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Full Length Research Paper

Plasmodium falciparium transmission intensity in Nyabushozi County, Kiruhura district, Uganda

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The prevalence of malaria in human beings and *Plasmodium falciparum* sporozoite infection in *Anopheles* mosquitoes were studied for seven months in eight villages in Nyabushozi County, Kiruhura District, Uganda. The aim of the research work was to assess *Plasmodium* sporozoite infection rates using enzyme-linked immunosorbent assay and link this with malaria prevalence. A total of 2566 female *Anopheles gambiae* s.l. and 270 *Anopheles funestus* group were collected in 24 households using pyrethrum spray catches, and from goats and cattle housing (kraals), using CDC light traps. The densities of female *An. gambiae* s.l. and *An. funestus* group in all the eight villages studied were significantly influenced by rainfall. *P. falciparum* sporozoite rate for *An. gambiae* s.s. ranged from 0.84 - 5.26%. A total of 4000 people were involved in four epidemiological rounds of malaria surveys. The mean prevalence of parasitaemia was 17.4% for all ages combined and 22.8% for the 5 - 9 year age group. The four separate surveys gave ranges of 12.5 - 22.2% for all ages combined and 17.8 - 25.8% for the 5 - 9 year old children. It is evident from this study that malaria transmission in Nyabushozi County is fairy moderate and perennial, and maintained predominantly by *An. gambiae* s.s.

Key words: *Plasmodium falciparum*, sporozoite rate, seasonal dynamics, Uganda.

INTRODUCTION

In Uganda, malaria ranks as the leading cause of morbidity and mortality, especially in children under five years (Langi et al., 2001). In 2006, Uganda had an estimated 10.6 million malaria cases (WHO, 2008). It is estimated that over 90% of the population in Uganda lives in highly endemic areas with perennial transmission, while only 10% live in low transmission areas that are prone to malaria epidemics (MOH, 2002). Records show that malaria accounts for 25 - 40% of outpatient visits to health facilities, 20% of hospital admissions and 9 - 14% of inpatient deaths (MOH, 2000). Of the deaths, 11 and 23% occur among children under 5 years in low and high transmission areas, respectively.

The most common vectors of malaria in Uganda are *An. gambiae* s.l. and *An. funestus* group with *An. gambiae s*.l. being the dominant species in most places. *Anopheles funestus* group are common in high altitude areas and during the short dry seasons when permanent water bodies are the most common breeding sites

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Table 1. Sample size of the target communities in the four sub-counties of Nyabushozi County, Kiruhura district.

Sub-county	Parish	Village	Total Number of households in each village	Number of Households sampled
Kenshunga	Nshwerenkye	Mugore	92	3
	Rushere	Akatongore	96	3
Nyakashashara	Nyakahita	Katooma	62	3
	Rurambira	Kakyeera	74	3
Sanga	Rwabarata	Kiribwa	113	3
	Nombe II	Ntuura	40	3
Kikatsi	Kanyanya	Ifura	58	3
	Kayonza	Rugaaga	55	3
Total			590	24

Source: SIMA-Uganda (2004): Household census survey in Nyabushozi County.

(Uganda malaria control strategic plan 2005 - 2010 unpublished document). From the baseline survey by System-wide Initiative on Malaria and Agriculture (SIMA) program Uganda in 2002, the community alleged that settlement in Nyabushozi County had led to increase in malaria and that nomadic pastoralists were less affected by malaria. However, the causes of increase in the occurrence of malaria and the level of vectors infection were not known. This research work aimed at assessing malaria transmission intensity (*Plasmodium* infection rates) in relationship to malaria prevalence in Nyabushozi County.

MATERIALS AND METHODS

Study area

The Kiruhura District (formerly part of Mbarara District) is located in southwest of Uganda. The district is part of the great East African rift valley system. The altitude is generally 200 m above the sea level and there is a wide variation in topology and climatic conditions. The terrain comprises flat areas with rolling and undulating hills interspersed by wide valleys. The major pre-occupation of the population is animal farming. Subsistence crop farming mainly of food crops (bananas, maize, sorghum, irish potatoes, and plantains) is also undertaken. The district has a population of approximately 120 persons per square kilometer.

Nyabushozi County is located in the North and Northeast of the Lake Mburo National Park within the Lake Victoria catchment, but lies in the rain shadow of the Kabula hills, which imparts a semi-arid type of climate. It receives a substantial amount of rainfall twice a year (1,000 - 2,777mm) between April and June, and November and December.

