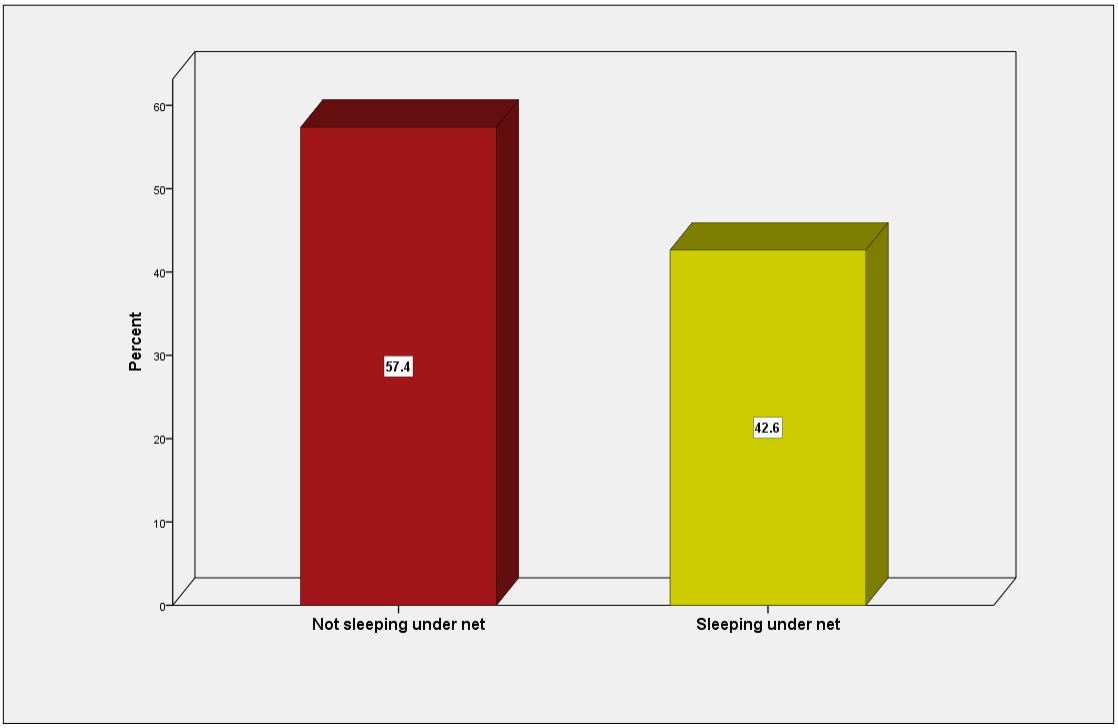
Fig. 11. Source of knowledge about malaria according to the students included in the study.



4.4 Reported mosquito net usage

57.4% of the students reported to sleep without mosquito net (insecticide treated and untreated net; Fig. 12). Among the students who slept without mosquito net, 89.3% stated that they could not afford a mosquito net and lack of financial resources was the main reason for not sleeping under a net. 8.6% did not use a net because they did not believe in the effectiveness of mosquito nets for malaria prevention. 2.1% reported other reasons why they did not use mosquito nets and mentioned that sleeping under a net makes them feel hot during the night.
42.6% of the students reported to sleep under a mosquito net. Among these students 94.5% used the net every night, 3.7% stated to sleep under the net two to three nights per week and 1.8% reportedly used it less than once a week.

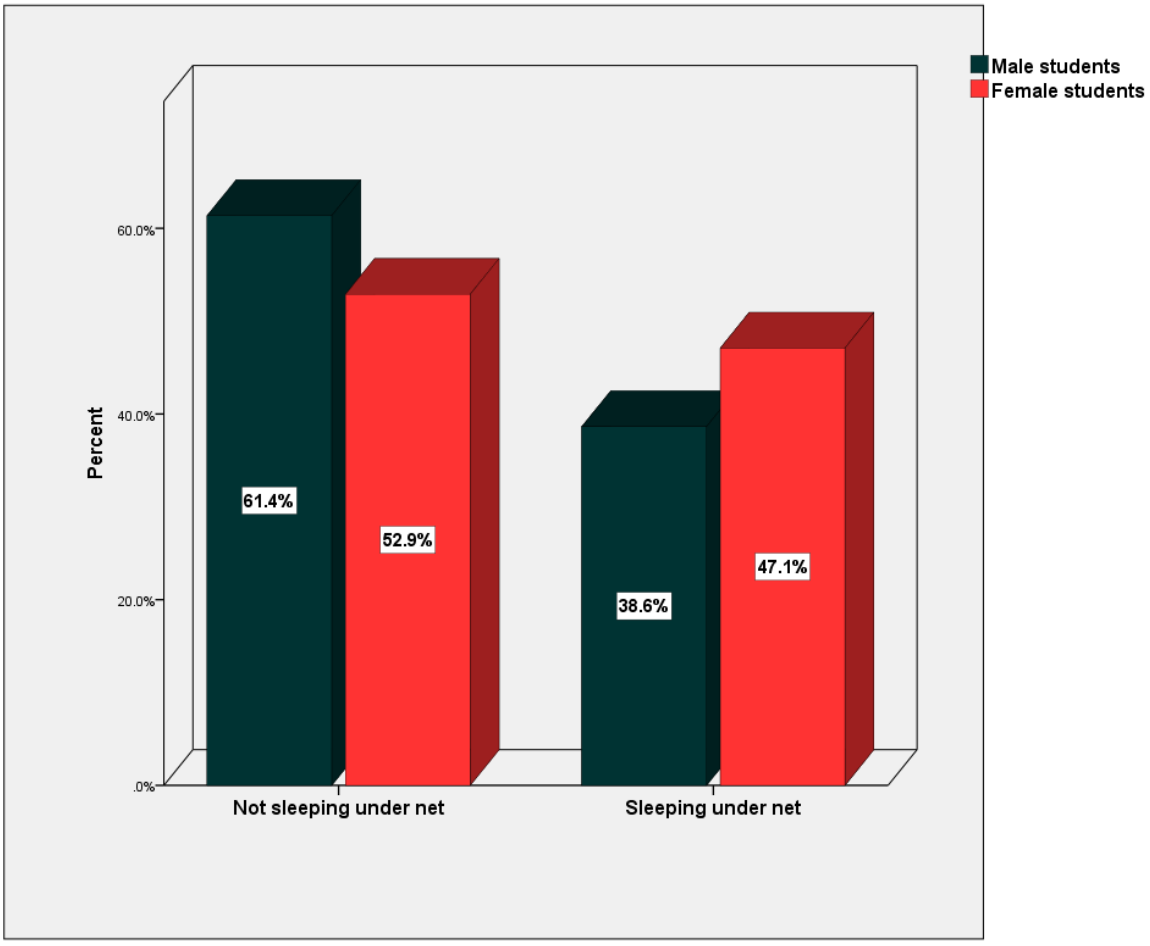
Fig. 12. Reported mosquito net usage among the students included in the study.



4.4.1 Sex and mosquito net usage

61.4% of males and 52.9% of females reported to sleep without a mosquito net (Fig. 13). In contrast, 38.6% of male and 47.1% of female students stated to use a mosquito net for malaria prevention.

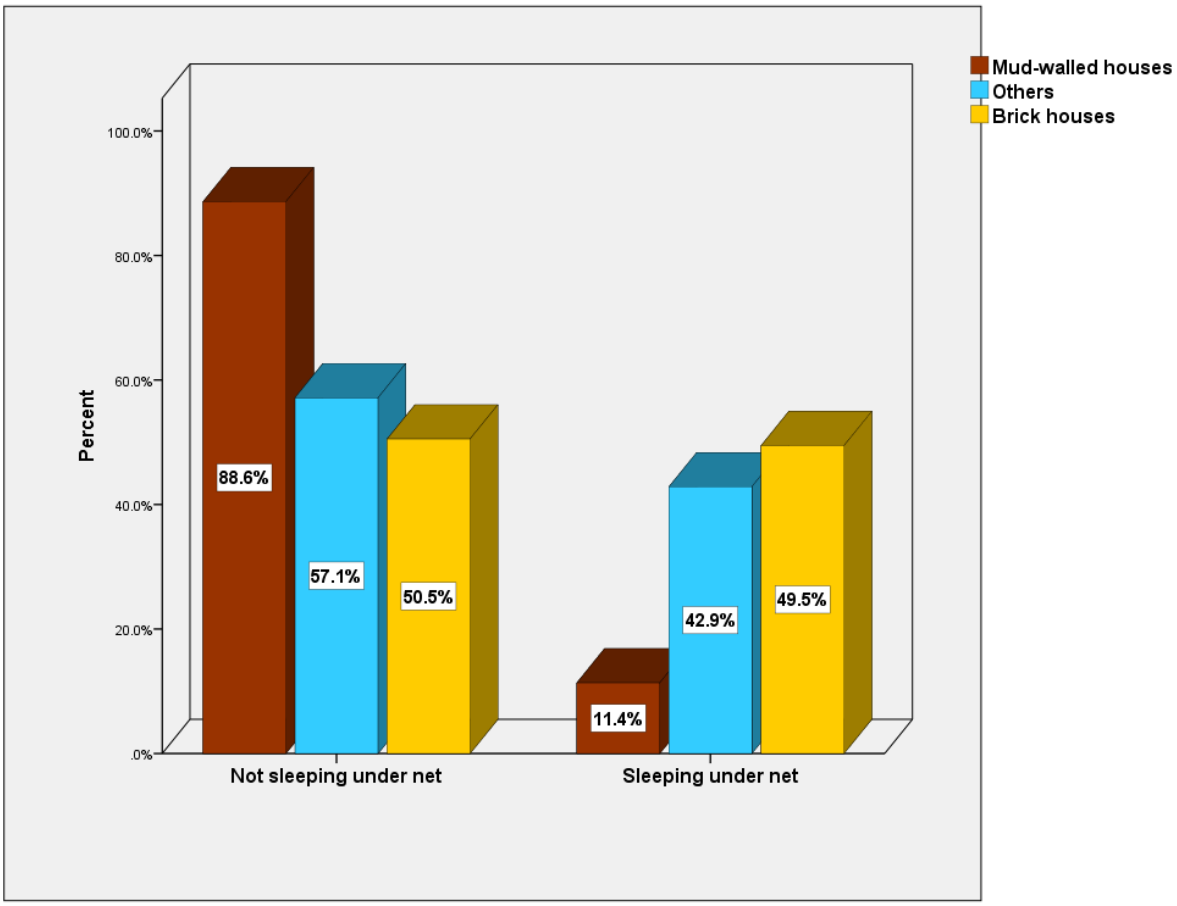
Fig. 13. Correlation between sex and mosquito net usage among the students included in the study.



