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Full Length Research Paper

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in non-human primates at a primate center at Franceville, Gabon

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Toxoplasma gondii **can cause fatal disease in both humans and non-human primates.** ***Neospora caninum*** **can also cause economic loss and disease to livestock. The distribution of antibodies against these parasites in non-human primates bred at the CIRMF Primate Center in Franceville, Gabon was determined. For their annual medical examination,** ***T. gondii*** **antibodies were identified using a modified agglutination test (MAT). Twenty-one percent were positive with antibody titers varying from 1:40 to 1:4000.** ***Pan troglodytes*** **(*n*=38; 42.1%) had the highest seroprevalence followed by** ***Mandrillus sphinx*** **(*n*=139; 16.5%). Only one** ***Gorilla gorilla*** **out of the four examined and one** ***Cercopithecus solatus*** **out of 12 were positive. At the same time, the general seroprevalence of** ***N. caninum*** **determined by competitive enzyme linked immunosorbent assay (ELISA) was 68.67% in the four species tested.** ***M. sphinx*** **(*n*=139; 66.12%),** ***P. troglodytes*** **(*n*=38; 16.75%),** ***C. solatus*** **(*n*=12; 4.75%), and** ***G. gorilla*** **(n=4; 1.10%) had the highest prevalence. Co-infection was noted in 24.07% of the positive cases. This study suggests that these primates may constitute different reservoirs for** ***T. gondii*** **and** ***N. caninum*** **in the cystic form and high distribution of these parasites in this environment.**

Key words: *Toxoplasma gondii*. *Neospora caninum*, antibodies, old world monkeys.

INTRODUCTION

Toxoplasma gondii is a Protozoa Apicomplexa intracellular parasite that infects most homeothermic animals (Nicolle and Manceau, 1909). This zoonosis can cause significant economic loss, as well as a significant public

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health problem (Dubey and Schares, 2011; Gharekhani, 2013). Some of the clinical features induced by *T. gondii* are abortion in humans and animals, neurological involvement, fetal malformation, and encephalitis in immunocompromised individuals (Sahwi et al., 1995; Israelski and Remington, 1988). The definitive host is felid. Different animals are infected via oocysts in the soil-contaminated oocyst (Frenkel et al., 1975) or ingestion of infected meat with cysts or bradyzoites. *T. gondii* is responsible for substantial mortality in New World primates (Central and South America) (Dubey et al., 1985). The disease is characterized by pulmonary involvement, diarrhea, and hypothermia (Salant et al., 2009; Carme et al., 2009). Old World primates (Africa, Southeast Asia) seem to be asymptomatic (Dietz et al., 1997; Dubey, 1986), and it is not clear whether they are resistant or susceptible but clinically asymptomatic.

Neospora caninum is a parasite discovered in 1984 (Bjerkas et al., 1984) and identified in 1988 (Dubey et al., 1988). It shares general morphological traits with *T. gondii* and clinical manifestations such as abortion in cattle (Dubey and Lindsay, 1996). The definitive hosts are dogs and coyotes (Canidae) and intermediary hosts are mainly cattle, but many warm-blooded animals have been reported to be infected (Shannon et al., 2015). The parasite is acquired congenitally or when carnivores ingest tissues containing bradyzoites; other animal species may be infected by ingesting food or drinking water containing sporulated oocysts (Dubey et al., 2007). Perinatal mortality has been reported in axis deer (Basso et al., 2014). However, some species seem resistant to *N. caninum* tachyzoites (Uillians et al., 2013).

In general, recent reports have shown that *T. gondii* and *N. caninum* are present in wild birds (Darwich et al., 2012), dogs (King et al., 2012), kangaroos (Mayberry et al., 2014), and rodents (Meerburg et al., 2012), but a recent review did not mention any report on old world primates (Shannon et al., 2015). Although there are recent reports of *T. gondii* in new world primates (Pires et al., 2012), there is no recent report on African non-human primates. In Gabon, timber exploitation has reduced the size of the primate habitat. Human-animal contact has therefore become frequent because of the use of common water sources and forest resources for such uses as agriculture, fishing, hunting, timber, and ecotourism. This situation will increase zoonotic disease prevalence or transmission. Many non-human primates are endangered species due to human activities or sometimes disease outbreaks. Many disease outbreaks of environmental origin have been reported in humans, specifically *T. gondii* (Carme et al*.,* 2002) and in Brazil (Vaudaux et al., 2010).

The outbreak of these diseases has not been reported in Gabon, suggesting that the distribution and the mode of dispersion of the pathogenic agent in nature need investigation. The conservation of biodiversity has become an urgent necessity. Due to difficulties in accessing wildlife primates, the International Centre for Medical Research of Franceville (CIRMF) primate center offers the opportunity for studies to be conducted on the distribution and dispersion of certain pathogens because it has animals in a semi-free-ranging enclosure natural forest and in cages. The prevalence of *T. gondii* and *N. caninum* among endemic primates in Gabon is not known. Therefore, the aim of this study was to provide preliminary results to make this information available.

MATERIALS AND METHODS

Sociodemographic characteristics of animals

*Gorilla gorilla*s are ground-dwelling herbivorous animals living in the forest of central Africa; males weigh 180 kg and females weigh 110 kg, and they are about 1.7 to 1.8 m tall. They live in groups. Females become mature at 10 to 12 years of age and males at 11 to 13 years of age; their lifespan is about 35 to 40 years.

Pan troglodytes (chimpanzee) live naturally in the forests and savannas of Central and Western Africa, weighing 40 to 65 kg; females are 1.3 m tall and males are 1.6 m tall. They reach puberty at 8 to 10 years of age and enjoy a 50-year lifespan. They live in a group of 15 to 150 members and are mostly frugivorous.

Cercocebus torquatus are also called red-capped mangabey and live in the coastal forest from Nigeria to Gabon, where they are limited geographically by the Ogooué river in Gabon. They live in groups composed of 14 to 23 members. *Cercopithecus solatus* are located only in Gabon; characterized by their “sun tail”, are generally frugivorous and live in groups of 10 to 20 members.

Mandrillus sphinx lives in the rainforest in central West Africa from Cameroon to Gabon and Congo Brazzaville. They are omnivorous. Two of the most common traits of these species are that they are endangered species due to the destruction of evergreen forest and commercial bush meat hunting. Two others species present in primatology center are: *Macaca mulatta*, also called macaque rhesus monkeys. They move naturally from Afghanistan to India, measuring 64 cm and weighing 5.3 to 7.7 kg with a 30-cm-long tail, and generally live in arid areas or uncovered areas.

They are herbivorous and live in groups of up to 180 individuals. *Chlorocebus pygerythrus,* also called vervet monkeys, live in southern and eastern Africa, they are herbivorous, males are 50 cm tall and females 40 cm, males weigh 3.9 to 8 kg and females 3.4 to 5.3 kg. They live in groups.

Five out of the seven species analyzed were endemic to the region: *M. sphinx* (*n*=139), *P. troglodytes* (*n*=38), *C. solatus* (*n*=12), *G. gorilla* (*n*=4) and *C. torquatus* (*n*=2); two were imported species: *C. pygerythrus* (vervet monkey, *n*=4) and *M. mulatta* (rhesus monkey, *n*=1). Animals of both genders were tested. Their mean age was 26.06 years, ranging from 1 to 30 years. No clinical signs suggested that *T. gondii* or *N. caninum* infection were recorded during a retrospective analysis of the animals’ medical file.

Primate population and sample collection

The primatology center houses primates either in cages or in a semi-free-ranging enclosure, with potential contact with free-living animals (Figure 1). Food and water are provided to those in cages (Figure 1, numbers 1 to 6) or the aviary (Figure 1, label Q and B), while those in the forest (Figure 1, number 7) use food available in their environment, complemented by fruits and cakes stocked daily by a guardian at different fixed points in the forest. The colonies are formed by animal founders brought to the CIRMF 30 years ago and animals born at the CDP since then. No outbreak of toxoplasmosis

Figure 1. Primatology Center of CIRMF (CDP). The figure shows the map of the CDP with cages (numbered from 1 to 6), aviary (Q and B), and natural forest surrounded by fence (number 7).

or neosporosis has been observed in the colony. During their annual health check-up, the animals were bled under anesthesia, and plasma or serum was obtained after centrifugation of total blood at 3000 rpm for 10 min at room temperature. Aliquots of plasma were kept at -20°C or used immediately in the modified agglutination test (MAT) or cELISA for *N. caninum* testing. No data existed before this study on the animals.

Ethical approval

This study was conducted in accordance with normative procedures as defined by the National Ethics Committee of Gabon, reference PROT N°0005/2013/SG/CNE, and Gabonese government authorization, reference RG/MINEF/00468.

Modified agglutination test for *T. gondii*

The Toxoscreen DA MAT (Biomerieux, Lyon, France) was performed on samples from animals diluted up to 1/4000 as recommended by the manufacturer, in 96-well plates containing formalin-treated *T. gondii* trophozoite and 0.2 M of 2-mercaptoethanol and left for incubation at room temperature overnight. A positive sample was characterized by agglutination of the *Toxoplasma* in a mat covering half of the well base, while negative samples were characterized by sedimentation of *Toxoplasma* in a button.

Competitive ELISA for *N. caninum* (cELISA)

To detect *N. caninum* antibodies, a competitive ELISA was performed according to the manufacturer's protocol (LSI Vet, Lissieu, France). Briefly, 50 µl of test serum from primates and controls was incubated in 96-well plates pre-sensitized with *N. caninum* antigen. After 1 h incubation at room temperature, the plate was washed with 300 µl of a wash solution and a monoclonal anti-*N. caninum* combined with peroxidase was added. After 20 min incubation, the plate was washed again three times with 300 µl wash solution followed by the addition of 50 µl of chromogenic substrate. The reaction was stopped by addition of 50 µl of stop solution. The intensity of the coloration was measured on a spectrophotometer at 620 nm. A lack of coloration or weak coloration indicated the presence of *N. caninum* antibodies. The measurement was validated when the optical density (OD) of the negative control was within 1.6 to 0.600 and the percentage of inhibition of the positive control was greater than 40%. Negative and positive control was included in each plate. The percentage of inhibition of each sample was determined using the formula:

%inh = OD negative control - OD tested sample/OD negative control × 100

Samples were considered positive when the percentage of inhibition was 30% or greater.

Statistical analysis

These analyses were done by comparing median values of groups of data using the Mann-Whitney U-test, the Chi-square test for groups of samples, and the Pearson correlation for relationships between data. These analyses were carried out using SPSS software. Results with a probability less than or equal to 0.05 were considered significant.