The county has seven subcounties: Kenshunga, Nyakashashara, Sanga, Kikatsi, Kinoni, Kashongi and Kanyareru, covering 647 square kilometers. Current statistics (UBOS, 2005) give a figure of 100,630 people in Nyabushozi according to the 2002 population and Housing Census Main Report. It has the largest cattle population in the district, dominated by the indigenous Ankole breeds. The households have mainly grass thatched, igloos, semi permanent (mudded or un burnt blocks with iron sheets) and permanent houses (burnt bricks and iron sheets). Recently, the county started experiencing some changes in livelihood patterns from a pastoral to an agro-pastoral production system, which involved a shift from living in traditional huts (grass-thatched and mudded), usually sited very close to kraals to living in permanent structures located far from animal housing.

Sample size

Field surveys were conducted in four subcounties: Kenshunga, Nyakashashara, Sanga and Kikatsi, for seven months from October 2004 to April 2005. Household Census survey in Nyabushzi County was previously done by SIMA-Uganda project, 2004. Ethical approval of this study was obtained from the Uganda National Council for Science and Technology. Household heads were informed about the study objectives, and informed consent was obtained from them before being recruited into the study. Household heads agreed for their homes to be sprayed with knock-down sprays to immobilize mosquitoes. Collections of adult *Anopheles* mosquitoes were done twice during rainy season, that is, October to November and November to December 2004 (with a spell of dry season in December) and twice during the dry season, that is, January and February 2005 in order to get the general impression of sporozoite infection situation. Two villages from each sub-county were chosen purposively for sampling of mosquitoes as shown in Table 1. From each village, three households pastoralist (nomads), transition farmer and a settled farmer were sampled throughout the study period except when circumstances required substitution of households.

Sampling of adult mosquitoes

Collection of adult mosquitoes in households was done between 8 a.m. and 12 noon. Latitude and longitude data were recorded for the sampled sites (household, cattle kraals and goats houses) using a hand-held navigational system, Global Positioning System (GPS). Adult mosquitoes were collected from the inside of selected households using pyrethrum spray collection (PSC) method (WHO, 1975). Information was also collected on household number; number of people who slept in the house the previous night, types of houses, and use of indoor residual spraying.

For outdoor catches , Centre for Disease Control and Prevention

(CDC) light traps were installed in goat's houses and cattle kraals throughout the night between 6:00 p.m. and 7:00 a.m. We used CDC light traps on outdoor catches so that we could get mosquitoes in the goat's dip and around cattle kraals so as to ascertain whether they are potential vectors of malaria. The traps were deployed in the same households on the same day they were sprayed. The collected mosquitoes were processed and identified according to Gillies and Coetzee (1987). Samples of *Anopheles* female mosquitoes were preserved individually in a 1.5 ml eppendorf tubes having silica gel separated with cotton wool and stored at ambient room temperature at the field station. The preserved mosquitoes were later transferred to National Livestock Resources Research Institute, Tororo for sporozoite ELISA testing.

P. falciparum sporozoite enzyme-linked immunosorbent assay

Preserved cephalic and abdominal parts of mosquitoes were screened, using a *P. falciparum* sporozoite ELISA procedure (Wirtz et al., 1987). The assay identifies the Circumsporozoite (CS) antigens of *P. falciparum.* The sensitivity and specificity of the procedure is based on the monoclonal antibodies (Mabs) used. The ELISAs detect CS proteins, which can be present in the developing oocyst, in haemolymph and on the sporozoite present in the haemocoel or salivary glands. Horse–Radish Peroxidase (HRP) conjugated with monoclonal antibodies was used as detector for the CS monoclonal antibody-antigen complex and substrate, Azino-bis-3-ethyl-benzthiazoline sulfonic acid (ABTS) provided in the ELISA kit (CDC, Atlanta, USA) with hydrogen peroxide as an indicator. Each test plate was read at 405 nm using ELISA plate reader (Dynatech). After measuring the optical density values, the standard curves were made and each reaction evaluated. Samples with an optical density value of 2 X the mean of negative controls were considered positive. Positive controls equivalences were determined to be 200 pg of CS antigen amounting to 3,200 *P. falciparum* sporozites.