4.4.2 Housing conditions and mosquito net usage

88.6% of participants residing in mud-walled houses, 57.1% of students sleeping in tents (others), and 50.5% of students living in brick houses did not use mosquito nets (Fig. 14). In contrast, 49.5%, 42.9%, and 11.4% of students living in brick houses, tents, and mud-walled houses reported to use mosquito nets for malaria prevention, respectively.

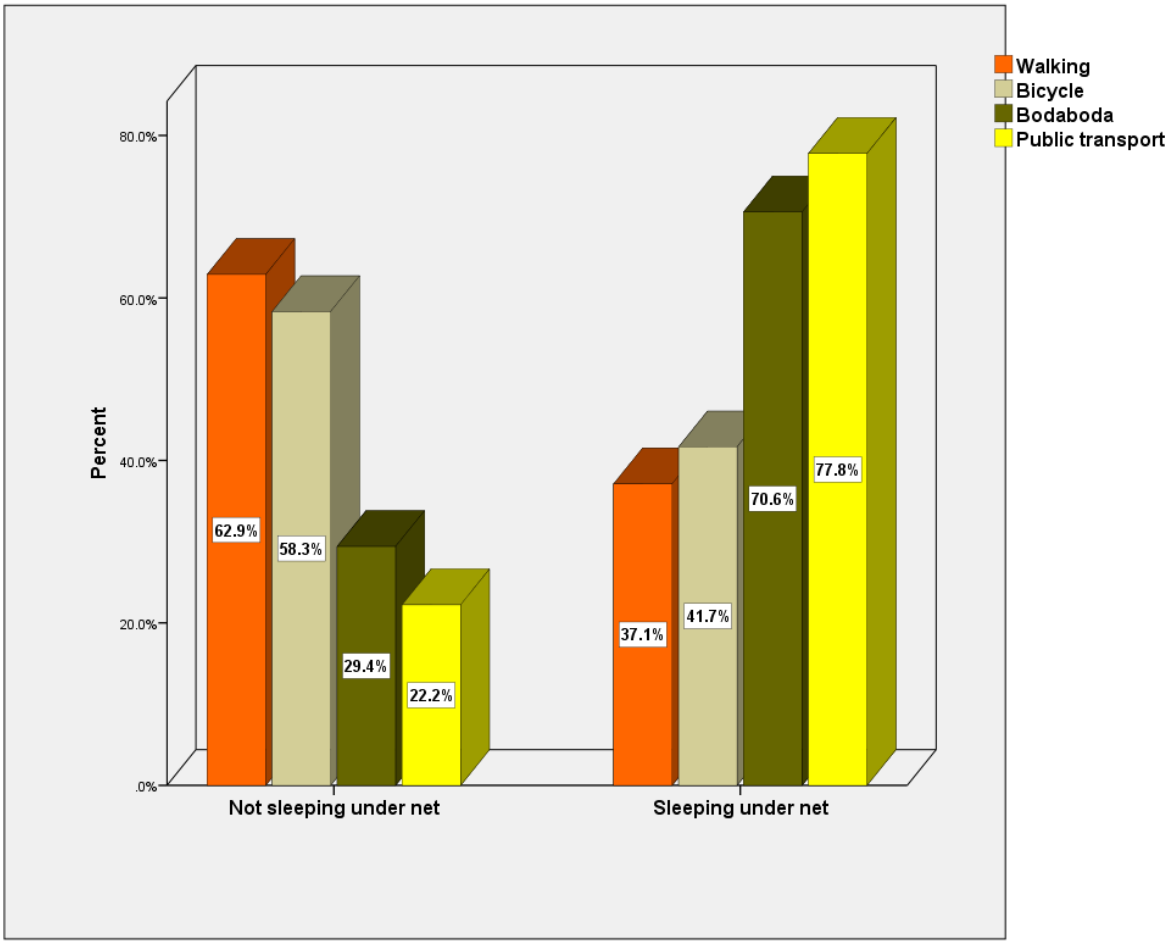
Fig. 14. Correlation between housing and mosquito net usage among the students included in the study.



4.4.3 Transportation and mosquito net usage

62.9% and 58.3% of students who generally walked and who rode their bike to school reportedly slept without a mosquito net, respectively (Fig. 15). 29.4% and 22.2% of students who commonly took the moped taxi ("bodaboda") and who used public transport reportedly slept without mosquito nets, respectively.
In contrast, 37.1% of students who usually walked, 41.7% of students who rode their bike, and 70.6% of students who took the "bodaboda" to school stated to sleep under a mosquito net. Students using public transport had the highest percentage of reported net users (77.8%).

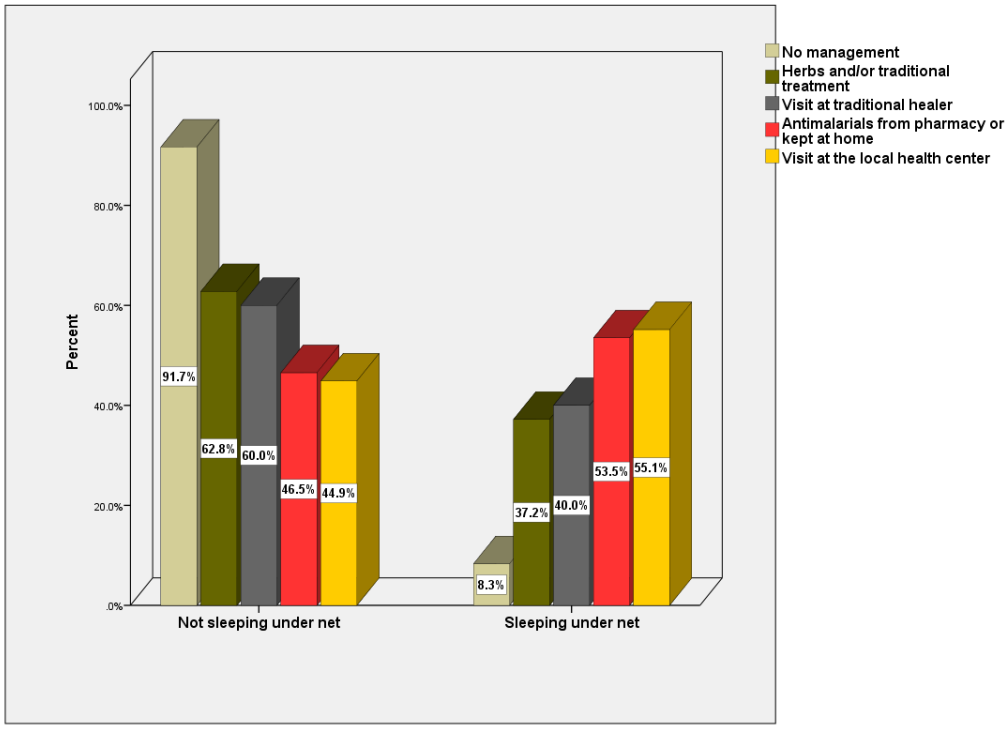
Fig. 15. Correlation between transportation to school and mosquito net usage among the students included in the study.



4.4.4 Disease management and mosquito net usage

91.7% of students who did not report a specific malaria management plan, also reportedly slept without a mosquito net for malaria prevention (Fig. 16). 62.8% of students usually treating malaria with herbs and/ or other traditional treatments and 60.0% of students routinely visiting a traditional healer for malaria treatment reportedly slept without a mosquito net. 46.5% of students who normally treat malaria with antimalarial drugs bought from a pharmacy or stored at home and 44.9% of students usually seeking malaria treatment at the local health center did not use a mosquito net. In contrast, 55.1% and 53.5% of students who routinely visit the health center and who generally treat malaria with antimalarial drugs from a pharmacy slept under a mosquito net, respectively. 40.0% of students seeking malaria treatment from traditional healers and 37.2% of students relying on herbs and/ or other traditional means of malaria management used mosquito nets. Only 8.3% of students who did not follow a specific treatment plan slept under a net.