RESULTS

Seroprevalence of anti-*T.gondii*

Table 1 shows that 21% of animals were positive for IgG

Table 1. Seroprevalence of *Toxoplasma gondii* in the primates at the Franceville Centre of Primatology.

Primates spp.	Number	Positive	Prevalence	CI (%)
Mandrillus sphinx	139	23	16.5	11-23
Pan troglodyte	38	16	42.1	16.7-68
Cercopithecus solatus	12	1	8.3	-
Gorilla gorilla	4	1	25	-
Cercocebus torquatus	2	1	50	-
Chlorocebus sabaeus	4	0	-	-
Macaca mulatta	1	0	-	-
Total	200	42	21	23.83-21.16

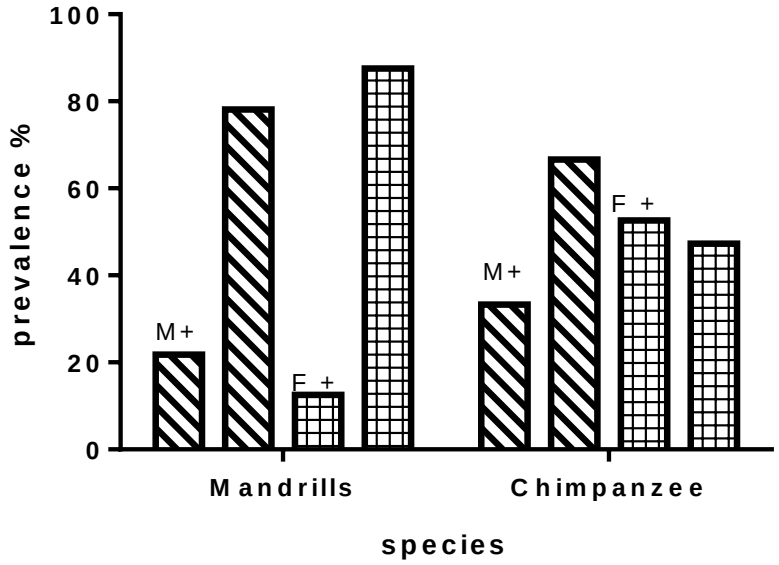

Figure 2. Distribution of *T. gondii* according to gender and animal species. *T. gondii*-positive animals were recorded as prevalence (%); this prevalence was plotted on a histogram for positive males (M+) and positive females (F+) compared to their negative counterparts.

against *T. gondii* (42/200). Seroprevalence according to species (Figure 2) shows that *P. troglodytes* had the highest prevalence (42.1%; 16/38) followed by *M. sphinx* (16.5%; 23/139), with a significant difference between the two species (42.1% versus 16.5%; p=0.001). Seroprevalence according to gender (Figure 3) showed that the prevalence was affected by gender in *M. sphinx*, with males being more infected than females (23.4% versus 10.6%; p = 0.043). However, in *P. troglodytes*, despite high prevalence (50%) in females compared to males (33.3%), this difference was not significant (p = 0.229). Furthermore, in *M. sphinx* the carriage of anti-*T. gondii* was not affected by age (Figure 3); although this carriage was high between 5 and 10 years, there was no correlation between age and seroprevalence (r = −0.534; p = 0.237). In contrast, the age of *P. troglodytes* (Figure3) affected the seroprevalence with increasing seroprevalence as age increased (r = 0.839; p = 0.037).

Seroprevalence did not vary according to habitat (Table 2), as shown by the distribution of IgG among the main species studied: *M. sphinx* (p = 0.214) and *P. troglodytes* (p = 0.513). It was noticed however that caged mandrills had no anti-*T. gondii* antibodies but only forest-dwelling animals (free-ranging) had these antibodies. The antibody titer rose to 1/4000 for all positive animals.

Seroprevalence of *N. caninum*

A total of 83 primates were tested and an overall prevalence of 68.67% was observed. A detailed distribution shows that *M. sphinx* (66.12%; 41/62), *P. troglodytes* (75%; 12/16), *C. solatus* (75%; 3/4), and one *G. gorilla* out of one (100%; 1/1) were the most infected

Figure 3. Progression of prevalence of *T. gondii* according to age. The prevalence of positive animals (%) per group of animals from 0–5 years up to 30–35 years recorded and plotted against prevalence (%) on a histogram for each species.

Table 2. Seroprevalence according to habitat.

Species	Habitat (*n*)	Positive (%)	Negative (%)
Mandrillus sphinx	Free-ranging (126)	23	103
	Cage (8)	0	8
Pan troglodyte	Cage (25)	10	15
	Aviary (9)	4	5

Table 3. Distribution of *N. caninum* among some primate species and coinfection with *T. gondii.*

Species	Number	Positive	Prevalence (CI 95%)	Co-infection (%)
Mandrillus sphinx	62	41	66.12 (54.34-77.9)	6 (14.63)
Pan troglodyte	16	12	75 (53.78-96.22)	6 (50)
Gorillagorilla	1	1	100 (-)	1 (100)
Cercopithecussolatus	4	3	75 (32.57-117.43)	0
Total	83	57	68.67 (58.69-78.65)	24.07

(Table 3). *T. gondii* and *N. caninum* co-infection was seen in 24.07% of the entire population of primates examined, that is, the percentage of individuals positive for both *T. gondii* and *N. caninum* (14/57 individuals). This was most prevalent in *P. troglodyte* (50%; 6/12), *G. gorilla* (100%; 1/1), and *M. sphinx* (14.63%; 6/66). No co-infection between these two parasites was seen in *C. solatus* (Table 3).

DISCUSSION

This study is the first to present seroprevalence of *T. gondii* and *N. caninum* in non-human primates in Gabon. Many reports are from outside Africa, with few coming from Africa (Ekanayake et al., 2004; Garcia et al., 2005; Sedlàk and Bàrtovà, 2006; McConnell et al., 1973). Primates are known as indicators of outbreaks of some

zoonoses in humans, such as Ebola (Georges et al., 1999). It is therefore necessary to study these parasites in primates, since these parasites might be a threat for human health or wildlife in general. Gabon is geographically well located for such studies (with the world's second largest forest).

The present study has shown that both *T. gondii* and *N.caninum* are prevalent among non-human primates but not distributed uniformly among different species of primates nor their habitats. In both cases, mandrills living in contact with the wild had more anti-*T. gondii* and anti-*N. caninum* antibodies than those in cages, suggesting the existence of these species of parasites in the wild. The prevalence of co-infection (24.07%) of these parasite species seems to indicate that both are distinct species despite certain biological and clinical similarities. In addition, they do not necessarily follow the same dynamics in their evolution and dispersion. Furthermore, it was shown that *T. gondii* and *N. caninum* differed in their host and transmission strategies (Reid et al., 2012). While age seems to be an important criterion for seropositivity in chimpanzees, in mandrills it was noted that these animals aged between 5 and 10 years had a very high prevalence of *T. gondii*. It was also observed that males had a higher prevalence than females. The prevalence of *T. gondii* at that age and in male mandrills seems to corroborate with their social behavior, characterized by fighting for male dominance (Setchell et al., 2006), resulting in severe injuries with bleeding or death, exposing males to infected blood.

This report also indicates a level of environmental contamination. The existence of a sylvatic cycle implicating wild canids for *N. caninum* or felids for *T. gondii* is suggested by these results on animals living in the forest. The fact that *T. gondii* DNA has been detected in fruit and vegetables (Lasso et al., 2012) and interaction with other wild species in the forest, makes the hypothesis of environmental contamination plausible. Estimation of antibodies gives a good indication of the exposure of animals to *N. caninum* and *T. gondii*. For *N. caninum*, although a confirmatory test such as Western blot or the immunofluorescence antibody test (IFAT) was not carried out, a competitive ELISA does not need specific secondary antibodies. However, the principle of competition indicates that the test can be used in different species (McCann et al., 2008). The retrospective examination of medical files did not allow us to relate clinical signs to *T. gondii* or *N.caninum* in these primates due to the nonspecific symptoms observed. However, the fact that antibodies remained high, as shown by the titer 1/4000, suggests that Old World primates may harbor the parasite either in a cystic form (bradyzoite) or as reactivation of an old infection (due to incoming tachyzoites). Reinfection (by trophozoites from a new infection) can occur quite often in a contaminated environment. The subsequent immune reaction may help contain the infection, resulting in mild or transient clinical signs (Drapper et al., 1971), which is substantiated by the fact that in chimpanzees experimental infection showed that a naive animal remains ill for a few days with a rise in the antibody titer for 6 months, while the other animals that had previous contact with *T. gondii* remain well. This suggests that old world primates may control their infection quickly, either by their immune system or other mechanisms that require clarification.

No studies have been conducted on this aspect on the other primates examined in this study. This suggests that the means of spreading the disease in primates require further study to determine their potential role in dispersion of these parasites.

Conclusion

This study suggests that old world primates are possible reservoirs of *T. gondii,* with variability depending on the species. Although antibodies against *N. caninum* were found, no parasite material was detected.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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Prevalence of malaria and anaemia during the dry season in North Central and South Western Nigeria

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Malariometric surveys provide information on epidemiological parameters used in assessing malaria burden for evidence-based decision making. This study provides information on some malariometric indices in two ecologically distinct areas in Nigeria. The study was conducted in New Bussa in Niger State (Sudan savannah) and Ijede in Lagos State (tropical rain forest). Study participants were screened for malaria, fever, anemia and their demographic characteristics recorded. A total of 1,648 participants (813 from New Bussa and 835 from Ijede) were recruited. Majority of the participants in New Bussa were in age group of 5-15 years, (39.9%) while in Ijede they were in age group >35 years, 68.6%. Overall, malaria prevalence by mRDT was 19.4% (95% CI: 17.4-21.5) while microscopy was 11.9% (95% CI: 10.3-13.6). Malaria prevalence in New Bussa by mRDT and microscopy were 32.5% (95% CI: 28.8-36.4) and 18.8% (95% CI: 16 -21.9) respectively while in Ijede malaria prevalence by mRDT was 9.6% (95% CI: 9.7-11.9) and microscopy was 5.7% (95% CI: 4.2 -7.7) respectively. Malaria prevalence was higher in children within 5–15 years of age than other age groups (P<0.05). Fever rate reduced with age and malaria was more prevalent in participants with fever than those without fever (P<0.01). Anaemia prevalence in the two study sites were similar, 21.5%. (P = 0.28). Malaria was hypoendemic and mesoendemic in Ijede and New Bussa respectively during the dry season. There was association between malaria and fever. However, malaria was not a major cause of fever.

Key words: Malaria, fever, anaemia, prevalence, endemicity, Nigeria.