Ribosomal DNA-polymerase chain reaction assay

The legs and wings of individual adult mosquitoes were plucked out and put in separate labeled tubes. Each mosquito leg or wing was homogenized in a sodium-Tris-edetic acid (EDTA) buffer (0.1M NaCl, 10mM Tris, 1mM EDTA, pH 8.6) and incubated at 94°C for 10 min. Cell debris was precipitated by centrifuging at 800 g for 1 min and 1 µl of DNA was used for PCR. Polymerase chain reaction (PCR) amplification of Deoxyribonucleic acid (DNA) from legs and wings was performed for *An. gambiae* s.l. complex as described by Scott et al. (1993) and for *An. funestus* group as described by Koekemoer et al. (2002). Primers used were specific for *An. gambiae* s.s. and *An. arabiensis*, and the universal primer for *An. gambiae* complex was also used to detect any other species other than the aforementioned two species. Primers for *An. funestus, An. leesoni, An. parensis,* and *An. rivulorum* were also used for detection of the species (Loekemoer et al., 2002) and the universal primer for *An. funestus* group was also used.

Amplification conditions for *An. gambiae* s.l. complex was carried out in a total volume of 25 µl of reaction mixture containing 1.5 mM $MgCl_2$, 1X PCR buffer, 200 µM each of dATP, dGTP, dTTP, dCTP, and 0.625 units of *Taq* DNA polymerase (Promega Corporation, Madison, USA) and sufficient sterile water. Test samples and reagents were appropriately dispensed into each tube after which the tubes were capped and run in the PCR machine (GeneAmp PCR system 9700 made by PE Biostems Foster, California USA). The amplification for *An. gambiae* s.l. complex involved a denaturation step at 94°C for 5 min, followed by 30 cycles each at 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, with a final extension step at 72°C for 10 min. The PCR amplification conditions for *An. funestus* group involved denaturation step at 94°C for 2 min, followed by 30 cycles each at 94°C for 30 s, 45°C for 30 s, 72°C for 40 s, with a final extension step at 72°C for 5 min. 1.5 and 2.5% of agarose gels (Sigma-Genosys, United Kingdom) were used for PCR products of *An. gambaie* s.l. and *An. funestus* group, respectively.

Malaria prevalence in human

The baseline epidemiology study aimed to determine the age profile of malaria parasitaemia in the inhabitants of the eight study villages in Nyabushozi County. Surveys were carried out by household visits for four times throughout the year and involved 4000 people. All the members in the household were screened by taking thick and thin blood slides and read promptly so that results and appropriate therapy could be provided to the people.

Data analysis

Data was entered into a computerized database SPSS version 6.0 and then exported to Microsoft Excel and verified to identify data entry errors. Verified data was analyzed using descriptive statistic.

RESULTS

A total of 24 households were sprayed using PSC method in eight villages in Nyabushozi County between October 2004 and April 2005. The total number of female *An. gambiae* s.l. and *An. funestus* group mosquitoes caught in indoor spraying in all the four rounds of surveys, in all the eight villages, were 1544 and 186, respectively. A total of 430 *An. gambiae* s.l. and 164 *An. funestus* males were also caught. However, some *Culex* sp. (n = 12), *Mansonia* sp. (n = 3) and *Ae. aegypti* (n = 1) were also collected during the four rounds of this study.

The average numbers of *An. gambiae* s.l. females caught per night varied greatly among the eight villages, ranging from 0.6 - 68.3 females per household. Mugore village recorded the highest average number of 68.3 females per household in November- December 2004, while Kakyeera had the lowest catch of only 0.6 females per household in January – February 2005.

Looking at the different surveys, the number of mosquitoes collected showed seasonal variations, with the highest catch of female *An. gambiae* s.l. and *An. funestus* group being recorded in the wet season or soon after the rains (Table 2). Mugore village had the highest female *An. gambiae* s.l. catch in the second survey when compared to other villages, while Kakyeera village recorded the highest female *An. funestus* group catches throughout the study.

P. falciparum sporozoite rate in *An. gambiae* and *An. funestus* group

A total of 811 (51.8%, n = 811/1566) *An. gambiae* s.l. and

Table 2. Results of ELISA sporozoites rates in *An. gambiae* and *An. funestus* in Nyabushozi County, Kiruhura district.