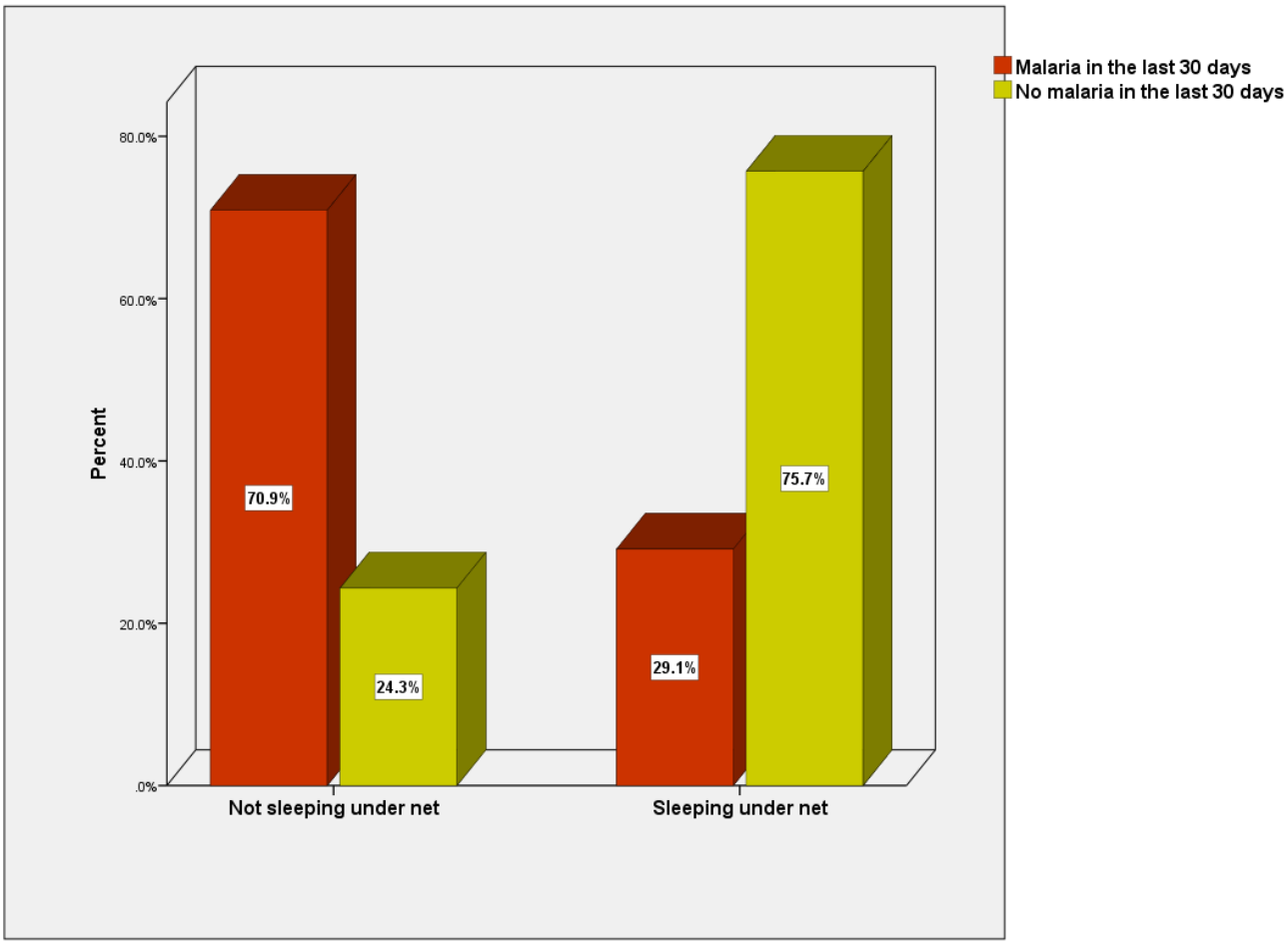
Fig. 16. Correlation between disease management and mosquito net usage among the students included in the study.



4.4.5 Reported malaria morbidity in the previous 30 days and mosquito net usage

70.9% of students who reported that they had been sick of malaria in the previous thirty days did not sleep under a mosquito net (Fig. 17). 24.3% of students who reportedly did not fall sick of malaria in the last month did not sleep under a net. On the contrary, 75.7% of students who reportedly had not been sick of malaria in the last thirty days used a mosquito net for prevention and 29.1% of students who stated that they had suffered from malaria in the last month slept under a net.

Fig. 17. Correlation between malaria morbidity in the previous 30 days and mosquito net usage among the students included in the study.

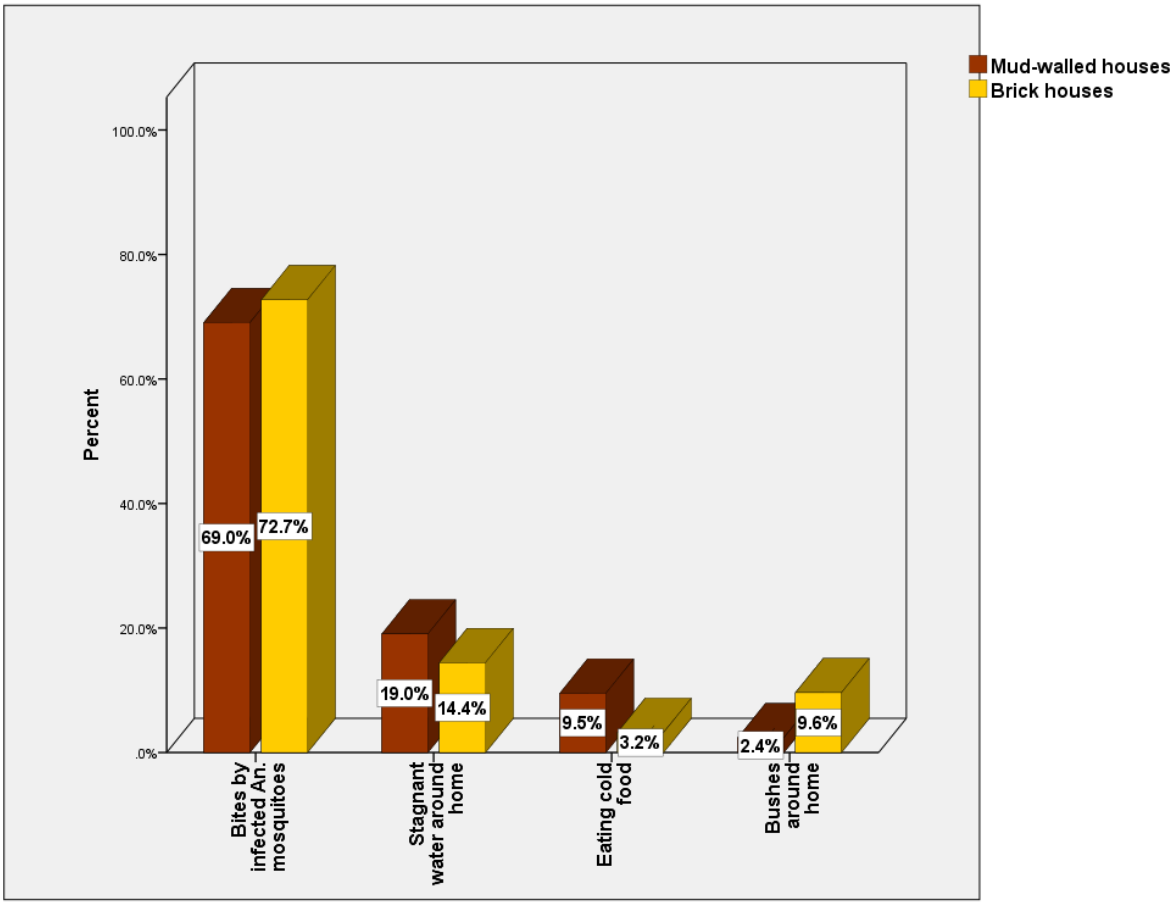


4.5 Housing

4.5.1 Housing and knowledge

72.7% of students living in brick houses and 69.0% of participants residing in mud-walled houses stated correctly that malaria is transmitted through bites of infected *An.* mosquitoes (Fig. 18). 19.0% and 14.4% of students from mud-walled and from brick houses believed that stagnant water around their home is causing malaria, respectively. 9.5% and 3.2% of students from mud-walled and from brick houses attributed the disease to eating cold food, respectively. 9.6% brick house students and 2.4% of students from mud-walled houses shared the belief that bushes around their home cause malaria.

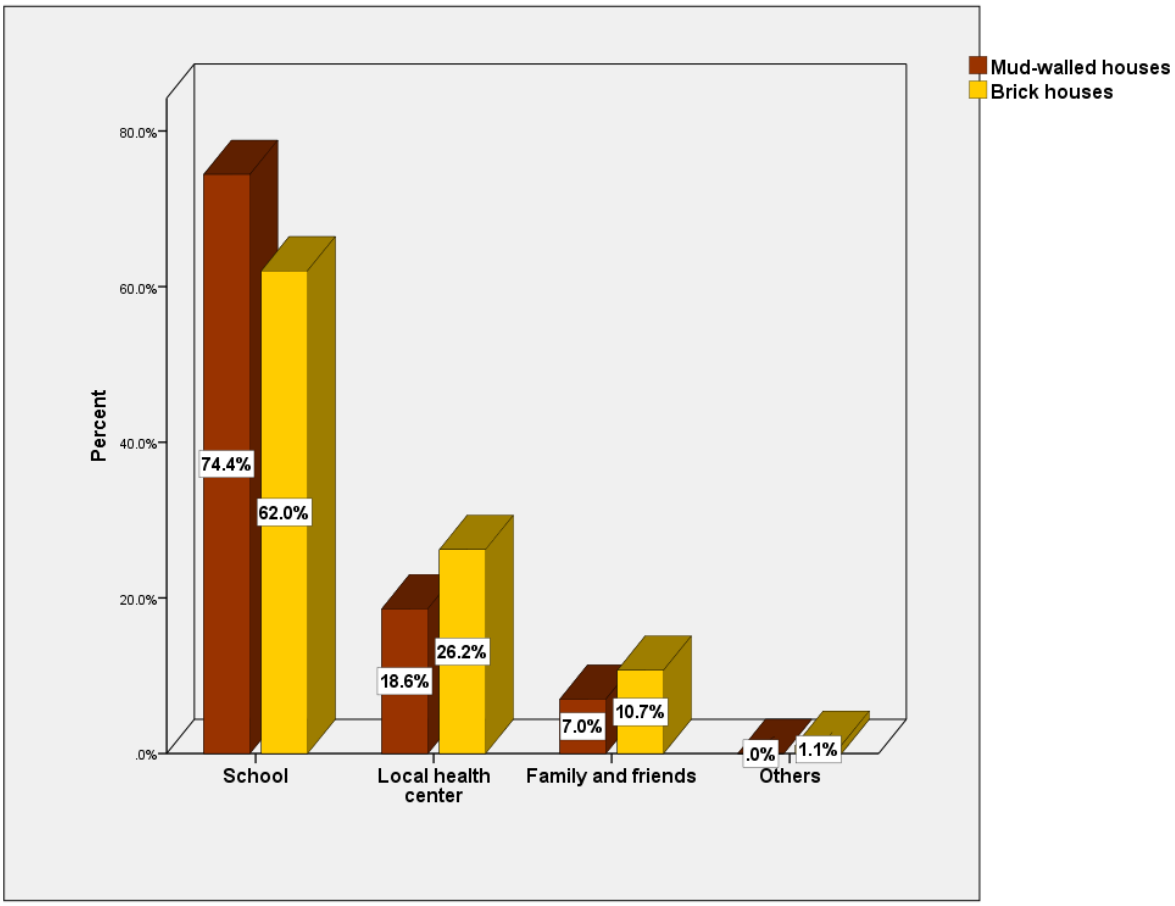
Fig. 18. Correlation between housing and knowledge about malaria transmission among the students included in the study.