INTRODUCTION

Significant progress has been made in curtailing the menace of malaria since the launching of Roll Back Malaria in the year 2000 (WHO, 2017), yet the disease continues to receive attention as a public health issue. The latest estimates report that 3.2 billion people are at the risk of being infected globally while 212 million malaria cases and 429,000 deaths occurred in year 2015 (WHO, 2017). As at 2010, 85% of Nigerians lived in

areas supporting mesoendemic transmission, 15% lived under conditions of hyper- to holo-endemicity with some areas showing hypo-endemicity (Federal Ministry of Health, 2014). Of the 91 countries that have on-going transmission of malaria, Nigeria contributed 29% of total malaria cases in 2015 (WHO, 2017). Thus, Nigeria has a potential to contribute substantially to the global efforts at eradicating malaria.

Seasonal variation has been known to affect malaria transmission such that there is an increase in malaria transmission in the rainy season compared to wet season. This is mainly due to increase in breeding sites of mosquitoes in the rainy season translating to increased mosquito density and therefore increase malaria infection rate (Midekisa et al., 2015; Reiner et al., 2015). Conversely, in the dry season, there is a reduction in malaria transmission and therefore reduction in malaria prevalence.

Although the causes for anaemia are multifactorial, parasite diseases such as malaria and helminths have been linked to anaemia in children living in malaria-endemic areas (Athuman et al., 2007; WHO, 2011). Similar seasonal patterns have been observed in malaria and anaemia and cases of anaemia increase when malaria cases increase (Koram et al., 2003; Okebe et al., 2016).

The major mechanisms are those of red cell destruction and decreased red cell production (Phillips and Pasvol, 1992). Potential causes of haemolysis include loss of infected cells by rupture or phagocytosis, removal of uninfected cells due to antibody sensitization or other physicochemical membrane changes, and increased reticuloendothelial activity, particularly in organs such as the spleen (Phillips and Pasvol, 1992). Analysis of anemia prevalence and status is therefore a relevant indices of malaria epidemiology.

Most of the current interventions including use of long-lasting insecticidal nets (LLIN) and artemisinin-based combination therapy (ACTs) have resulted in reduction of the malaria burden. However, reliable data on progress made in reducing malaria burden are not exhaustive. Up to date, information will be required as malaria landscape changes to determine appropriate interventions needed. Therefore it is necessary to determine baseline malariometric information of communities that will serve as models for intervention studies. Malariometric survey can provide time-point and seasonal information on parasitological and entomological indices, pattern of drug use and efficacy, socio-behavioural and economic determinants of malaria incidence, prevalence and endemicity in defined geographical locations. This study provides information on parasitological aspect of malariometric characterization of two ecologically distinct sites in Nigeria.

METHODOLOGY

Study sites

This cross-sectional study was carried out in the dry season, January 2014, in New Bussa (N09° 54.476', E004° 33.876'), Borgu LGA, Niger State and Ijede (N06° 34.076 E003° 35.637'), Ikorodu LGA, Lagos State. New Bussa has an average elevation of 171 m above sea level. It is sparsely populated with 14 people per km^2, small settlements separated by short distances. New Bussa has a dry sub-humid climate which is classified as a Sudan savannah and landscape mostly covered with closed to open shrub land. The hottest period in the year is in March with an average temperature of 37.9°C at noon, preceding the rains in April. The coldest temperatures are experienced in December with an average temperature of 16°C at night. Annual average rainfall is 1010 ± 180 mm, while humidity averages 80% in the year. Rainfall and other precipitations peak in May/June and August/September. The time around November is driest. The four rural communities studied in New Bussa including Tada, Monai, Masana, and Yuna are serviced by a primary health post located in Tada. There is a public primary school and a secondary health facility located in New Bussa which serves a population of 234,718 people, the estimated population of New Bussa in 2014 (Projections from 2006 Census). The people of New Bussa speak varieties of Mandig language, Boko bussa, Baatcnum, Boo Bussa, Boko Baru, Dendi, Fulfulde, Yoruba, and Hausa. They are mostly fishermen, farmers and petty traders. The schools, churches and big shops are located in the main town away from easy reach of the villagers who commute on dirt roads by the occasional vehicle, but mostly on bikes and on foot.

Ijede has an average elevation of 42 m above sea level. The area is very densely populated with 6,174 people per km^2. Ijede has a secondary forest vegetation of mostly broadleaved evergreen or semi-decidious forest, with swampy areas. It also has two peaks of rainfall in June and September with mean annual rainfall of about 1900 ± 250 mm and humidity averaging 77%. March is warmest with an average temperature of 32.4°C at noon. August is coldest with an average temperature of 22.6°C at night. The time around January is driest. Ijede is categorized as semi urban by the Ikorodu Local Government Area to which it belongs in Lagos State. It is one of the five Local Government Development Council Areas (LCDA) of Ikorodu and lies about 36 km North East of Lagos State, a coastal community with veritable undulating lowland, its population was put at 133,317 in 2014 (Projections from 2006 Census). The locals are Ijebu Yorubas and are mostly petty traders with fishermen and a few farmers. Ijede has a public primary and two public secondary schools. There are several private primary and secondary schools, with well served electricity by a 1320MV Egbin thermal station, the biggest in West Africa just next to the community.

Field study

All study participants were screened for fever (axillary temperature≥ 37.5°C), malaria by microscopy (WHO, 2010) and malaria rapid

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Table 1. Demographic characteristics of study participants.

Characteristic	Ijede [n (%)]	New Bussa [n (%)]	Total	P
Number of participants	813	835	1648	
Sex				
Male	257 (31.6)	433 (51.9)	690	<0.001
Female	556 (68.4)	402 (48.1)	958	
Age (years)				
Median (range)	30 (0.42-100)	12 (0.4-90)	20 (0.4-100)	<0.001
Age group				
<5	134 (16.5)	143 (17.1)	277 (16.8)	<0.001
5-15	108 (13.3)	333 (39.9)	441 (26.8)	
16-35	244 (30)	209 (25)	453 (27.5)	
>35	327 (68.6)	150 (18)	477 (28.9)	
Weight (kg)				
Median (range)	59 (5-124)	33 (5-97)	48 (5-124)	<0.001
Height (m)				
Median (range)	1.67 (0.6-1.95)	1.43 (0.57-1.87)	1.55 (0.57-1.95)	<0.001
Temperature (°C)				
Mean±SD	36.6±0.6	36.9±0.5	36.7±0.6	<0.001
≥37.5	32 (3.9)	84 (10.1)	116 (7.1)	<0.001
<37.5	779 (96.1)	750 (89.9)	1529 (92.9)	

n: Number of participants; %: percentage; P: Probability value; SD: Standard deviation.

diagnosis test (mRDT) using Carestart™ (Access Bio Inc., USA). All persons diagnosed to have malaria were treated with artemether-lumefantrine. Blood spots were made on filter paper for molecular studies on malaria parasite characteristics such as level of resistance to various antimalarial drugs (findings to be published elsewhere). Anaemia status of the participants were defined using the WHO haematocrit cut-off for mild, moderate and severe anaemia based on age and sex (WHO, 2011). Demographic information of the participants was also recorded.

Data analysis

Malaria endemicity was classified based on prevalence of malaria in children between the ages of 2 and 9 years (Metselaar and Van Theil, 1959). In this method, an area with a malaria prevalence in 2 to 9 years old children <10% is hypoendemic, 11 to 50% is mesoendemic, 51 to 75% is hyperendemic, while >75% is holoendemic. Data were analyzed using SPSS 20.0 for Windows. Variables considered in the analysis were related to the presence and densities of malaria parasites, fever, anemia and participants demographics. Proportions were compared by calculating Chi-square, Fisher's exact or Mantel Haenszel tests as appropriate. Normally distributed, continuous data were compared by t test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U tests and the Kruskal Wallis tests (or by Wilcoxon ranked sum test). P values less than 0.05 were considered statistically significant.

Ethical consideration

The study protocol was approved by the Institutional Review Board of Nigerian Institute of Medical Research, Yaba, Nigeria. Local permissions to conduct the study were obtained from community heads, while individuals gave written informed consent before participating in the study.

RESULTS

A total of 1,648 participants were recruited into the study (813 from New Bussa and 835 from Ijede). The demographic characteristics in the two study sites were different, more males were seen in New Bussa, 433 (51.9%) than in Ijede, 257 (31.6%). The median ages of participants were 12 and 30 years in New Bussa and Ijede, respectively. Majority of the participants in New Bussa were in the age group 5 to 15 years, 333 (39.9%) while in Ijede they were in the age group >35 years, 327 (68.6%). The median height and weight of participants in New Bussa were 1.4 m and 30.6 kg, while in Ijede they were 1.7 m and 59 kg, respectively. About 10% of the study participants were febrile in New Bussa, while less than 4% in Ijede were febrile (Table 1).

Table 2. Malaria indicators in Ijede and New Bussa sites.

Characteristic	Ijede [n (%)]	New Bussa [n (%)]	Total	P
Malaria				
Prevalence (mRDT)	78 (9.6)	197 (32.5)	275 (19.4)	
95% CI	7.7-11.9	28.8-36.4	17.42-21.53	<0.001
Prevalence (microscopy)	43 (5.7)	129 (18.8)	172 (11.9)	
95% CI	4.2-7.7	16-21.9	10.33-13.6	
Plasmodium species				
P. falciparum	39 (90.7)	118 (91.5)	157 (91.3)	
P. malariae	0	4 (3.1)	4 (2.3)	
P. ovale	1 (2.3)	1 (0.8)	2 (1.2)	<0.001
P. falciparum+ P. malaraie	3 (7.0)	4 (3.1)	7 (4.1)	
P. falciparum + P. ovale	0	2 (1.6)	2 (1.2)	
Parasite density/µl blood				
Geometric mean (Range)	277 (34-50833)	342 (28-30891)	325 (28-50833)	0.180
Parasite density groups				
1-500	28 (66.7)	80 (64.5)	108 (65.1)	
501-1000	6 (14.3)	19 (15.3)	25 (15.1)	0.968
>1,000	8 (19.0)	25 (20.2)	33 (19.9)	
Axillary temperature (°C)				
Mean±SD	36.6±0.6	36.9±0.5	36.7±0.6	0.935
Range	35-40	35.6-40.5	35-40.5	
≥37.5	32 (3.9)	84 (10.1)	416 (7.1)	<0.001
<37.5	779 (96.1)	750 (89.9)	1529 (92.9)	
PCV (%)				
Mean±SD	39.3±6.0	38.4±5.2	38.9±5.6	<0.001
Range	15 - 55	13 - 53	13 - 55	
Normal PCV	593 (79)	517 (77.9)	1110 (78.5)	
Mild Anaemia	71 (9.5)	78 (11.7)	149 (10.6)	0.28
Moderate anemia	72 (9.6)	62 (9.3)	134 (9.5)	
Severe anaemia	15 (2)	7 (1.1)	22 (1.5)	

Anaemia was determined according to WHO classification for different categories*. n: Number of participants; %: percentage; P: Probability value; SD: Standard deviation.