Village	*An. gambiae*				*An. funestus*			
	Total No. Collected	Number Tested	Number Positive	Sporozoite rate (%)	Total No. Collected	Number Tested	Number Positive	Sporozoite rate (%)
Mugore	318	135	5	3.7	19	19	0	0
Akantogore	143	104	4	3.85	14	14	0	0
Katooma	162	119	1	0.84	3	3	0	0
Kakyeera	166	60	2	3.33	198	64	1	1.56
Kiribwa	248	124	4	3.23	23	6	0	0
Ntuura	207	95	5	5.26	9	9	0	0
Ifura	205	86	1	1.16	2	2	0	0
Rugaaga	115	88	1	1.14	2	2	0	0
Total	1566	811	23	2.84	270	119	1	0.84

The highest and lowest sporozoite infection rates in *An. gambiae* s.l. were recorded in Ntuura and Katooma villages respectively. Sporozoite infection in *An. funestus* group was only recorded in Kakyeera village.

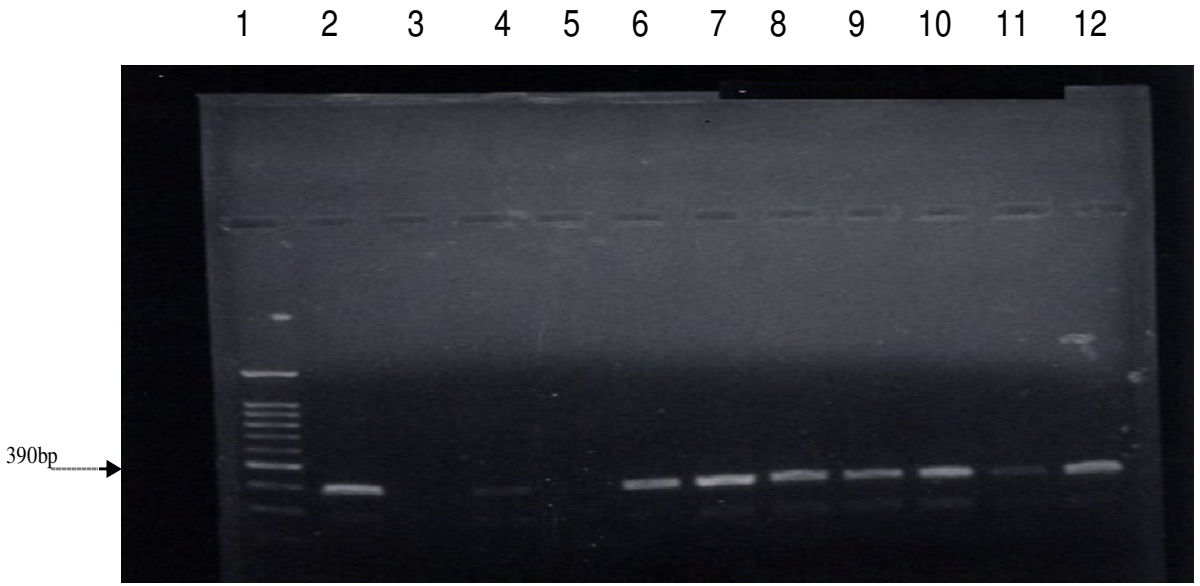

Figure 6. 1.5% Agarose gel electrophoresis of *An. gambiae* PCR product. Lane 1, Molcular marker, Lanes in mosquito samples amplified by rDNA primers, Lane 2 positive control, lane 3 negative control, lane 5, 6, 7, 8, 9, 10, 11, and 12 are products of *An. gambiae* s.s.

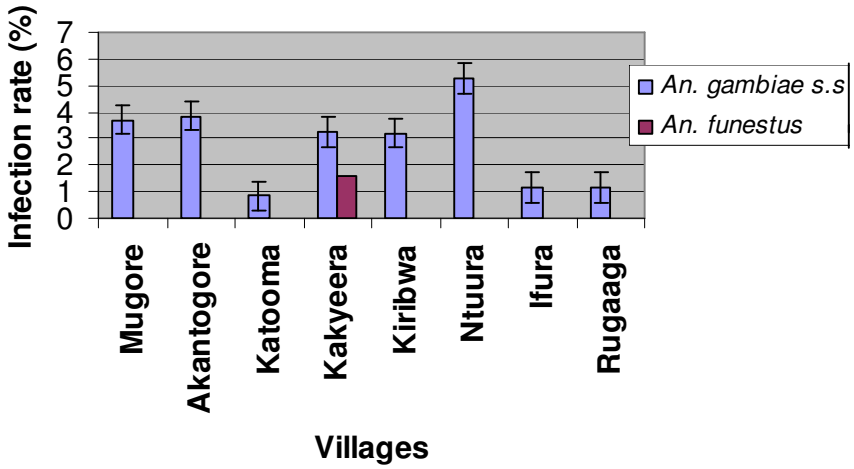

Figure 1. Comparison of sporozoite infection rates among eight villages in Nybushozi County. There were no significant differences in sporozoite infection rates in *An. gambiae* s.l. across the eight villages. The highest and lowest sporozoite infection rates in *An. gambiae* s.l. were recorded in Ntuura and Katooma villages respectively. Sporozoite infection in *An. funestus* group was only recorded in Kakyeera village.