4.5.2 Housing and source of information

74.4% of students living in mud-walled houses and 62.0% of individuals staying in brick houses reported that school was their prime source of information about malaria (Fig. 19). 18.6% and 26.2% from mud-walled and from brick houses stated that the local health center is their prime source of information, respectively. 7.0% of students living in mud-walled and 10.7% living in brick houses reported that family and friends were their prime source of information about the disease. Very few student and only those living in brick houses (1.1%) mentioned radio and newspaper as their source of information.

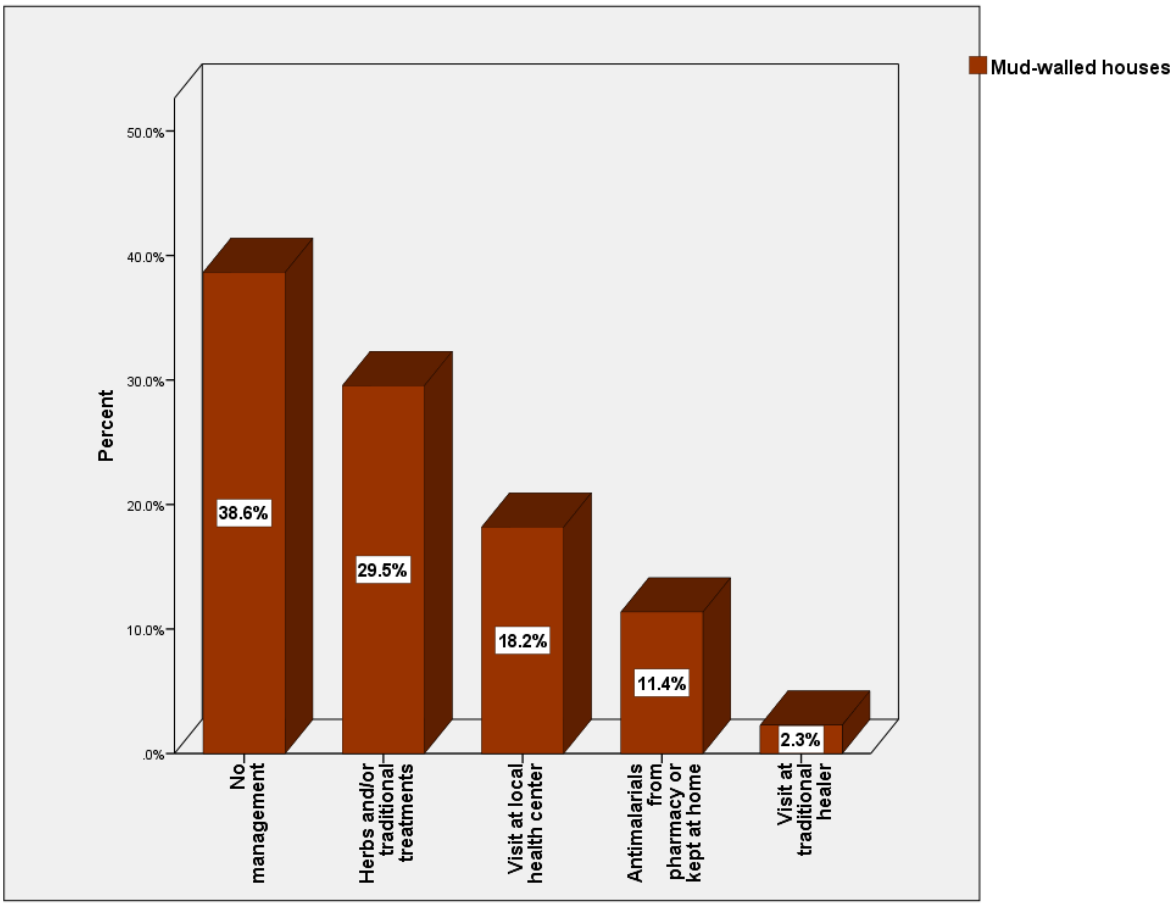
Fig. 19. Correlation between housing and prime source of information about malaria among the students included in the study.



4.5.3 Housing and disease management

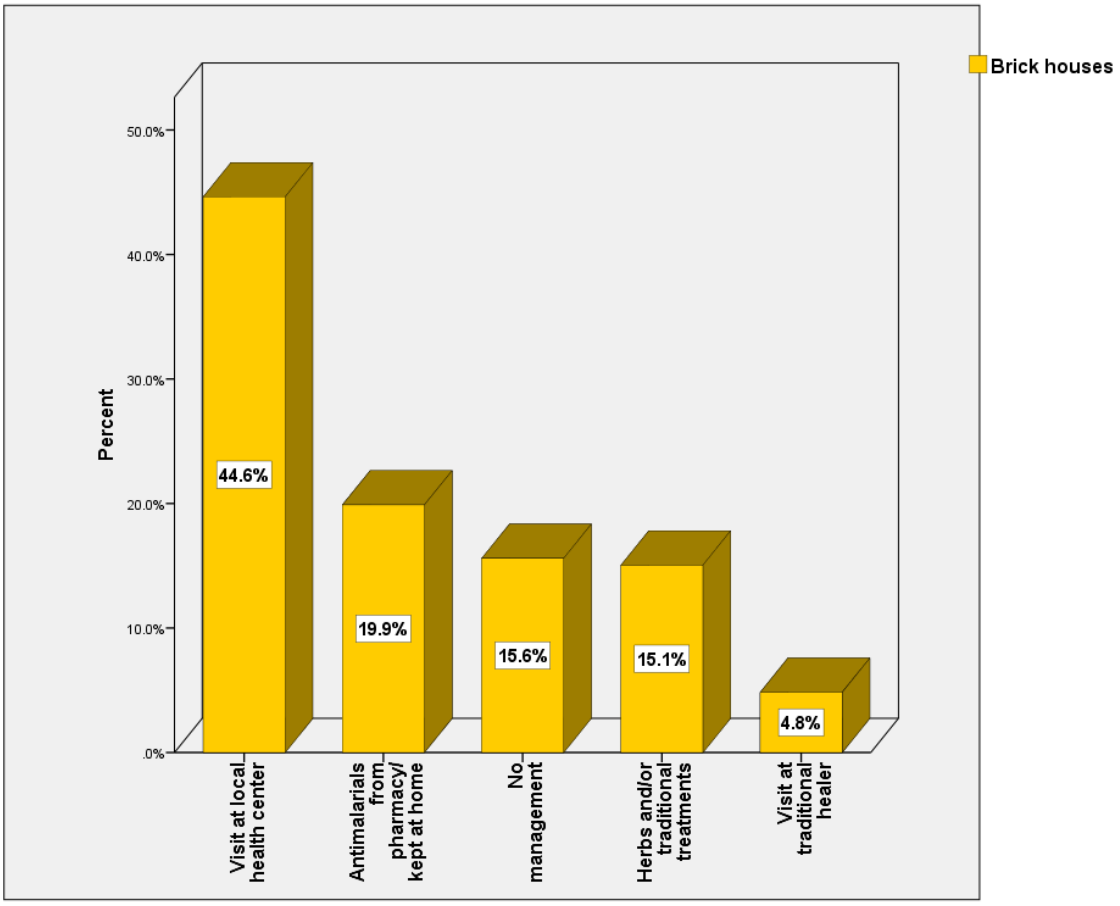
When looking at the relationship between residing in a mud-walled house and malaria management, 38.6% of students living in mud-walled houses reported that they commonly don't follow a specific management when being sick of malaria (Fig. 20). 29.5% of students staying in mud-walled houses answered to treat malaria with herbs and/ or other traditional treatments. 18.2% of the same group reported to visit the local health center and 11.4% stated to cure malaria with antimalarial drugs from a pharmacy or stored at home. 2.3% of students from mud-walled houses visit a traditional healer for malaria management.

Fig. 20. Correlation between living in a mud-walled house and management of malaria among the students included in the study.



In contrast, when looking at the malaria management of students living in brick houses, 44.6% reported to visit the local health center for treatment (Fig. 21). 19.9% of this group stated to treat the disease themselves with antimalarial drugs from a pharmacy or stored at home. 15.6% of brick house students replied that they generally do not follow a specific management plan when sick of malaria and 15.1% reported to use herbs and/ or other traditional regimens for treatment. 4.8% of participants living in brick houses answered to seek malaria treatment from a traditional healer.