Overall, malaria positivity rate was 19.4% (95% CI: 17.4-21.5) and 11.9% (95% CI: 10.3-13.7) by mRDT and microscopy, respectively. Malaria prevalence in New Bussa by mRDT and microscopy were 32.5% (95% CI: 28.8-36.4) and 18.8% (95% CI: 16-21.9), respectively while in Ijede malaria prevalence by mRDT and microscopy were 9.6% (95% CI: 9.7-11.9%) and 5.7% (95% CI: 4.2-7.7), respectively. *Plasmodium falciparum* was the predominant species in both sites with prevalence of over 95%. Other species (*Plasmodium malariae* and *Plasmodium ovale*) were also seen either as mono infection or mixed infection with *P. falciparum.* Geometric mean parasite density in the two sites were similar [New Bussa, 342 Parasite/µl of blood; Ijede, 277 parasite/µl of blood; P=0.18]. Fever rate was higher in New Bussa (10.1%) compared to Ijede (3.9%). The mean packed cell volume of the study participants in New Bussa (38.4±5.2%) was significantly lower than in Ijede (39.3±6.0%). However, the proportions of study participants with mild, moderate or severe anemia were similar in both sites (P=0.28) (Table 2).

Overall, malaria positivity by mRDT and microscopy was associated with fever (P<0.01). However, in the individual sites this was not the case (P>0.05) though the proportion of malaria positive cases were higher in persons with fever than in those without fever. In Ijede,

Table 3. Comparison of fever rate and malaria positivity by MRDT and microscopy in the study sites.

Character	Ijede		New Bussa		Overall	
	mRDT (%)	Microscopy (%)	mRDT (%)	Microscopy (%)	mRDT (%)	Microscopy (%)
Temperature (°C)						
<37.5	74/779 (9.5)	39/722 (5.4)	170/545 (31.2)	112/617 (18.2)	244/1324 (18.4)	148/1339(11.0)
≥37.5	4/32 (12.5)	4/31 (12.9)	26/60 (43.3)	17/69 (24.6)	30/92 (32.6%)	21/100 (21.0)
Total	78/811 (9.6)	43/753 (5.7)	196/605 (32.4)	129/686 (18.8%)	274/1416 (19.4)	169/1439 (11.7)
P	0.371	0.094	0.056	0.191	**0.0008**	**0.003**

MRDT: Malaria rapid diagnostic test; P: Probability value.

Table 4. Malaria positivity according to age groups and sex in Ijede and New Bussa.

Age (years)	mMRDT			Microscopy		
	Ijede [n/N (%)]	New Bussa [n/N (%)]	P	Ijede [n/N (%)]	New Bussa [n/N (%)]	P
<5	14/134 (10.4)	25/105 (23.8)	0.001	4/126 (3.2)	21/107 (19.6)	<0.001
5-15	23/108 (21.3)	119/236 (50.4)	<0.001	13/102 (12.7)	65/275 (23.6)	0.020
16-35	15/244 (6.1)	36/156 (23.1)	<0.001	12/224 (5.4)	24/168 (14.3)	0.002
>35	26/327 (8.0)	17/109 (8.6)	0.02	14/303 (4.6)	19/137 (13.9)	0.001
P	0.0001	<0.0001		0.0088	0.0336	
Endemicity						
Prevalence in children 2-9 years	27/148 (18.2)	85/223 (38.1)		10/131 (7.1)	56/249 (22.5)	
95%CI	12.9-25.2	32.0-44.6	<0.001	3.9-12.6	17.7-28.1	<0.001
Classification	Mesoendemic	Mesoendemic		Hypoendemic	Mesoendemic	
Gender						
Male	30/257 (11.7)	117/314 (37.3)	<0.001	13/247 (5.3)	73/367 (19.9)	<0.001
Female	48/556 (8.6)	80/292 (27.4)	<0.001	30/508 (5.9)	56/320 (17.5)	<0.001
P	0.171	0.01		0.721	0.423	-

MRDT: Malaria rapid diagnostic test; P: Probability value; CI: Confidence interval.

malaria positivity by mRDT in persons with and without fever were 4 of 32 (12.5%) and 74 of 779 (9.5%) while in New Bussa it was 26 of 60 (43.3%) and 170 of 545 (31.2%), respectively. In Ijede, malaria positivity by microscopy in persons with and without fever were 4 of 3 (12.9%) and 39 of 722 (5.4%), while in New Bussa it was 17 of 69 (24.6%) and 112 of 617 (18.2%), respectively (Table 3). Study participants in the age group 5 to 15 years had significantly higher malaria positivity rate than other age group in both study sites irrespective of the diagnostic method used. Gender was not associated with malaria positivity except in New Bussa using mRDT, where the males had higher malaria positivity rate of 117 (37.3%) and females 80 (27.4%) (P=0.01). Malaria prevalence rate in children aged 2 to 9 years, which is used for the classification of malaria endemicity (Metselaar and Van Theil, 1959; Metselaar and Van Theil, 1959) was 7.1% in Ijede and 22.5% in New Bussa based on microscopy result. This is an indication that malaria is hypodemic in Ijede and mesoendemic in New Bussa in the dry season (Table 4).

Fever among study participants with malaria decreased with age, the higher the age the lower the fever rate among malaria positive participants (Figure 1). Malaria contributed more to fever rate in New Bussa than in Ijede. The difference was greatest in 15 years and below in 16 years and above (Figure 2). Malaria cases with fever in children under 5 years were lower in Ijede than in New Bussa. However, the reverse was the case in above 5 years in both sites (Figure 3).

P. falciparum species was the only species observed in children under 5 years in both study sites, while other species were observed in participants above 5 years (Figure 4). *Plasmodium falciparum* species was the only species observed in children under 5 years in both study sites while other species were observed in participants above 5 years (Figure 5).

Overall, there was a significant decline in the

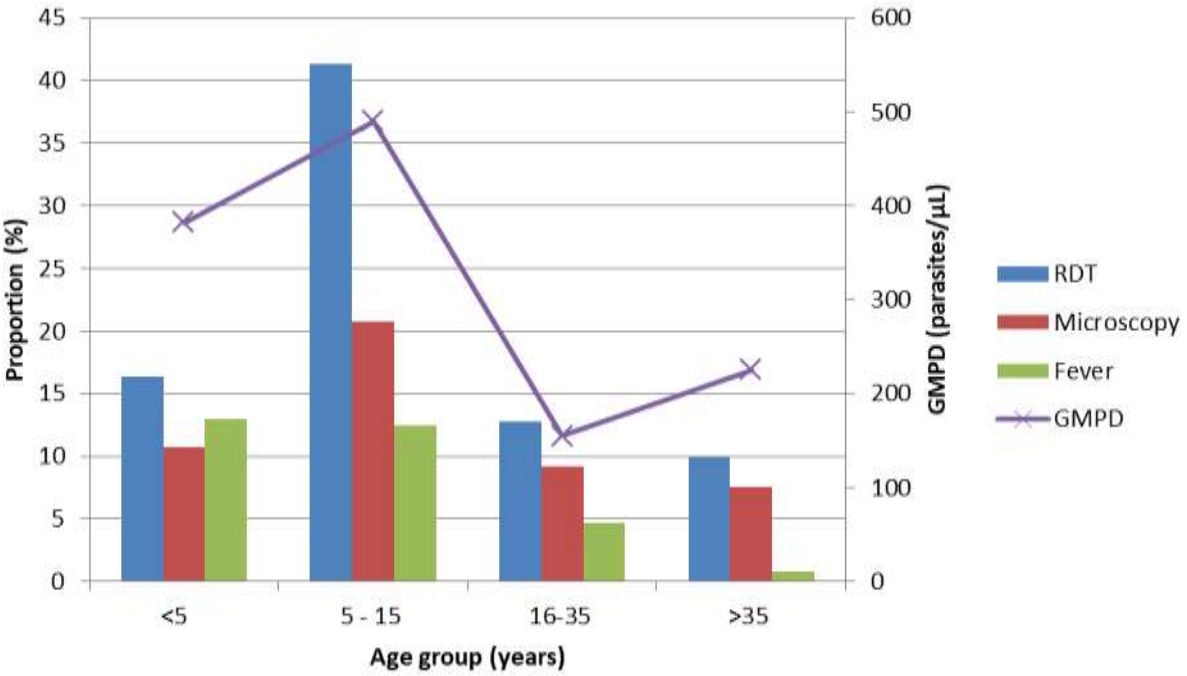

Figure 1. Prevalence Distribution of persons with fever, prevalence of malaria and parasitemia amongst the malaria positive study participants in different age groups.

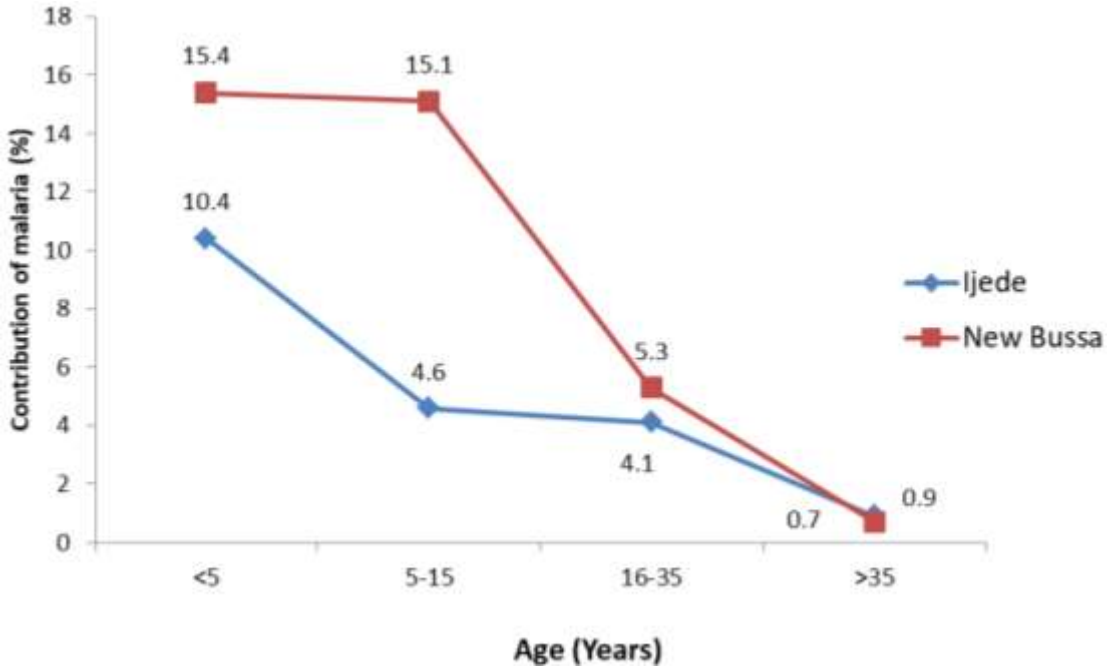

Figure 2. Contribution of malaria to fever by age in the two study sites.

prevalence of anaemia as age increased (P = 0.036) withchildren less than 5 years having the highest prevalence of anaemia (30.5%). This trend was observed in both sites but the decline was not statistically significant in either Ijede (P=0.704) or New Bussa (P=0.085). Comparison of proportion of children with anaemia in the different age groups showed that specific age groups had similar anaemia rates (P>0.05). There was no association between anaemia and gender either overall or in any of the two sites (P>0.05) (Table 5).