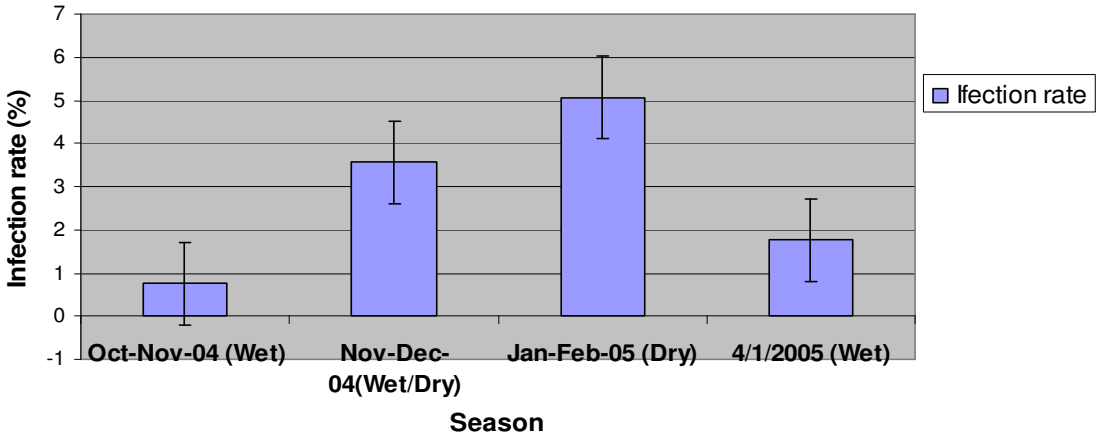

Figure 2. Seasonal comparison of malarial sporozoite infection rates. There was seasonal variation in malaria sporozoite rates with dry seasons (November, December, January and February) having highest infection rates. The standard error bars indicate significant different in infection rates in dry season as compared to rainy season.

119 *An. funestus* group (44.1%, n = 119/270) mosquitoes were analyzed using ELISA technique for malaria sporozoite detection. The circumsporozoite antigen of *P. falciparum* was detected in 2.84% (23 of 811) of *An. gambiae* s.l. samples tested. Using the technique described by Wirtz et al. (1987), we estimated the number of sporozites in 24 positive mosquitoes. Based on optical density values obtained with an ELISA, sporozite estimates in infected mosquitoes ranged from 500 - 17,700. Out of the 23 positive samples, 6 (26.1) were identified as *An. gambiae* s.s. by PCR test (Figure 6). Only 0.84% (1/119) of *An. funestus* group was found positive. The mean sporozoite rates for both *An. gambiae* s.l. and *An. funestus* group in the eight villages are shown in Table 2.

Significant difference was recorded in the mean *P. falciparum* sporozoite rates for *An. gambiae* s.l. (2.84%, 23/811) and *An. funestus* group (0.84%, 1/119) over the study period ($p < 0.05$). However, the highest sporozoite rate (5.26%) for *An. gambiae* s. l. was recorded in Ntuura village (Figure 1). This was closely followed by Akatongore (3.85%) and Mugore (3.7%) villages, but with no significant difference in infection rates between them ($p = 0.445$).