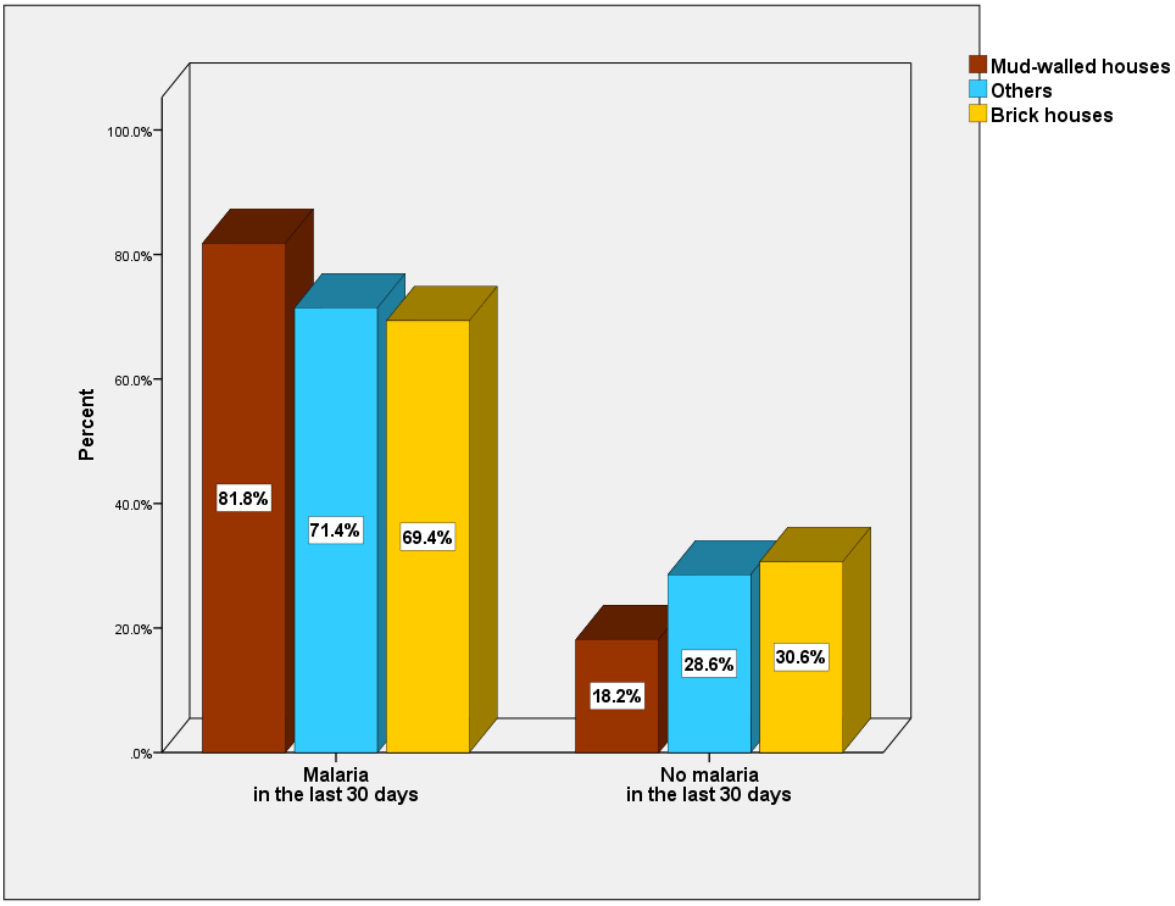
Fig. 21. Correlation between living in a brick house and management of malaria among the students included in the study.



4.5.4 Housing and reported malaria morbidity

81.8%, 71.4%, and 69.4% of participants living in mud-walled houses, tents, and brick houses reported that they had suffered from malaria in the previous thirty days, respectively (Fig. 22).
In contrast, 18.2%, 28.6%, and 30.6% of students residing in mud-walled houses, tents, and brick houses reported that they had not been sick of malaria in the previous thirty days, respectively.

Fig. 22. Correlation between housing and reported malaria morbidity in the previous 30 days among the students included in the study.

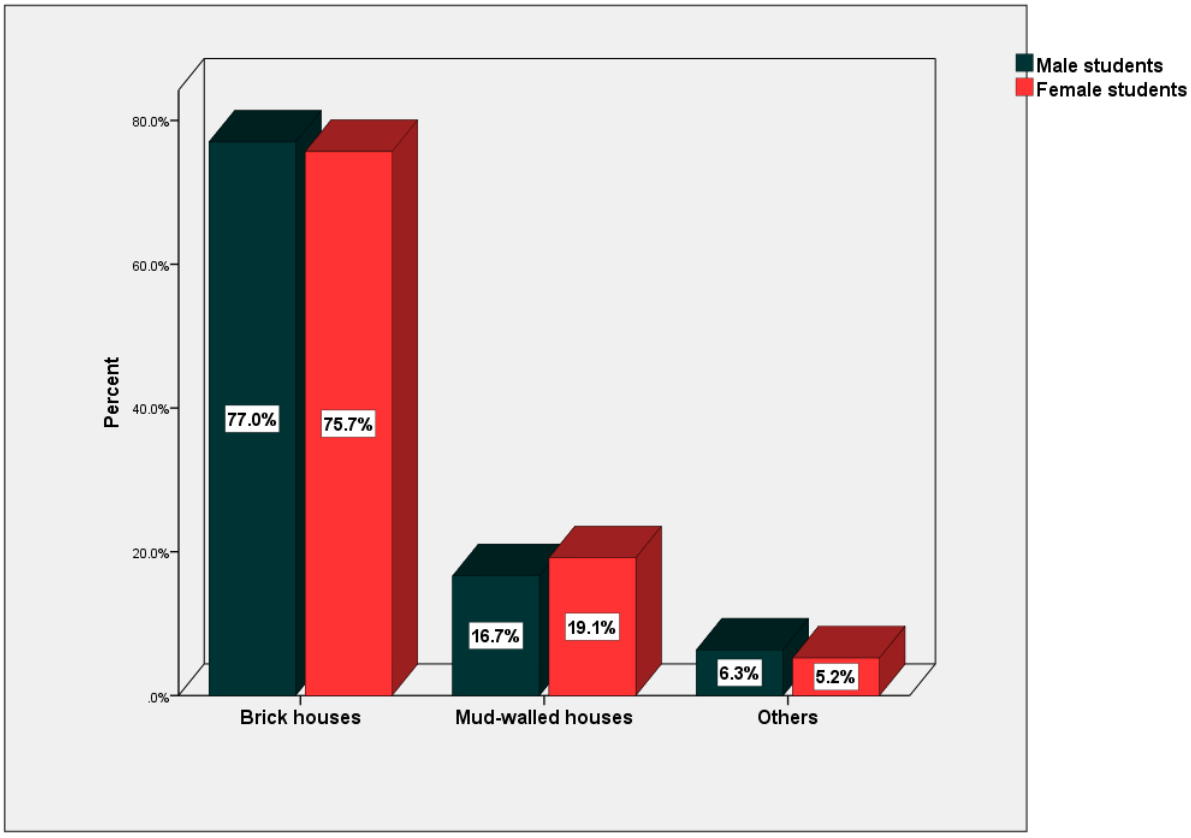


4.6 Gender differences

4.6.1 Gender differences: housing

n the survey, 77.0% of male and 75.7% of female students lived in brick houses (Fig. 23). 16.7% and 19.1% of males and females resided in mud-walled houses, respectively, and 6.3% of males and 5.2% of females lived in tents.

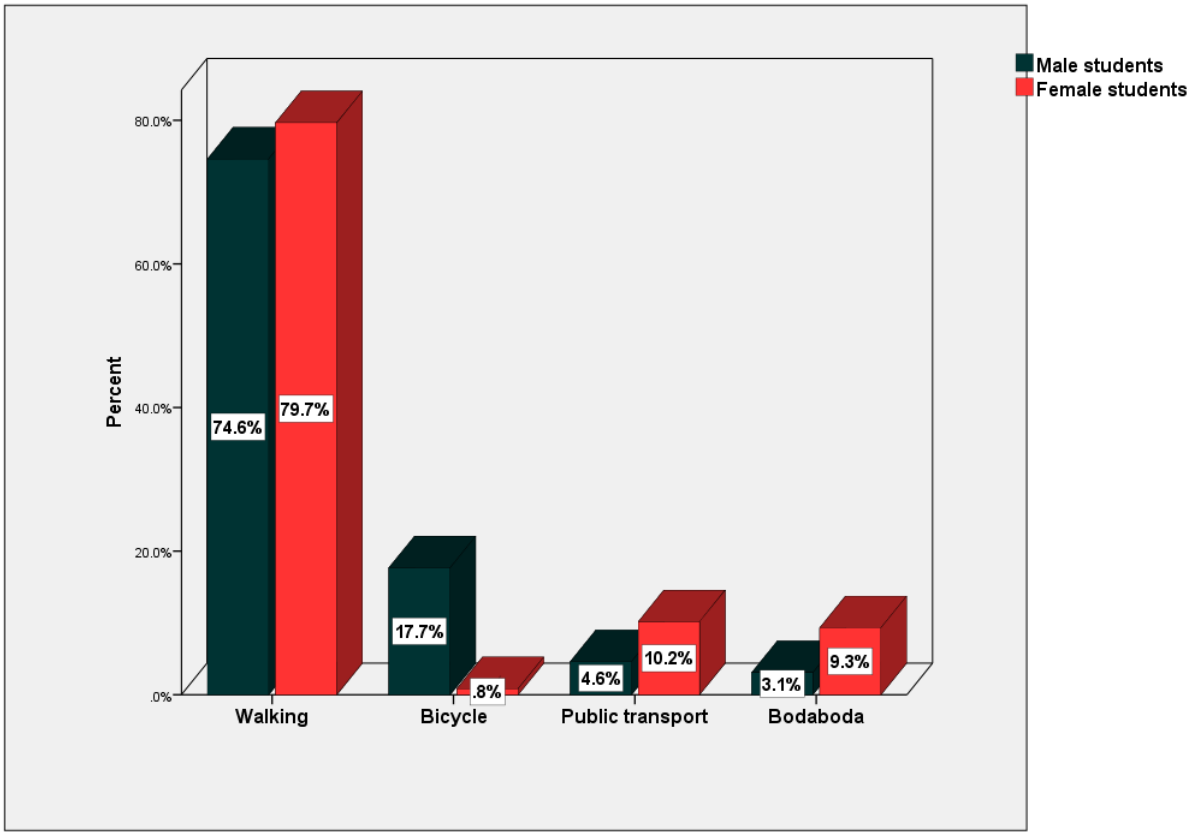
Fig. 23. Gender differences: housing conditions among the students included in the study.



4.6.2 Gender differences: transportation to school

79.7% and 74.6% of female and male students generally walked to school, respectively (Fig. 24). 17.7% of male and only 0.8% of female students usually rode their bike to school. 10.2% of females and 4.6% of males used public transport to school. 9.3% and 3.1% of female and male students were usually brought to school on a “bodaboda”, respectively.