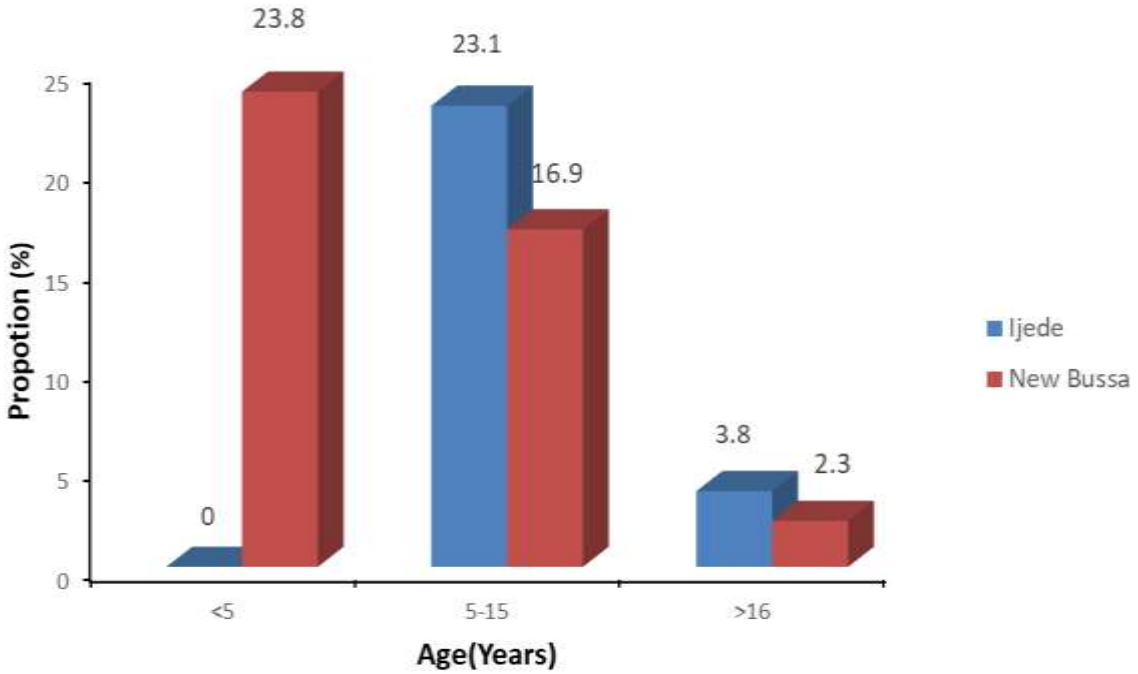

Figure 3. Malaria cases with fever.

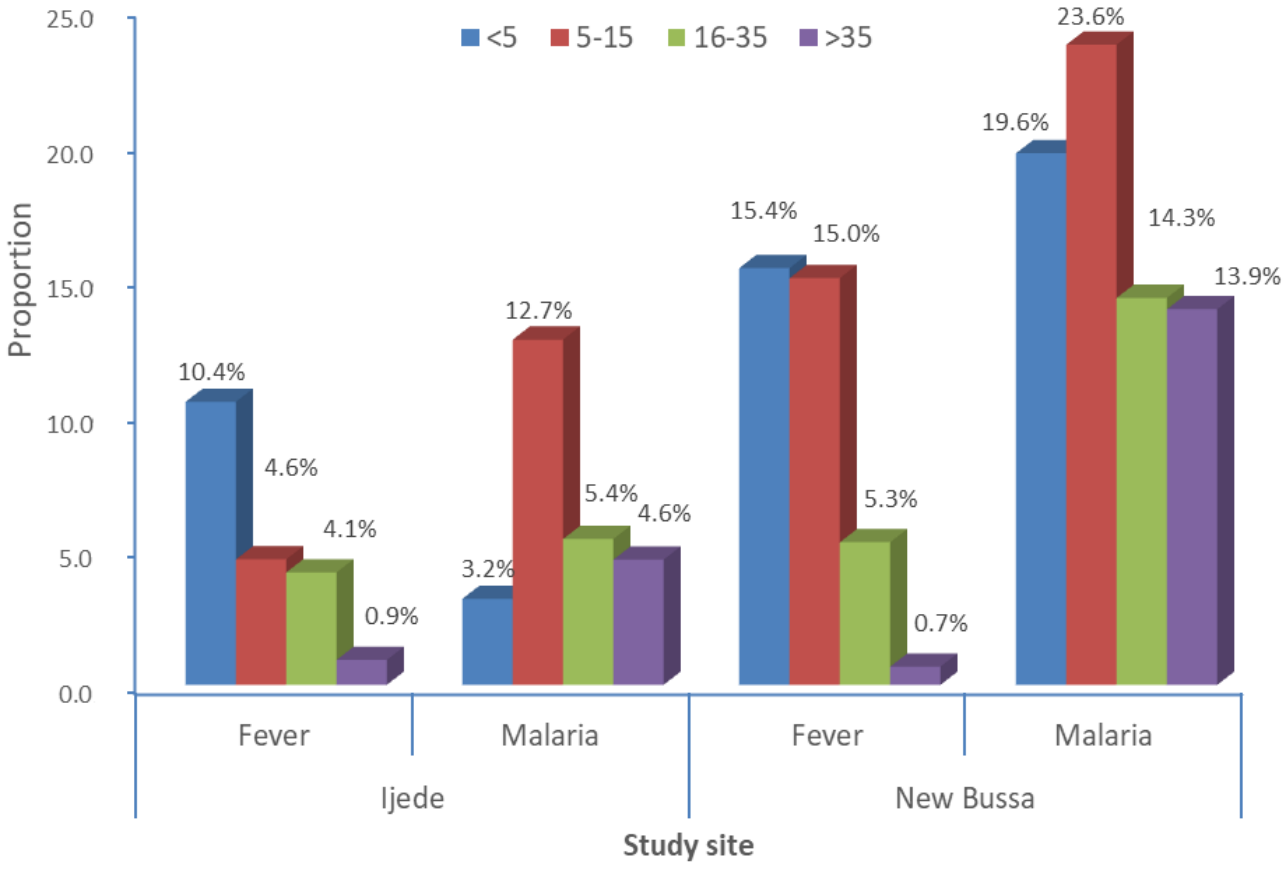

Figure 4. Prevalence of malaria and fever by age group.

Table 5. Prevalence of anaemia by age and gender in the two study sites.

Parameter	Anaemia			
	Ijede	New Bussa	ALL	P
Age				
<5	18/68 (26.5)	21/60 (35.0)	39/128 (30.5)	0.295
5-15	15/62 (24.2)	44/144 (30.6)	59/206 (28.6)	0.354
16-35	29/135 (21.5)	24/90 (26.7)	53/225 (23.6)	0.369
>35	36/180 (20.0)	12/72 (16.7)	48/252 (19.0)	0.542
Total	98/445 (22.0)	101/366 (27.6)	199/811 (24.5)	0.664
P	0.704	0.085	0.036	-
Gender				
F	61/308 (19.8)	52/186 (28.0)	113/494 (22.9%)	0.037
M	38/138 (27.5)	49/180 (27.2)	87/318 (27.4)	0.95
Total	99/446 (22.2)	101/366 (27.6)	200/812 (24.6)	0.076
P	0.069	0.875	0.148	-

P: Probability value.

P. falciparum was the only species observed in the age group of <5 and >35 years. Mononfection of each of the three plasmodium species and co-infection of *P. ovale* and *P. malariae* with *P. falciparum* was observed in age group 5 to 15, while age group 16 to 35 years had *P. malariae* and *P. falciparum* species infections

DISCUSSION

The difference in the demographic characteristics in the two sites especially having more males in New Bussa than in Ijede was likely due to the limited access to females due to religious reasons in some of the communities in New Bussa that were predominantly muslims. This study reports a lower prevalence of malaria in the South Western than in the North Central part of Nigeria. Similar findings have been reported by other workers showing that prevalence of malaria has been consistently higher in the Northern than Southern parts of Nigeria (Dawaki et al., 2016; Umaru and Uyaiabasi, 2015; Oguche et al., 2014). In the study by Oguche et al. (2014), the hospital prevalence of malaria was 25% in Lagos (South west) and in Barkin Ladi (North Central) it was 38%. The two sites in our study are close to large water bodies, River Niger in New Bussa and Lagos lagoon in Ijede, thus providing sufficient breeding ground for mosquitoes. The difference in prevalence could be attributed to the implementation of malaria control programme in Lagos State since 2010, starting with free diagnosis and malaria treatment programme in public health facilities, household distribution of LLINS to Indoor Residual Spray (IRS) of houses while this integrated malaria control interventions are low in Niger State. Furthermore, the living conditions in New Bussa are poorer and more conducive with transmission than in Ijede.

Malaria was hypodemic in Ijede but mesoendemic in New Bussa in the dry season based on malaria microscopy assessment in 2 to 9 year-old children. This is the first report showing that malaria drops to hypoendemic levels in Lagos during dry seasons. This is an indication that malaria burden in Lagos State is declining. A previous study carried out earlier in Lagos in a location close to and with similar environmental characteristics to the present study site reported malaria to be mesoendemic in the dry season (Aina et al., 2013). The mesoendemicity of malaria in New Bussa reported in this study is similar to previous reports in the region (Federal Ministry of Health, 2016; Adedoja et al., 2015).