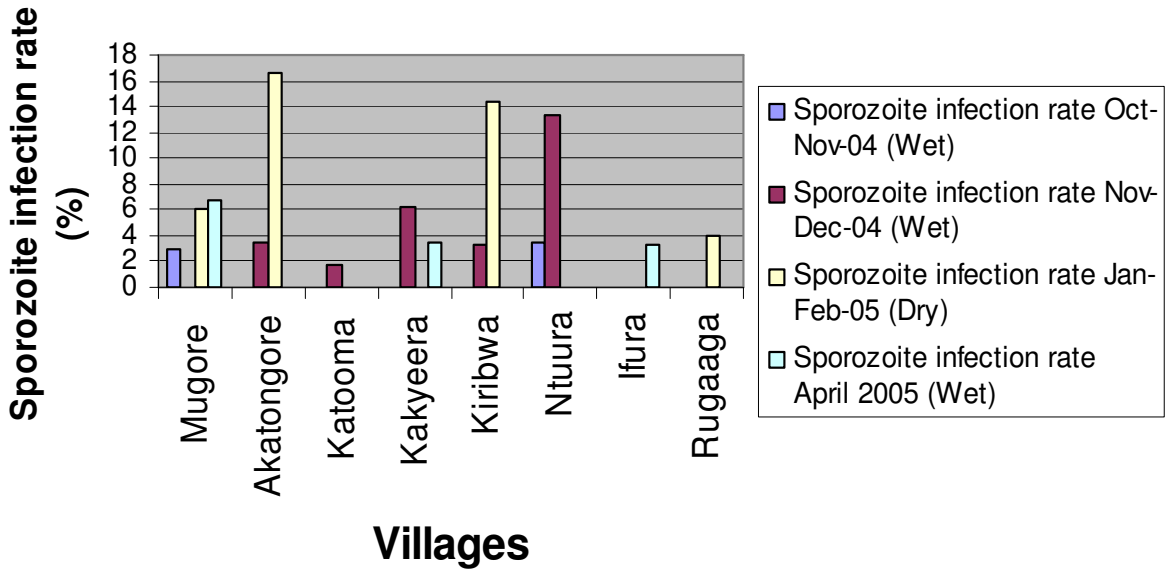

Figure 3. Comparison of sporozoite infection rates across eight villages in Nybushozi County. There were variations in sporozoite infection rates from season to season across the eight villages. Akatongore and Kiribwa villages recorded highest infection rates in dry season (January to February) while Ntuura recorded highest infection rates in rainy season mostly in the month November.

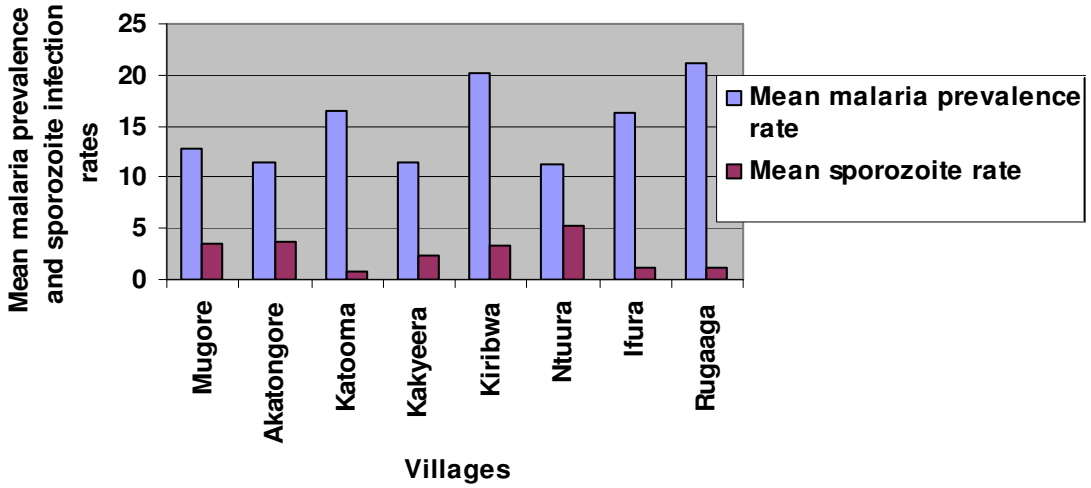

Figure 4. Comparison of mean malarial prevalence and sporozoite infection rates in eight villages in Nybushozi County. The high malaria prevalence across the eight villages is able to be maintained by low sporozoite infection rates in *Anopheles* mosquitoes in Nyabushozi County. Rugaaga and Kiribwa recorded highest malaria prevalence.

An. funestus group had the lowest sporozoite rate of 1.54% (1/65) and it was only recorded in Kakyeera. However, the mean *An. funestus* group sporozoite rate across all the villages was 0.84% (1/119).

Seasonal variation in sporozoite rates

Sporozoite infection rates showed seasonal variations, with the highest mean infection rates of 5.1 and 3.5% recorded in dry season (January - February, 2005) and at the beginning of dry season spell (November - December, 2004) respectively, (Figure 2). The lowest sporozoite infection rates of 0.8 and 1.8% were recorded in wet seasons, October-November 2004 and April 2005, respectively. Significant differences in sporozoite infection rates occurred between dry and wet seasons ($p < 0.05$).