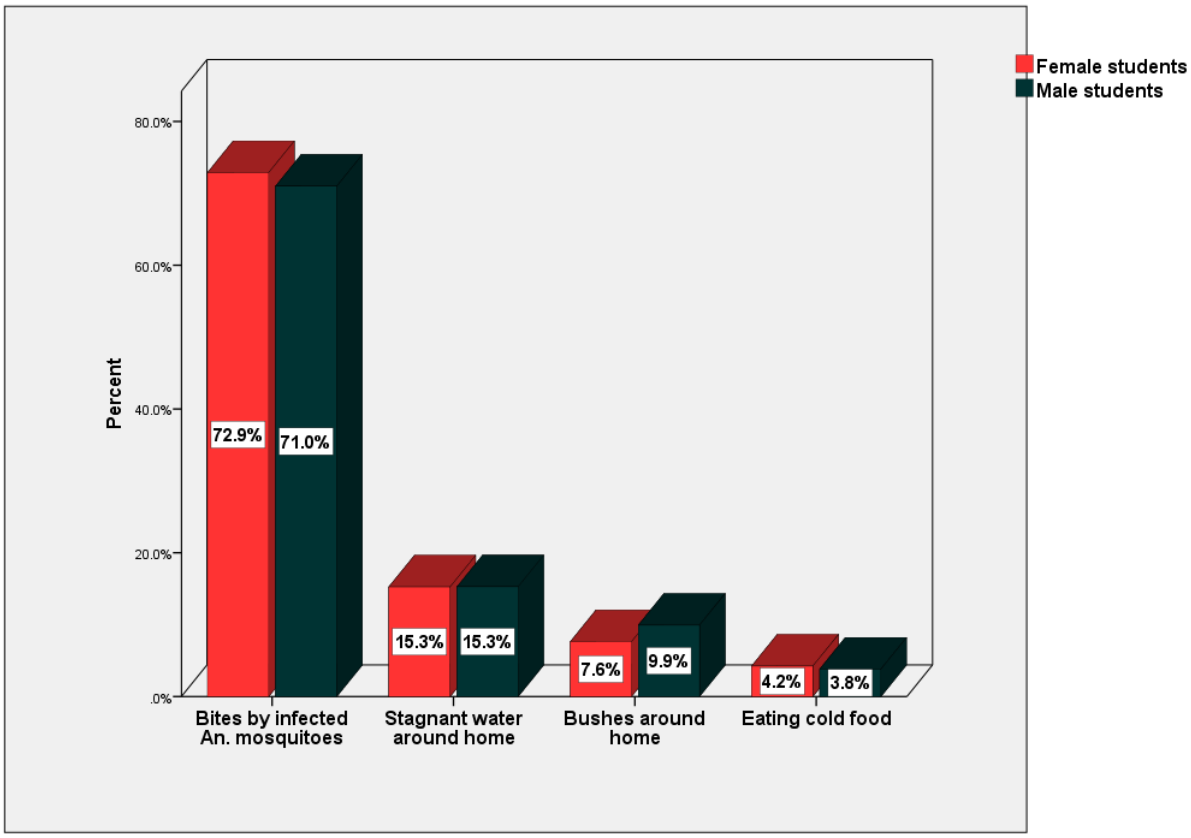
Fig. 24. Gender differences: transportation to school among the students included in the study.



4.6.3 Gender differences: malaria knowledge

72.9% of female and 71.0% of male students stated correctly that malaria is transmitted through bites of infected *An.* mosquitoes (Fig. 25). 15.3% of both sexes believed that stagnant water around their home is causing the disease. 7.6% and 9.9% of females and males thought that bushes around their home were causing malaria, respectively, and 4.2% of females and 3.8% of males attributed the disease to eating cold food.

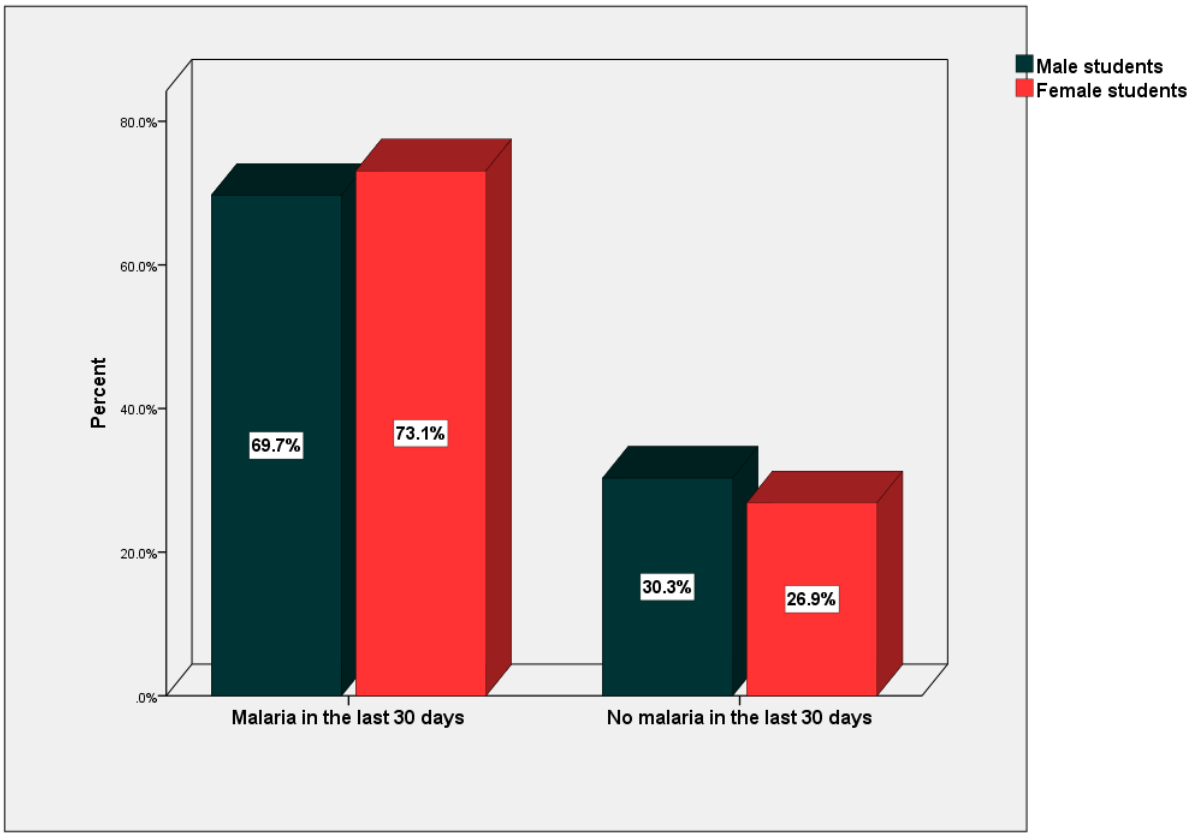
Fig. 25. Gender differences: knowledge about malaria transmission among the students included in the study.



4.6.4 Gender differences: reported malaria morbidity

69.7% of males and 73.1% of females reported that they had been sick of malaria in the last thirty days (Fig. 26).
In contrast, 30.3% and 26.9% of male and female students reported that they had not suffered from malaria in the previous thirty days, respectively.

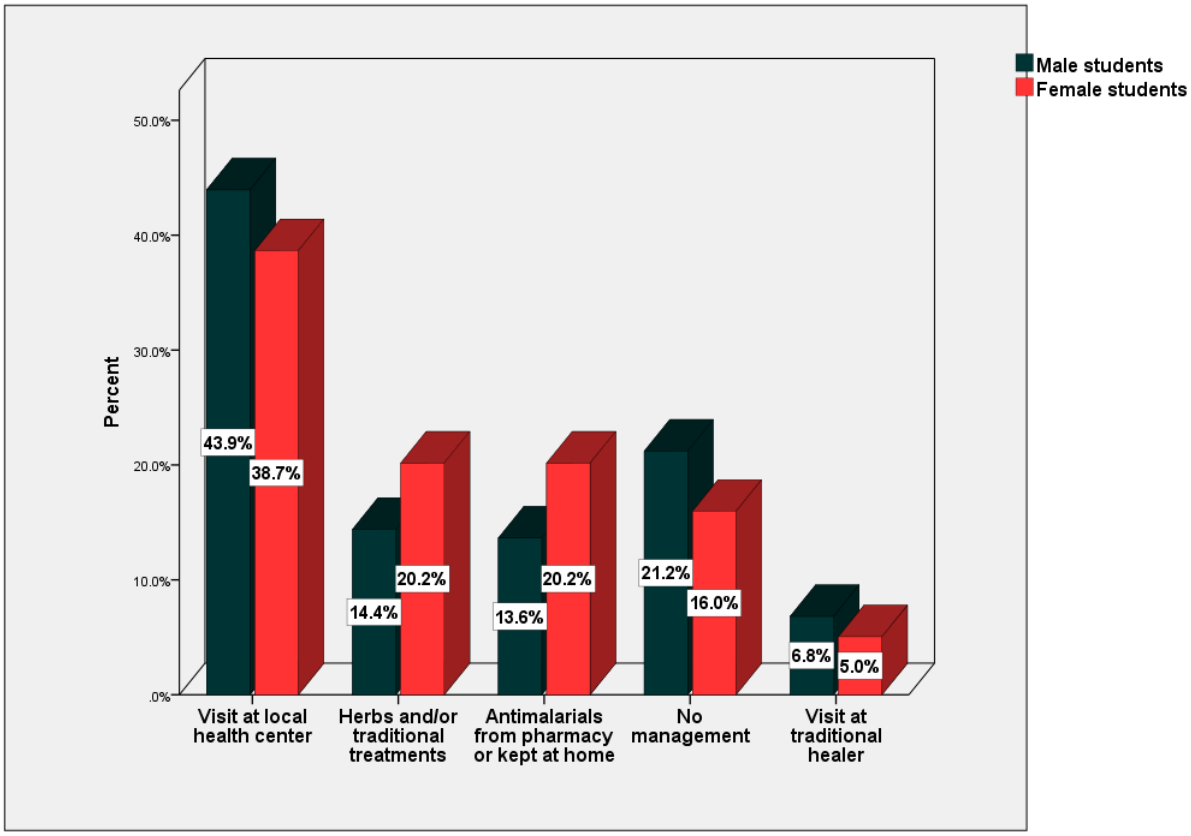
Fig. 26. Gender differences: reported malaria morbidity in the previous 30 days among the students included in the study.



4.6.5 Gender differences: malaria management

43.9% of male and 38.7% of female students stated to visit the local health center for malaria management (Fig. 27). While the same percentage of female students reported to treat malaria with herbs (20.2%) and antimalarial drugs (20.2%), the percentage of males choosing these two options for malaria treatment was lower (14.4% and 13.6%, respectively). 21.2% and 16.0% of male and female students stated to not follow a specific management plan for malaria, respectively. 6.8% and 5.0% of males and females answered to visit a traditional healer for malaria treatment, respectively.