The trend in malaria positivity rate (irrespectively of diagnostic method) and level of parasitaemia in the different age groups was similar in the two study sites, with age range 5 to 15 years having the highest prevalence and geometric mean parasite density. Our finding that children above 5 years are more at risk of malaria infection than under 5 year olds, has also been reported by other workers (Aina et al., 2013; Oladosu and Oyibo, 2013; Mawili-Mboumba et al., 2013; Umaru and Uyaiabasi, 2015; Ceesay et al., 2008). However, Nmadu et al. (2015) reported the highest prevalence of malaria in children between 2 to 5 years of age in a General Hospital in Abuja. The lower parasite rate in under 5-year olds than older children may be attributed to the impact of previous malaria interventions in Nigeria which focused on the protection of under 5-year olds and pregnant women at the time of the study (NPC, 2012). Currently, malaria intervention activities in Nigeria are targeted at all persons irrespective of level of susceptibility to malaria, while in a more distant past year 2005, efforts at renewed

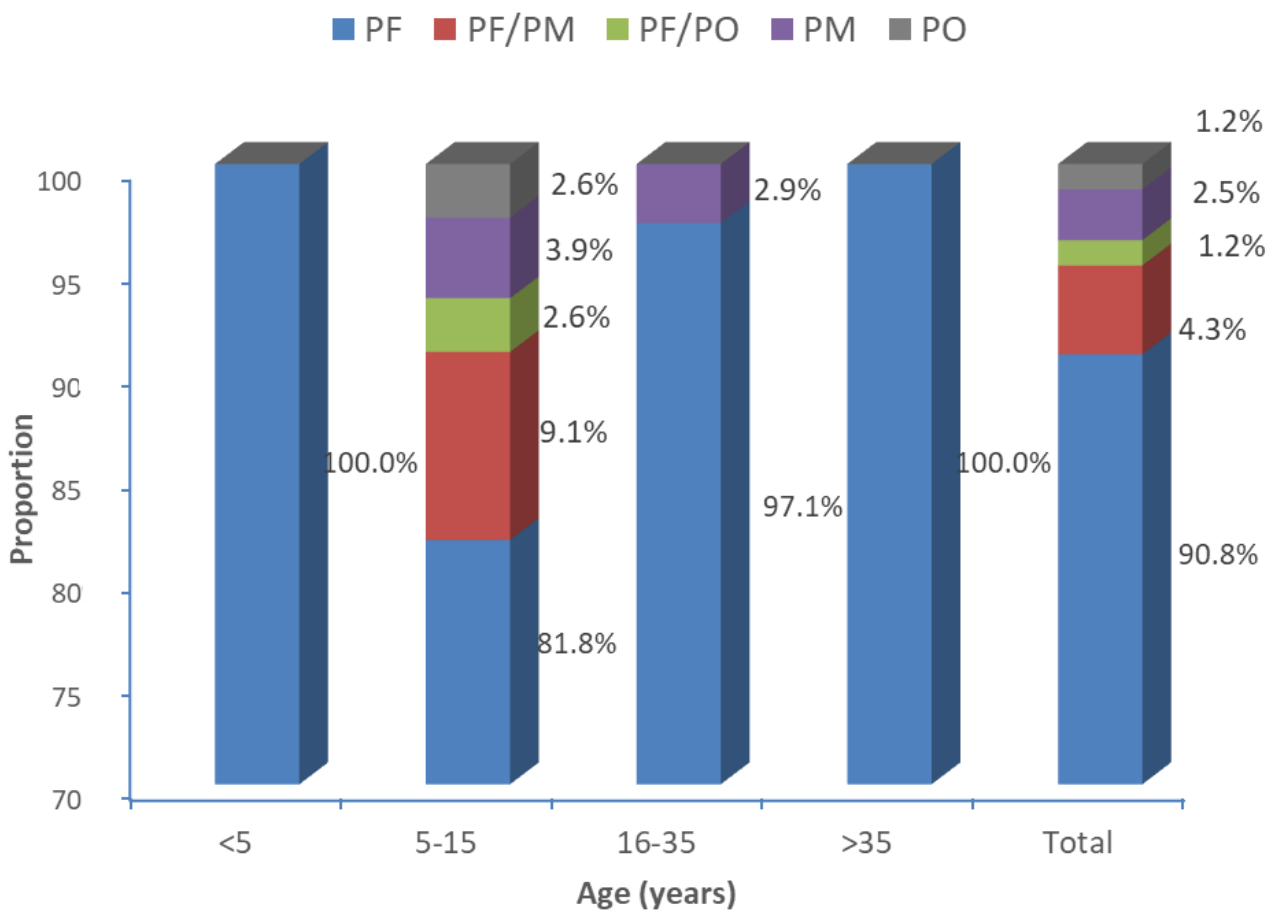

Figure 5. Plasmodium species distribution by age.

malaria control were effected through policy change in the drug of choice for treatment of malaria from the ineffective chloroquine and sulfadoxine pyrimethamine to Artemisinin Combination Therapies (Federal Ministry of Health, FMH 2005). Gains have accrued in an estimated20 to 40% decrease in malaria incidence and mortality rates between 2010 and 2015 (WHO, 2017) but the factors responsible for these gains are not all known (NMCP, suMAP and Project, 2013). It is apparent that there is delay in the acquisition of immunity to *P. falciparum* because children encounter the disease much latter and less frequently, a quality that describes pattern of malaria burden in meso-endemic regions as compared with situations when malaria transmission was hyper and holoendemic (Molineaux and Gramiccia, 1980; Carter and Mendis, 2002; Doolan et al., 2009). On the other hand, progressive decline in fever rate as age increased is a natural consequence of repeated exposure to infectious diseases, as age increases (Ladeia-Andrade et al., 2009).

The *Plasmodium* species distribution is similar in both study sites with *P. falciparum* having prevalence of over 95% (either as mono or mixed infection). It was noted that children under the age of five had only *P. falciparum* (100%) in both study sites unlike older age group where other *Plasmodium* species were observed albeit at very low levels. This is in agreement with studies by Sitali et al. (2015), but contrary to observation of inverse correlation of age with mixed infection by (Guerra-Neira et al., 2006). It may be attributed to the low immunity in the under 5 years and the high virulence of *P. falciparum* such that once the children under 5 are infected with *P. falciparum* it results quickly in acute malaria cases. However, in children above 5, their anti-disease immunity allows asymptomatic carriage of *P. falciparum* infection, thereby providing opportunity for multiple infections with malaria parasite species. This is the first report documenting different species in different age groups in the study sites.

The prevalence of anaemia was similar in both sites and there was no association between anaemia and malaria positivity unlike observed in a near-universal LLIN coverage study in Zambia which showed a strong positive correlation between the prevalence of malaria parasite infection and severe anemia. The predominantly low parasitaemia (1-500 parasite/µl of blood) was not

sufficient to cause a significant difference in anaemia levels [Anaemia prevalence: New Bussa 22%; Ijede 21%]. Although, the mean packed cell volume (PCV) values were significantly different, the relatively wide band width for normal PCV (approximately 17 points) (WHO, 2011) may have accounted for the similarity in anaemia levels in both sites. The lower mean PCV observed in New Bussa than in Ijede may be attributed to the higher parasite prevalence in New Bussa. The study did not assess other causes of anaemia apart from malaria.

CONCLUSION

The level of endemicity of malaria was different in both study sites, hypoendemic in Ijede and mesoendemic in New Bussa, during the dry season. Malaria prevalence was lower in children less than five years than in above 5-year old children. Fever rate reduced as age increased. However, malaria was not a major cause of fever in the two study sites. Anaemia prevalence was similar in both sites and was not associated with malaria.

RECOMMENDATION

Community based malaria study should not be based on fever rather it should be based on screening for malaria parasite. Malaria control intervention should be deployed to everyone and not only children under 5 years of age and pregnant women.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Low prevalence of soil transmitted helminths among children in rural areas in Senegal: A cross sectional survey

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Soil transmitted helminthiasis (STH) represents a major public health problem in tropical regions. In many countries including Senegal, STH control strategies usually involve mass deworming campaigns. This study was carried out to assess the prevalence and distribution of intestinal parasites among children, several years after the initiation of mass deworming campaigns with mebendazole in Senegal. A cross sectional survey was conducted in 8 villages located in the Southeastern part of Senegal. Children younger than 10 years old were sampled using a two level random sampling technique. Stool samples were collected from each participant after clinical assessment. Parasites detection was done by light microscopy using a modified Ritchie technique. Among the 1,163 surveyed children, 353 were found with at least one intestinal parasite species representing an overall prevalence of 30.4% (IC95%: 27.3 to 33.7). Proportion of children with protozoan infections was 29.6% (95%CI: 26.9 to 32.3); a small fraction of children were found with helminthic infestations (0.8%) (95%CI: 0.3 to 1.4). The identified parasites were represented by *Giardia intestinalis* (17.7%), *Entameoba coli* (14%), *Endolimax nana* (0.86%), Hookworm (0.52%), *Ascaris lumbricoides* (0.17%), and *Hymenolepis nana* (0.34%). This study revealed a low prevalence of helminthic infestations while protozoan infections remained high. This changing profile in the epidemiology of intestinal parasitic infections among children may require revision of the current deworming policy programme. However, extensive data at the national level are needed to support modification of strategy.

Key words: Helminths, protozoan, children, Senegal.

INTRODUCTION

Intestinal parasitic infections are still a public health concern despite reported declines in Sub-Saharan and Asian countries (de Silva et al., 2003; Pullan and Brooker, 2012). Globally 1.45 billion people are infected

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worldwide with at least one species of a nematode (Pullan et al., 2014). In sub-Saharan countries, 866 million individuals are infected by Soil Transmitted Helminth (STH), with the majority of these infections occurring among pre-school and school children (Pullan et al., 2014). These paediatric infections can lead to adverse effects on nutrition, growth and cognition and contribute to the global burden of childhood anaemia (Moore et al., 2012; Balarajan et al., 2011; Bethony et al., 2006).

The control strategies of STH in many endemic countries usually involve mass drug administration (MDA) programmes with single oral dose of Mebendazole or Albendazole periodically administered to pre-school and school children (Levecke et al., 2014; Gabrielli et al., 2011; WHO, 2012). The word health organisation (WHO) has advocated for countries to roll out anti-helminthic drugs with a long term goal of eliminating STH as a public health problem by 2020 (WHO, 2015). Consequently, in the last 10 years, there has been increased scientific and financial support for the control of STH, with a strong focus on mass deworming campaigns targeting pre-school and school children (Bundy, 2011; WHO, 2015).

In Senegal, mass deworming programmes have being implemented since 2005 as a national initiative to reduce morbidity related to STH. The national deworming initiative implemented by the Ministry of Health in Senegal consists of administering a single dose of mebendazole (500 mg) twice yearly to pre-school children (6 to 59 months). In 2010, a study conducted by the Department of Parasitology, University of Dakar, revealed a high coverage of MDA (95%) with mebendazole and a low prevalence of STH (1.5%) among children living in rural areas in the central part of the country (Tine et al., 2013). However, more epidemiological and parasitological data are required to confirm reported findings as well as assess the situation in other places such as the southern part of the country, where STH have been considered in the past years, as major public health problem (Diouf et al., 2000). Such a data will contribute to further guide the existing STH control programs. The present study aimed at assessing the prevalence and distribution of intestinal parasites species in children under the age of 10 years living in southern rural areas of Senegal, several years after the implementation of mass deworming initiatives.