There are also variations in sporozoite infection rates during different seasons across the villages. Akatongore

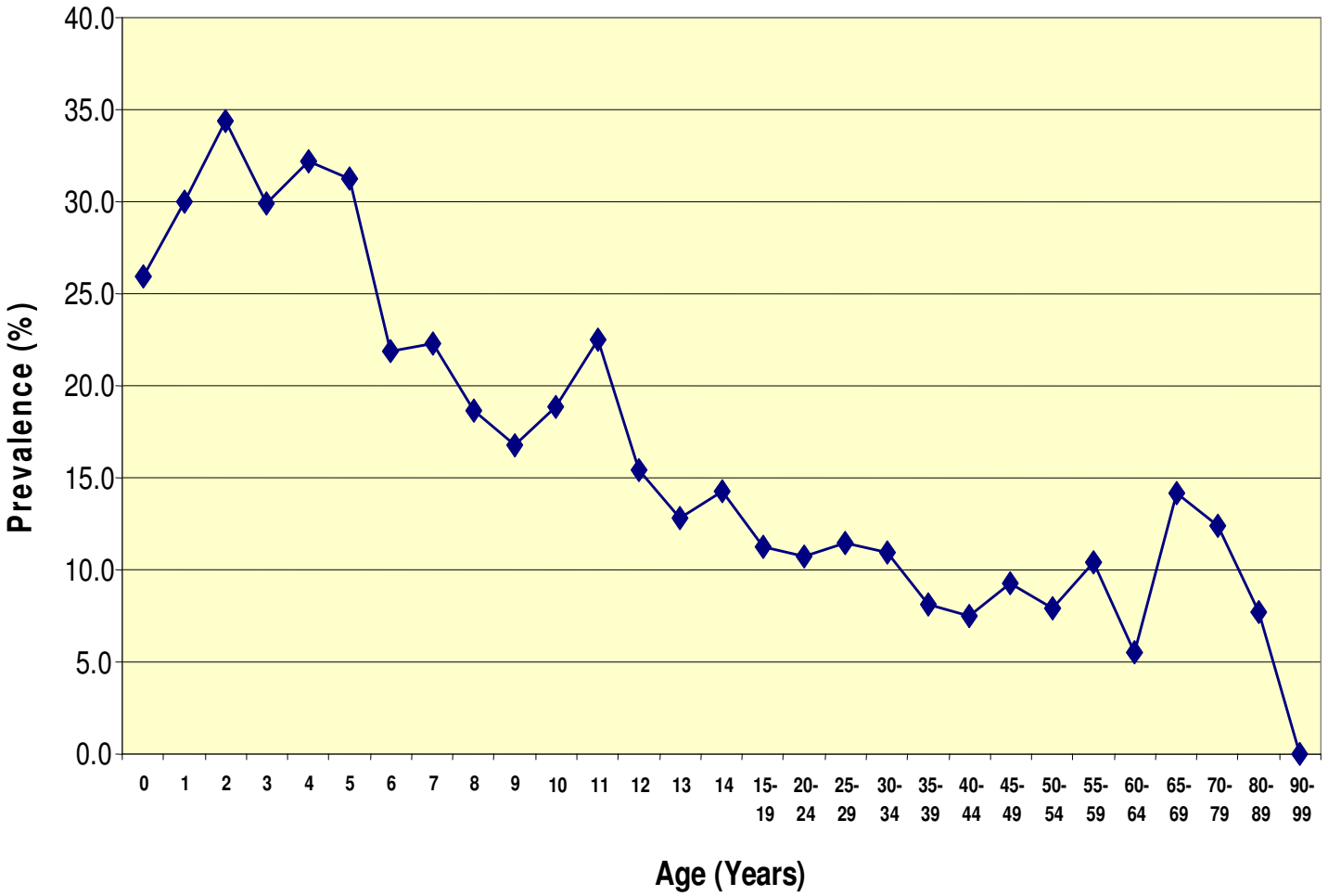

Figure 5. Malaria prevalence rate by Age for the overall data set (n = 7988). Children under the ages of 5 years old had the highest malaria prevalence rate of up to 34.6%. Persons of age group 65-69 years old also indicated high prevalence of up to 14%.

and Kiribwa villages had the highest sporozoite infection rates of 16.7 and 14.3%, respectively, which differed significantly ($p < 0.05$) from the other villages (Figure 3). During the interface of rain season and dry spell in November – December, 2004, the highest infection rate (12.5%) was recorded in Ntuura village, which was significantly different from the rest of the villages ($p < 0.05$).