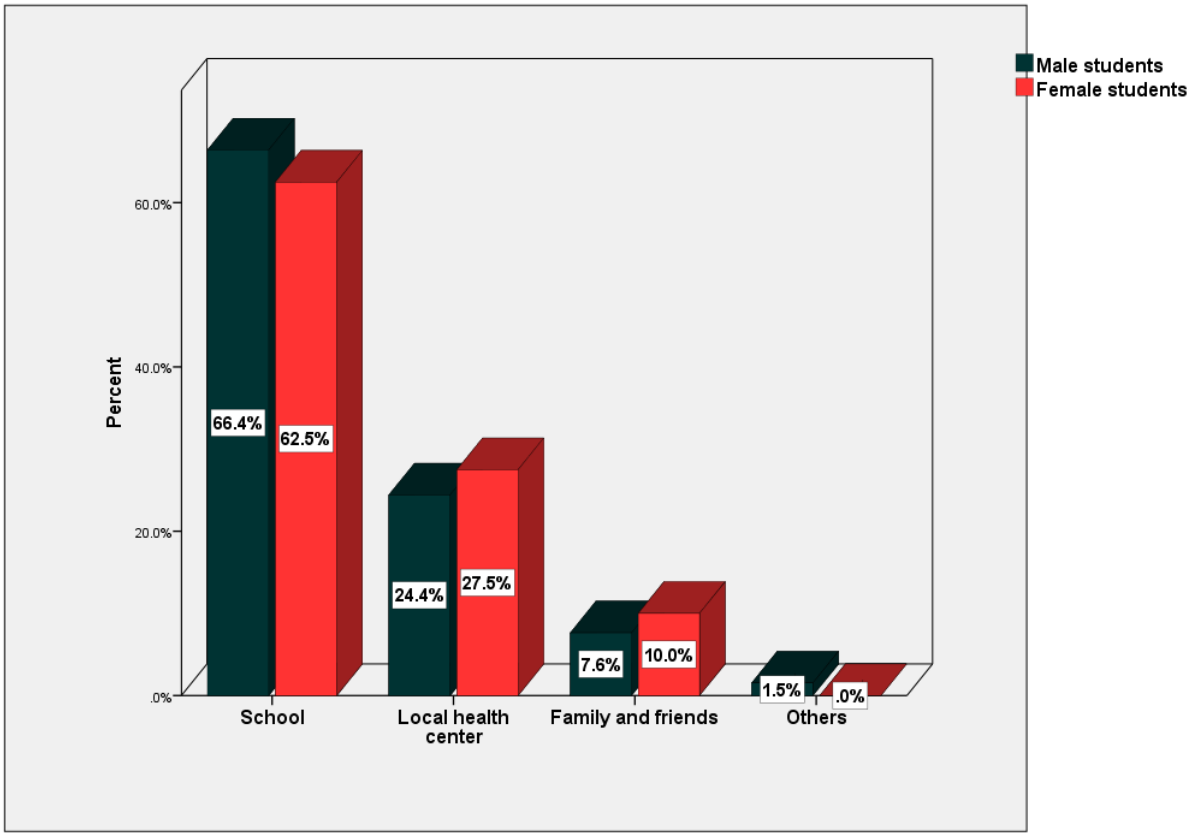
Fig. 27. Gender differences: malaria management among the students included in the study.



4.6.6 Gender differences: source of information

66.4% and 62.5% of male and female students stated that school is their prime source of information about malaria, respectively (Fig. 28). 24.4% and 27.5% of males and females indicated that their local health center was their prime information source about the disease, respectively, and 7.6% of males and 10.0% of females reported to primarily rely on family and friends for information about malaria. Very few students and among those only boys (1.5%) reported that radio and newspaper was an important source of information about malaria.

Fig. 28. Gender differences: source of information among the students included in the study.



5 Discussion

Mosquito nets, especially those treated with insecticides, are a well-established and evidence-based vector control method (Lengeler 2004). Pregnant women and children <5 years of age benefit most (Diallo *et al.* 2004, Gamble *et al.* 2007, Schellenberg *et al*. 2001).

In the present study, mosquito net usage among students in an east central Ugandan rural setting with stable, year-round malaria transmission was investigated (Uganda Bureau of Statistics 2010). This population is known to be at high risk for not using mosquito nets to prevent malaria - teenagers and males >15 years (Baume *et al.* 2007). Children <5 and pregnant women are the two groups most susceptible to malaria infection and death and a meta-analysis from nine large-scale household surveys conducted in six African countries showed that these two groups were found to use mosquito nets most frequently (Baume *et al.* 2007). In contrast, children aged 5-14 and males >15 were the two groups with the lowest probability to sleep under mosquito nets within a household in the countries investigated, followed by non-pregnant females >15. Since the majority of students enrolled in the present study were 15 years and older, the population investigated was a high risk group for not using mosquito nets for malaria prevention.

In the present study, more than half of the students (57.4%) reported to sleep without mosquito net. These results are consistent with two investigations. The first study, a recent survey including nearly 50,000 students from 480 schools in Kenya, showed that a very similar percentage of students (55.1%) reportedly slept without mosquito net (Gitonga *et al*. 2012). The second investigation, the national Ugandan Demographic Health Survey (UDHS), conducted in 2011, showed that 53.9% of 15-34 year-old individuals slept without a mosquito net (treated with insecticides or untreated) (Uganda Bureau of Statistics 2012). When focusing on the east central region, where the present study was conducted, the percentage of the total population reporting to sleep under a net was the lowest in all of Uganda (Uganda Bureau of Statistics 2012). In the present study and that conducted in Kenya, more female students reported to sleep under a net than male ones

(Gitonga *et al.* 2012). These findings are confirmed by the study of Baume and colleagues where men aged >15 were among the least likely individuals within a household to sleep under a mosquito net (Baume *et al.* 2007). The Ugandan demographic health survey 2011 also showed a similar trend in male and female mosquito net usage (Uganda Bureau of Statistics 2012). It is encouraging that more females reported to sleep under a net in all studies including this one since women in the reproductive age might not know immediately about pregnancy and they might not want to make a pregnancy public for a certain period of time by changing their sleeping habits (Baume *et al.* 2007). However, universal mosquito net usage for all populations at risk needs to be achieved to meet the Roll Back Malaria and WHO targets of a 75% reduction of malaria cases by 2015 compared to levels in 2000 (Roll Back Malaria 2008).

Since the socioeconomic background has shown to influence mosquito net usage in Uganda (Uganda Bureau of Statistics 2010, 2012), the housing conditions of each student was evaluated in the present study and correlated with mosquito net usage. Not surprisingly, students living in mud-walled houses were less likely to sleep under a mosquito net than students residing in tents or brick houses. Students living in brick houses showed the highest percentage of mosquito net users among all students. The gender distribution for housing was fairly balanced in the present study with a slightly higher percentage of female students living in mud-walled houses compared to males. These findings are confirmed by a recent study investigating the indicators of mosquito net usage in 643 Ugandan households (Nuwaha 2001). That study found that living in a permanent house was the strongest indicator for mosquito net usage among all factors investigated, followed by the educational level of the participants and owning of certain household assets. Not only housing influenced the mosquito net usage in the present study but also a relation between housing and reported malaria morbidity was evident. A higher percentage of students living in mud-walled houses reportedly had been sick of malaria in the thirty days prior to the survey than students residing in tents or brick houses.

Another parameter that influenced the mosquito net usage among students in the present study was the means of transportation to school. Students routinely

walking to school showed a lower percentage of mosquito net users than those riding their bike or those who were brought to school on a moped taxi ("bodaboda"). Students usually getting to school by means of public transport showed the highest percentage of mosquito net users. Looking at gender differences regarding transportation to school, a higher percentage of boys rode a bike to school than their female colleagues, but a higher percentage of girls were brought to school on a moped-taxi, used public transport, or walked compared to boys. Means of transportation to school may indicate the socioeconomic background. However, it also must be taken into account that means of transportation to school is not only determined by the students' socioeconomic background but is also influenced by the distance to school. Students living in close proximity to school may own a bike or afford to pay for a moped taxi or public transport; however, they may prefer to walk to school.

It has been demonstrated that the education level positively correlates with mosquito net usage in the Ugandan population (Uganda Bureau of Statistics 2010, 2012, Nuwaha 2001). Although the majority of students knew the cause of malaria infection in the present study, some students had difficulties to differentiate between the actual cause of malaria infection and risk factors for infection (e.g., bites of infected *An.* mosquitoes vs. stagnant water or bushes). Stagnant water and leaves as well as leaf axils of certain plants or bushes where water accumulates may serve as mosquito breeding grounds but are not the actual cause of disease. There was no major gender difference in the knowledge about malaria transmission. The percentage of students who were correctly informed about the cause of malaria was slightly lower in the present study compared to the population investigated in the Uganda Malaria Indicator Survey (UMIS) 2009 (Uganda Bureau of Statistics 2010); however, that survey analyzed the total population and not students in particular. When looking at perceived malaria symptoms, it is surprising that cough was reported most frequently, followed by headache, flu-like symptoms, fever, and weakness. Diagnosing malaria based on symptoms alone can be challenging even for a clinician, given the variety of presentations related to the disease. Therefore, parasitological confirmation is endorsed for all suspected cases if possible (WHO 2012a). However, the classic symptoms of fever and chills expected to be reported more frequently in the

present study, ranked only fourth and ninth, respectively, according to the frequency of each symptom stated. Headache and flu-like symptoms are indeed common presentations of malaria and were reported second and third most frequently. The fact that cough ranked first confirms that malaria may mimic respiratory tract infections and vice versa or even co-infections may occur (Thompson *et al.* 2012).