METHODOLOGY

Study area

The study was conducted at the Bonconto health post, located in the Velingara health district in the south-eastern region of Senegal, 500 km from the capital city of Dakar. The area is predominately rural and most residents live in villages as agriculturists and farmers. Mass deworming that was initiated in 2006 covers this area. A single oral dose of 500 mg mebendazole is administered in June and December each year. Drug delivery is done by community health workers under the supervision of district health staff.

Study design and population

A cross sectional survey was conducted within the 8 villages covered by the Bonconto health post. In each village, prior to the study, a census was done to enumerate all under 10-year-old children living in the study area. Children less than 10 years of age were sampled using a two level random sampling technique. At the first level, participating villages were randomly selected from the list of the 16 villages covered by the Bonconto health post. Study subjects were then randomly selected from the sampled villages using the list of enumerated children.

Sample size calculation

With 1163 children sampled, the study was powered at 90% to detect 5% variation of intestinal parasite prevalence, assuming a current prevalence of intestinal parasite carriage at 25% based on previous studies (Tine et al., 2013) with alpha at 0.05 (two sided).

Data collection methods and specimen examination

Data were collected using a structured questionnaire followed by a biological assessment. An initial physical examination was first conducted by a physician and all data obtained from physical examination and interviews were assigned on a case report form (CRF). A code was given to every child after parents' informed consent. For each enrolled child, the mother was interviewed directly concerning the child's symptoms as well as sociodemographic characteristics, history of mebendazole uptake using a standard questionnaire. Fresh stools samples were collected into wide mouth screw-cap clean containers. Faecal samples were examined for the intestinal parasite using direct microscopic examination and a modified Ritchie technique. Intestinal parasite was recorded as positive in the presence of helminthes and/or protozoans in the faeces.

Data management and data analysis

Data were double entered in Excel software and all analyses were conducted using Stata package (StataCorp, Texas). For categorical data, percentage was used to assess the frequency of each outcome with a 95% confidence interval. Characteristics of all children included in the study were tabulated. Proportions were compared using chi square test (univariate analysis). To assess the main factors associated with intestinal parasitic infections, a multivariate logistic regression was done with adjustment on covariates such as exposure to mebendazole in the previous 6 months, age group, gender, mother or care taker level of instruction. From the final model, adjusted odds ratios were derived with their 95% confidence interval. Model validity was tested using the Hosmer-Lemeshow goodness of fit test. The performance of the final model was assessed by the area under the curve and Akaike and Bayesian information criterion; in addition a test for multicolinearity between variables was done using the variance inflation factor. Significance level of the different tests was 0.05 (two sided).

Ethical considerations

The study protocol was approved by the Conseil National de Recherche en Santé in Senegal (N° 027/MSP/DS/CNRS). Prior to the study, a community sensitization was undertaken in villages surrounding the Bonconto health post and community consent was obtained from community leaders (religious guide, village head).

Table 1. Study participant's characteristics at enrolment (N=1163).

Parameter	Number	Percentage	95%CI
Age group (years)			
under 5	604	51.9	49.1 - 54.8
5 to 10	559	48.1	45.1 - 50.9
Sex			
Female	571	49.1	46.2 - 52.0
Male	592	50.9	47.9 - 53.8
Care taker's level of instruction			
Non educated	917	78.8	76.4 - 81.2
Primary school	152	13.1	11.2 - 15.1
Secondary school	33	2.8	1.9 - 3.9
Coranic instruction	16	1.4	0.8 - 2.2
Family size			
Less than 5 members	848	72.9	70.3 - 75.4
5 to 10 members	301	25.9	23.4 - 28.5
More than 10 members	14	1.2	0.6 - 2.0
Symptoms at enrollement			
Nausea	20	1.7	1.1 - 2.6
Vomiting	70	6.0	4.7 - 7.5
Anorexia	79	6.8	5.4 - 8.4
Palor	257	22.1	19.5 - 24.3
Diarrhea	110	9.5	7.8 - 11.3
Exposure to antihelminthic treatment within previous 6 months			
Under 5 years	479 / 604	79.3	75.8 - 82.4
5 to 10 years	173 / 559	30.9	27.1 - 34.9

On the day of survey, informed consent was obtained from mothers or children's care takers prior to their enrollement. The study was conducted in collaboration with the Velingara health district.

RESULTS

Study participant's characteristics

Overall, 1163 eligible children participated in the study. Children under 5 years of age represented 51.9%; proportion of female children was 49.1%. The majority of interviewed mothers or caretakers were illiterate (78.8%). The main symptoms presented by study participants on the day of survey were pallor (22.1%), diarrhoea (9.5%), anorexia (6.8%), vomiting (6%) and nausea (1.7%). Table 1 summarises study participants characteristics at enrolment.

Prevalence and distribution of intestinal parasitic infections among study participants

Overall, 353 children were found with at least one intestinal parasite species. The prevalence of intestinal parasites was 30.4%. Intestinal parasitic infections were predominantly caused by protozoas (29.6%), (95%CI: 26.9 to 32.3) relative to helminthics infections (0.8%) (95%CI: 0.3 to 1.4). Intestinal parasitic infections were mainly caused by *Giardia intestinalis* (14%), *Entamoeba coli* (17.7%), *Endolimax nana* (0.9%), Hookworm (0.5%), *Ascaris lumbricoides* (0.2%), and *Hymenolepis nana* (0.3%) (Table 2).

Intestinal parasitic infections were higher among children aged between 5 to 10 years (34.5%), as compared to children less than 5 years (26.5%) with a prevalence ratio of 1.3 (95%CI (1.1 to 1.6), p = 0.003). Protozoan infections were more frequent among children aged above 5 years (33.4%) compared to the youngest age group (25.9%) (prevalence ratio 1.3; 95%CI (1.1 to 1.6), p = 0.005)). No statistical difference was found in terms of helminthic infections between the two age categories (prevalence ratio: 2.2 95%CI (0.5 - 8.6); p=0.26)). Parasitic infections distributions by age groups are summarised in Table 3.

Among patients with protozoan infections, the most frequently reported symptoms were pallor (18.5%) and

Table 2. Overall prevalence of intestinal parasitic infections among study participants (N=1163).

Parameter	Number of positive	Prevalence (%)	95%CI
Overall intestinal parasite carriage	353	30.4	[27.7 - 33.1]
Overall protozoan infections	344	29.6	[26.9 - 32.3]
Overall helminthic infestation	09	0.8	[0.3 - 1.4]
Parasites especies			
Giardia intestinalis	163	14.0	[12.2 - 16.1]
Entameaba coli	206	17.7	[15.5 - 20.0]
Endolimax nana	10	0.9	[0.4 -1 .6]
Hookworm	06	0.5	[0.2 - 1.1]
Ascaris lumbricoides	02	0.2	[0.02 - 0.6]
Hymenolepis nana	04	0.3	[0.09 - 0.8]

Table 3. Prevalence of intestinal parasitic infections by age group and gender.

Parameter	Under 5 years (N=604) [n(%), (95%CI)]	5 to 10 years (N=559) [n(%), (95%CI)]	Prevalence ratio 95%CI	p value
Overall intestinal parasite carriage	[160(26.5), 23.0 - 30.2]	[193(34.5), 30.6 - 38.6]	1.3(1.1 - 1.6)	0.003
Overall protozoan infections	157(25.9), 22.5 - 29.7]	[187(33.4), 29.5 - 37.5]	1.3(1.1 - 1.5)	0.005
Overall helminthic infestations	[03(0.5), 0.1 - 1.4]	[06(1.1), 0.4 - 2.3]	2.2(0.5 - 8.6)	0.26
Parasites especies				
Giardia intestinalis	[87(14.4), 11.7 - 17.5]	[76(13.6), 10.8 - 16.7]	0.94(0.7 - 1.3)	0.69
Entameaba coli	[87(14.4), 11.7-17.5]	[119(21.3), 17.9 - 24.9]	1.5(1.1 - 1.9)	0.002
Endolimax nana	00	[10 (1.8), 0.8-3.3]	--	0.001
Hookworm	[01 (0.2), 0.00 - 0.9]	[05(0.9), 0.3 - 2.1]	5.4(0.6 - 46.1)	0.08
Ascaris lumbricoides	00	[2(0.4), 0.04 - 1.3]	--	0.14
Hymenolepis nana	[02 (0.3), 0.04 - 1 .2]	[02(0.4), 0.04 - 1.3]	1.1(0.1 - 7.6)	0.93
	Female (N=532) (n, %, 95%CI)	**Male (N=592) (n, %, 95%CI)**	**Prevalence ratio 95%CI**	**p value**
Overall intestinal parasite carriage	[177(33.3), 29.3 - 37.4]	[171(28.9), 25.3 - 32.7]	0.9 (0.7 - 1.0)	0.11
Overall protozoan infections	[175(32.9), 28.9 - 37.1]	[164(27.7), 24.1 - 31.5]	0.8 (0.7 - 1.0)	0.06
Overall helminthic infestations	[05(0.9), 0.3 - 2.2]	[04(0.7), 0.2 - 1.7]	0.7 (0.2 - 2.6)	0.62
Parasites especies				
Giardia intestinalis	[97(18.2), 15.0 - 21.8]	[64(10.8), 8.4 - 13.6]	0.6 (0.4 - 0.8)	0.0004
Entameaba coli	[92(17.3), 14.2 - 20.8]	[110(18.6), 15.5 - 21.9]	1.1 (0.8 - 1.4)	0.57
Endolimax nana	[05(0.9), 0.3 - 2.1]	[05(0.8), 0.3 - 1.9]	0.9 (0.3 - 3.1)	0.86
Hookworm	[04(0.7), 0.2 - 1.9]	[02(0.3), 0.04 - 1.2]	0.4 (0.1 - 2.4)	0.34
Ascaris lumbricoides	[02(0.4), 0.04 - 1.3]	00	00	0.14
Hymenolepis nana	[01(0.2), 0.00 - 1.0]	[03(0.5), 0.1 - 1.4]	2.7 (0.3 - 25.8)	0.37

diarrhoea (13.8%); other observed symptoms were anorexia (7.9%), vomiting (6.5%) and nausea (2.4%). Only one patient with helminthic infection was found with pallor. No other symptoms were described in patients with helminthic parasites. Episodes of diarrhoea was more frequently reported among patients with protozoan infections (13.8%) compared to patients with helminthic infections (0%) and non-infected participants (7.7%) p = 0.003. The frequency of reported symptoms by type of infection is presented in Table 4.