Low levels of sporozoite infections were recorded during rainy seasons in October - November, 2004. Ntuura and Mugore villages registered 3.5 and 2.9% sporozoite infection rates, respectively, while in April, 2005, infection rate of 6.7% was recorded in Mugore village.

Malaria prevalence and sporozoite infection rate

Comparison of malaria prevalence in humans and mean sporozoite infection rates in mosquitoes across all the eight villages, indicated higher malaria prevalence rates then sporozoite infection rates (Figure 4). Mean malaria prevalence rates ranged from 11.22 - 21.21% with the highest value recorded in Rugaaga and Kiribwa villages. Sporozoite infection rates ranged from 0.84 - 5.26%, with the highest values of 5.26% recorded in Ntuura village. This also registered the lowest malaria prevalence rate of 11.22%.

Children under the age of 5 had the highest malaria prevalence of up to 34%. The ages of 65 0 69 also showed high prevalence of 14% (Figure 5). However, the mean prevalence of parasitaemia was 17.4% for all ages combined and 22.8% for the 5 - 9 year age group. The four separate surveys gave parasitaemia ranging from 12.5 - 22.2% for all ages combined and 17.8 - 25.8% for the 5 - 9 year old children.

DISCUSSION

The total number of female *An. gambiae* s.l. and *An.*

funestus group in all the eight villages, were 1544, and 186, respectively. During the use of PCR, 223 out of 245 samples of *An. gambiae* s.l. analyzed by PCR were identified as *An. gambiae* s.s., suggesting that this could be the only member of the *An. gambiae* complex in Nyabushozi County, Kiruhura district. Increase in the population of *An. gambiae* s.s was observed to coincide with rainy seasons.

PCR identified three species of *An. funestus* group (*An. funestus*, *An. leesoni* and *An. parensis*). *An. funestus* is the most anthropophilic and endophilic member of the group and is a highly efficient vector of malaria. *An. funestus* was found to be in sympatry with the other members of the group, which agrees with the earlier findings of Gilles et al. (1968 and 1987). Most of the *An. parensis* were caught in the goat's houses, which is also in line with the findings of Gilles et al. (1968).

The mean sporozoite rates of 2.84% (23/811) obtained for *An. gambiae* s.l. over the 7 months' study period was fairly lower than those obtained by Egwang et al. (2003) in Kampala (at Kitebi, 10.2%; Kikulu, 9.5%) and Jinja (Police Barracks, 12.5% and Loco, 5.9%) and by Lindblade et al. (2000) in Kabale District (6.1%). This is mainly because Kampala, Jinja and Kabale areas have higher population of human beings as compared to the study area. However, these mean sporozoite rate in this study are similar to those obtained by Zulueta et al. (1963) in Masaka District; at 2.2% for *An. gambiae* complex and 0.5% for *An. funestus* group. This is mainly because Masaka district is a neighboring district to Kiruhura district and have similar environmental conditions. The *P. falciparum* sporozite load is higher than the range of 100 - 7000 per mosquito found by Wirtz et al. (1987) in Papua New Guinea, using similar method. The sporozite load of 500 - 17,700 in this study, which falls within the ranges of 130 - 245,760 and 82 - 77,270 were found in *An. gambiae* and *An. Funestus*, respectively, in the malarious areas of East Africa and the rest of Africa (Pringle 1966).

The mean sporozoite rate of 0.84% obtained for *An. funestus* was very low. This may not necessarily mean that *An. funestus* is not an important vector in the study area. In essence, *An. funestus* seems to play a relatively minor, but important role in malaria transmission, although the sporozoite rate is low. It is more important during the dry season than in the wet season. It is evident from this study that *An. gambiae* s.s. plays a major role in malaria transmission in Nyabushozi County, followed by *An. funestus*.

The relatively high malaria prevalence rate occurred at low levels of transmission. This implies that the low sporozoite rates can maintain a high incidence of malaria in most of the villages of Nyabushozi County. However, it is not clear whether the same malaria profile occurs in Kiruhura district. Nevertheless, this study has demon-strated that malaria transmission in Nyabushozi County is perennial with high inoculation rates occurring in dry season mostly by *An. gambiae* s.s.

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