Looking at different means of malaria prevention, sleeping under a mosquito net was the intervention method most students were aware of. This is an encouraging finding suggesting that most students knew well about the effectiveness of mosquito nets for malaria prevention. These results are consistent with data from Uganda's Malaria Indicator Survey 2009 which shows that most people are aware of mosquito nets for prevention, followed by destroying mosquito breeding sites, taking preventive medication, and spraying the walls of the house with insecticides (indoor residual spraying IRS) (Uganda Bureau of Statistics 2010). Although awareness for prevention was high, self-reported malaria morbidity in the month prior to the survey was high (71.1%) in the present study. Unfortunately, there is only limited data about malaria prevalence in the same age group and geographic location available. However, parasite prevalence estimates for children under 10 years of age for east central Uganda are extremely high exceeding 60% (Yeka *et al.* 2012). Uganda's national Malaria Indicator Survey 2009 investigated children < 5 in different regions throughout the country and demonstrated a malaria prevalence of 52% based on RDTs and 42.4% based on microscopy (Uganda Bureau of Statistics 2010). The rural pediatric population showed a significantly higher parasite prevalence than urban pediatric populations (56.0% vs. 28.6% based on RDT and 47.1% vs. 15.3% based on microscopy). Remarkably, the east central region, where the present study took place, showed the second highest prevalence in all of Uganda (65.2% based on RDT and 56.2% based on microscopy) (Uganda Bureau of Statistics 2010). A study looking at the parasite prevalence of 740 primary school children in the mid-eastern region of Uganda showed a lower prevalence rate of about 30% based on microscopy (Nankabirwa 2013). Another study comparing malaria prevalence rates throughout Uganda demonstrated significant differences in parasite prevalence ranging from 7% in South and Northwest Uganda up to 75% on the eastern shore of Lake Victoria

(Stensgaard *et al.* 2011). However, limited data about parasite prevalence in the same age group and region make a comparison difficult.

It is encouraging that about three quarters of students who reportedly had not been sick of malaria in the month prior to this study slept under a mosquito net. In contrast the majority of students who confirmed that they had been sick of malaria in the previous thirty days had not used a mosquito net for prevention. No major gender difference in reported malaria morbidity became evident.

When the health care seeking behavior of the students was investigated, it turned out that visiting the local health center for malaria treatment was fairly popular. In contrast, the percentage of students not following a clear management plan when experiencing malaria symptoms was relatively high. Self-treating malaria with antimalarial drugs was equally popular among students as using herbs or other traditional treatments. Interestingly, only a small percentage of students stated to visit a traditional healer for malaria treatment. Some gender differences in the malaria management were evident in the present study. While a slightly smaller percentage of female students reported to visit the local health center for malaria management, a higher percentage of females stated to use herbs and antimalarial drugs in the present study. A slightly higher percentage of male students reportedly did not follow a specific management plan. When looking at the correlation between malaria management and mosquito net usage, students without specific management plan had the lowest percentage of reported mosquito net use, followed by those treating malaria with herbs or other traditional treatments and those visiting a traditional healer. Students visiting the local health center for treatment showed the highest percentage of reported net use, followed by students taking antimalarial drugs from a pharmacy or stored at home. These findings are not surprising since students who visit the local health center or purchase antimalarial drugs from a pharmacy are usually from wealthier families and therefore can afford a mosquito net. Moreover, students seeking treatment at health centers or buying drugs from pharmacies are also more exposed to health information provided at such centers including malaria prevention.

Remarkably, the students' malaria management varied according to their housing condition in the present study. The highest proportion of students living in mud-walled houses did not report a specific management plan for malaria. Some of these students certainly are from very poor families and thus occupied by the struggle of daily life in rural Uganda. Among students from mud-walled houses, malaria treatment with herbs and or other traditional treatments were popular. However, the percentage of students who visited the local health center for treatment or taking antimalarial drugs from a pharmacy or stored at home was low in this group. Visiting a traditional healer was very uncommon in students from mud-walled houses. In contrast, the vast majority of students living in brick houses reported to visit the local health center for management of disease followed by management with antimalarial drugs from a pharmacy or stored at home. Just a few students did not have a management plan or used herbs and/ or other traditional treatments. The traditional healer ranked last. The percentage of students visiting the local health center was more than two times higher in the brick house group than in the mud-walled house group (44.6% vs. 18.2%). This may not only be related to different perceptions of and trust in various forms of malaria treatment but also due to the accessibility of a health center from remote rural dwellings. For students living in brick houses, it simply may be less difficult to afford the travel to a health facility than students from mud-walled houses. In contrast, the percentage of students treating malaria with herbs and/ or other traditional treatments was found to be almost two times higher in the mud-walled house group than in the brick house group (29.5% vs. 15.1%). The reason for this may be that students from mud-walled houses use the resource they have access to rather than being able to choose between various treatment options. Therefore, it is important to consider that malaria management is not necessarily driven by the students' believes regarding efficacy or safety of a treatment but more likely by the feasibility of a management option from an individual point of view.

When investigating where the students got their malaria education from, school was the main source of knowledge for almost to two thirds of students, followed by the local health center, and family and friends. The importance of other sources such as radio and print media was very limited. School was a particularly important provider of malaria information for students living in mud-walled houses because a

smaller percentage of those students had access to information provided in local health centers compared to students living in brick houses. Moreover, results obtained from the present study indicate that health topics are addressed more frequently in families living in brick houses. There were almost no gender differences regarding the source of information. A slightly higher percentage of girls reported that the health center and family and friends were important in providing information about malaria than boys.

Limitations

One of the limitations of the present study is that each student's household was not visited to confirm mosquito net usage at home. In rural Africa, this is time-consuming, logistically difficult, and expensive. Monitoring the impact and coverage of malaria intervention programs has proven extremely difficult in some African countries including Uganda (WHO 2012a, Yeka *et al*. 2012). While coverage of mosquito nets for malaria prevention has been estimated on the number of nets distributed in the public and private sector and in net distribution campaigns, relatively few studies investigate the actual use of prevention methods. The inability to monitor prevention methods on a regular basis has been attributed to the lack of rapid and simple assessment methods (Ndyomugyenyi *et al.* 2007). A study conducted in Uganda including more than 3600 school children from 39 schools tried to estimate community coverage of mosquito nets based on reports from school children provided in a questionnaire at school (Ndyomugyenyi *et al.* 2007). Simultaneously, almost 2800 households were visited in the catchment area of the schools and the mosquito net coverage was assessed. Interestingly, when comparing the school surveys with the household surveys, it became apparent that students provided a very accurate approximation of mosquito net coverage in the communities (±5% difference between the school and household surveys) (Ndyomugyenyi *et al.* 2007). Although households were not visited in the present study, results strongly correlate with Uganda's recent national demographic health survey (UDHS) 2011, one of very few surveys in Uganda to date that collected health data on a household level from a large,

nationally representative population (Uganda Bureau of Statistics 2012).

Another limitation of the present study is that the students were asked to provide a self-assessment whether they had been sick of malaria in the thirty days prior to the survey but invasive diagnostic testing was not performed. It is extremely difficult to assess the reliability of self-reported malaria. A study conducted in the Peruvian Amazon compared self-reports of malaria morbidity from a household survey with reports on the same individuals based on microscopy performed in the local health center. This study reported an accurate estimate of malaria prevalence, in particular for shorter periods of time (Maheu-Giroux *et al.* 2011). Although self-reports are not highly specific or sensitive, they provide a good estimate of malaria morbidity and are commonly used to assess disease burden as it is experienced by a population and to further plan intervention methods (Yé *et al.* 2007, Subramanian *et al.* 2009).

6 Conclusion

The mosquito net usage among students attending a secondary school in east central Uganda was found to be low. The highest percentage of mosquito net usage was among students who lived in brick houses, used public transport to school, routinely visited the local health center for malaria treatment, did not report malaria morbidity in the thirty days preceding the survey, and who were female. The lowest percentage of mosquito net usage was among students who lived in mud-walled houses, usually walked to school, did not follow a specific management plan when being sick of malaria, who reportedly had been sick of malaria in the thirty days preceding the survey, and who were male. These findings are consistent with the national data for mosquito net usage in Uganda in which the use of mosquito nets falls short of the recommended levels of usage. Consequently, a significantly increased distribution of mosquito nets is most urgently needed, especially in Uganda's rural areas, to decrease numbers of preventable deaths and protect all individuals at risk including secondary school students.

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TABLE OF FIGURES

Adnex 1

Dear student,
Please fill in the following questionnaire truthfully and without the help of your neighbor or teacher! The collected information does not influence your school grades in any way, has no consequence on your school performance or on you individually and is for informational use only! When there is a choice, please mark the circle (o) located in front of the answers. A correct answer looks like this: X (answer). Thank you very much for your effort and honesty! Webale nyo! – Benjamin Kraler

Surname:_____ **First name:**_____
age:_____ years **Sex:** **o** male **o** female
village/town you live in_____

1) You live in a: **o** brick house **o** mud-walled house **o** other: _____
2) With how many people do you live together in your house? _____ people.
3) How do you usually get to school? **o** walking **o** by bicycle **o** by public transport **o** by bodaboda
4) How much time does it take to get from your home to school every morning? ___minutes.
5) Do you know how you get malaria? **o** due to stagnant water around the house **o** due to bushes around the house **o** due to eating cold food **o** due to bites by infected anopheles mosquitoes
6) Do you know the signs or symptoms when you suffer from malaria? Please name signs you know (not more than 5):
_____
7) Do you know how you can protect yourself from getting malaria? **o** by wearing long clothes after dusk **o** by sleeping under a mosquito net **o** by staying indoors at night **o** I don't need any protection, because I'm strong **o** by removing stagnant water around the house

8) Do you sleep under a mosquito net? **o** Yes **o** No
9) If yes how often do you sleep under the net? **o** less than once a week **o** 2 -3 nights per week **o** every night.
10) If you don't sleep under a net, please give reasons: **o** I can't afford a net **o** I don't think mosquito nets prevent from malaria **o** I don't use a net, because_____
_____
_____

11) Did you suffer from malaria within the last month? **o** Yes **o** No
12) Did you suffer from malaria within the last two months? **o** Yes **o** No

13) When you suffer from malaria, what do you usually do? **o** nothing, I just wait until it gets better **o** I visit a traditional healer **o** I use herbs and traditional treatments kept at home **o** I take pills from a pharmacy or kept at home **o** I visit the local health center (if so, please add the name: _____)
14) Where did you learn about malaria? **o** in school **o** from my family and friends **o** at the local health center **o** others, please specify:_____

Thank you!