Factors associated with intestinal parasitic infections among study participants

Participants who had received mebendazole during the previous 6 months prior to the survey were less likely to carry intestinal parasites compared to those who did not

Table 4. Distribution of reported symptoms among study participants

Parameter	Non infected (N=814)	Helminthic infestation (N=9)	Protozoan infections (N=340)	p value
Nausea	12 (1.5%)	00	08 (2.4%)	0.53
Vomiting	48 (5.9%)	00	22 (6.5%)	0.70
Anorexia	52 (6.4%)	00	27 (7.9%)	0.45
Palor	193 (23.7%)	01 (11.1%)	63 (18.5%)	0.11
Diarrhea	63 (7.7%)	00	47 (13.8%)	0.003

Table 5. Adjusted analysis of intestinal parasitic infections among study participants.

Parameter	Intestinal parasites prevalence (95%CI)	Unadjusted prevalence ratio (95%CI)	Adjusted prevalence ratio (95%CI)	p value
Received mebendazole in previous 6 months				
No	37.4 (30.7 - 38.8)	1	1	
Yes	24.9 (21.6 - 28.3)	0.55 (0.43 - 0.71)	0.65 (0.48 - 0.92)	0.01
Gender				
Girls	31.9 (28.1 - 35.9)	1	1	
Boys	28.9 (25.3 - 32.7)	0.87 (0.68 - 1.11)	0.77 (0.59 - 0.97)	0.04
Mother's education level				
No formal education	31.6 (28.1 - 35.5)	1	1	
Coranic education	18.7 (3.9 - 54.8)	0.49 (0.14 - 1.76)	0.74 (0.20 - 2.69)	0.65
Primary school	30.9 (22.7 - 41.1)	0.97 (0.67 - 1.40)	1.01 (0.69 - 1.49)	0.95
Secondary school	18.2 (6.7 - 39.6)	0.48 (0.20 - 1.18)	0.35 (0.14 - 0.88)	0.02
Age group (years)				
Under 5	26.5 (23.0 - 30.2)	1	1	
5 to 10	34.5 (30.6 - 38.6)	1.46 (1.14 - 1.88)	1.10 (0.79 - 1.52)	0.57

Goodness of fit test: Hosmer-Lemeshow Chi (7df) =4.01; p=0.78. Area under the curve (AUC)=68.3%; Akaike Information Criterion (AIC): 1339; Bayesian Information Criterion (BIC): 1365; variance inflation factor: 1.17.

received any anti-parasite treatment during the previous 6 months: adjusted odds ratio at 0.65; 95%CI (0.48 to 0.92). Other factors significantly associated with intestinal parasitic infections were represented by: (i) gender: male children were less likely to be infected compared to female children (adjusted odds ratio: 0.77 ; 95%CI (0.59 to 0.97) ; p=0.04); (ii) education level of mothers or care takers: children whose mothers or care takers instruction level was equivalent to secondary school, were less likely to carry intestinal parasites compared to others (adjusted odds ratio: 0.35 ; 95%CI (0.14 - 0.88), p=0.02) (Table 5).

DISCUSSION

As part of a long-term goal of eliminating STH as public health problem by 2020, WHO recommended MDA with single oral dose of mebendazole or albendazole periodically administered to pre-school and school age children (Gabrielli et al., 2011; Levecke et al., 2014; WHO, 2012). This strategy is currently implemented in many countries including Senegal in order to control morbidity related to STH. Our study investigated the prevalence and distribution of intestinal parasitic infections among children less than 10 years of age in rural areas in Senegal, several years after the implementation of MDA campaigns.

One third of the study participants were infected with at least one intestinal parasite species and protozoan infections were predominant (29.9% of infected children) compared to the small fraction of children carrying helminthic parasites (0.8%). Contrary to previous studies conducted in Senegal, helminthic infections have become very rare, while protozoan infections are more common, with *Giardia* being the predominant pathogenic protozoa. Indeed, previous studies demonstrated a prevalence of helminthic infections of 42 to 56% (Faye et al., 2008; Faye et al., 1998a) with *A. lumbricoides* being the most

prevalent STH (22 to 34%) (Faye et al., 1998b), followed by *Trichuris trichiura* (15 to 20%) (Faye et al., 1998c). Protozoan infections ranged from 15 to 22.5% (Diouf et al., 2000).

The low prevalence of helminthic infections among children observed in this study is in line with recent findings from other rural areas in Senegal (Tine et al., 2013) and other sub-Saharan countries (Moser et al., 2017). Several years after the initiation of mass deworming campaigns, helminthic infections among children in this area of Senegal have significantly decreased to a point where they can no longer be regarded as a significant public health treat. Consequently a substantial modification in the spectrum of intestinal parasites is noted, with protozoan parasites predominating.

While helminthic infections remained at a low level, one third of the study participants were infected with protozoan species in which *Giardia* was the predominant pathogen. Single dose of mebendazole given during deworming campaigns has limited effect on Giardiasis (Olsen, 2003; Keiser and Utzinger, 2008), and may have induced a “replacement effect” on parasite species as described in other settings (Moore et al., 2012). Observed frequency of Giardia is however consistent with other findings (Tine et al., 2013; Azazy, 2002). The deleterious effect of *Giardia intestinalis* on growth and health of children has been shown by several studies (Al-Mekhlafi et al., 2005; Rosenthal, 1999). This parasite is known for its ability to induce diarrhoea (Polis et al., 1986) and mal-absorption syndrome. It can lead to protein energy malnutrition, vitamin A deficiency, iron deficiency anaemia and vitamin B12 deficiency (Gendrel et al., 2003).

Although, *Giardia* prevalence remained high, the potential source of contamination for children with regard to this parasite remained unclear. Distributions of Giardiasis and other intestinal parasites are often linked to lack of sanitation, lack of access to safe water and improper hygiene (Abossie and Seid, 2014). The study did not investigated the distribution of these known risk factors, but its was noted that children whose mothers or caretakers instruction level was equivalent to secondary school were less likely to carry intestinal parasites which is in line with findings from other studies (Okyay et al., 2004). Improving population awareness on Giardiasis risk factors and its modes of transmission could contribute to further reduce morbidity related to this parasite. Additional data for a better understanding of the epidemiology of *Giardia* and its determinants will be needed to optimise Giardiasis control strategies (Savioli et al., 2006).

Conclusion

This study revealed a low prevalence of helminthic infections while protozoan infections remained high. This changing profile in the epidemiology of intestinal parasitic infections among children may require revision of the current deworming policy programme. However, extensive data at the national level are needed to support modification of strategy.

CONFLICT OF INTERESTS

The authors have no conflicts of interests concerning the work reported in this paper.

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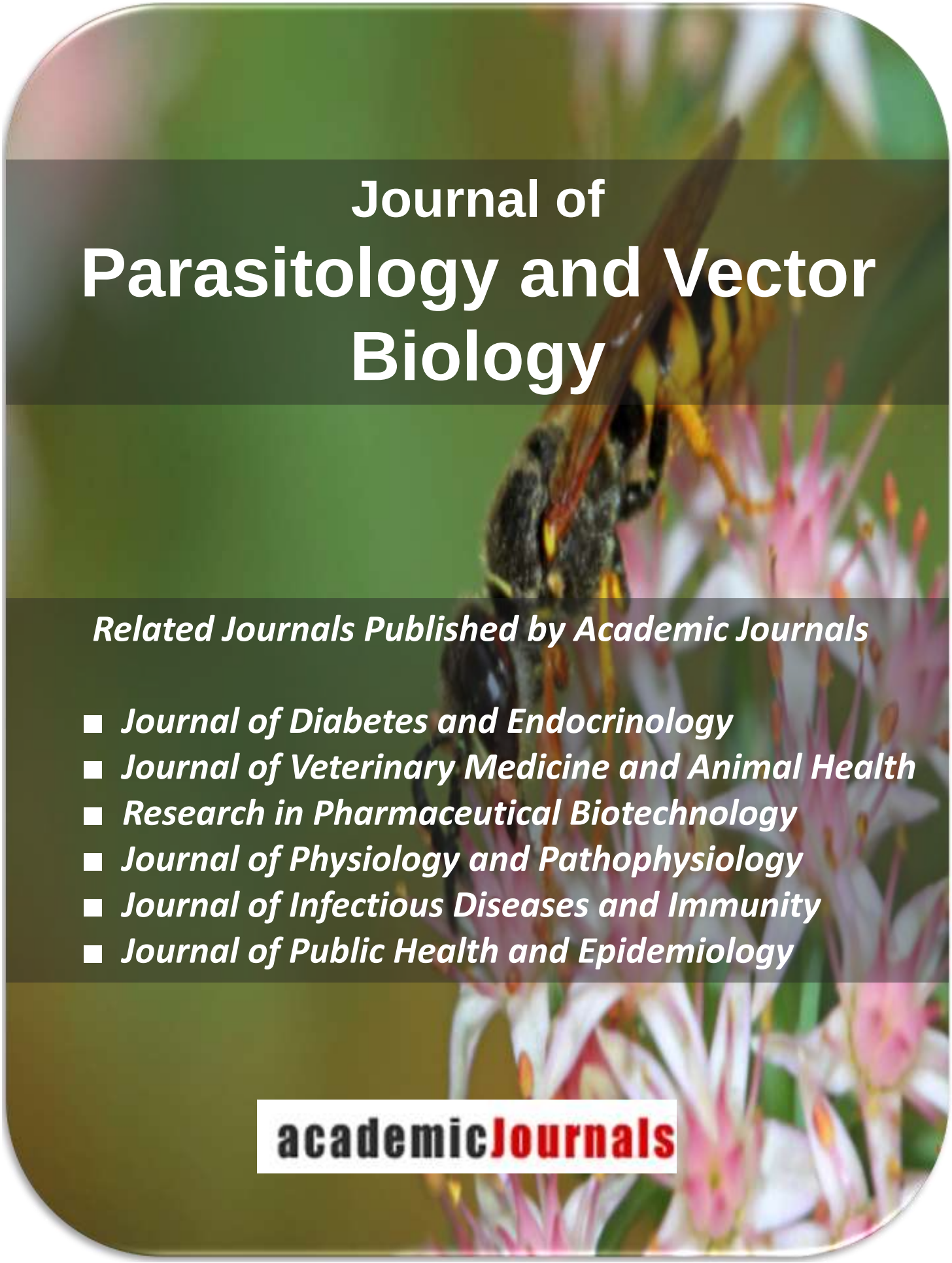

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