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The Association Between Malaria Parasitaemia, Intestinal Parasite and Anemia in Children Less Than 6 Month in Senegal: A Cross Sectional Survey

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Abstract: Introduction: Although malaria is declining in many countries in Africa, malaria and anaemia remain frequent in children. This study was conducted to assess the relationship between malaria parasitaemia, intestinal worms, and anaemia, in children <6 months living in low transmission area in Senegal. Methods: A survey was carried out in Lamarame in the central part of Senegal. A cross sectional survey was used to select study participant. Children <6 months were enrolled after informed consent. For each child, blood thick and smear tests were performed, haemoglobin concentration was measured with HemoCue, and stool samples were collected and examined using the Ritchie technique. Result: A total of 162 children were recruited. Malaria parasite prevalence was 0.75% (0.7–2.6); anaemia was found in 16.7% (11.3–23.3), while intestinal parasites and stunting represented 25.4% (18.5–33.4) and 22% (18.6–25.5), respectively. The association was found between intestinal parasites and anaemia OR=1.1 (0.3-3.4). Conclusion: Malaria and anaemia remain closely associated even when malaria is declining. Scaling up antimalarial interventions may contribute to eliminate malaria and reduce the occurrence of anaemia among children.

Keywords: Malaria Parasitimia, Intestinal Parasite, Anemia, Children

1. Introduction

Malaria remains major public health problem worldwide particularly in sub-Saharan Africa. According to the World Health Organization, an estimated 219 million malaria cases and 434,000 malaria deaths occurred worldwide. 92% of malaria cases and 93% of deaths are observed in Africa. Children under five are the most vulnerable group affected by malaria [1].

Also the morbidity related to intestinal parasitic infection caused by pathogenic protozoa and helminths is important worldwide, particularly in developing countries where children are most affected. It is estimated that 3.5 billion patients are affected and that 450 million of them are ill [2]. According to World Health Organization (WHO), children living in endemic are most affected with an estimated number of 270 million preschool children and over 600 million school children [3]. These pediatric 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; Bethonyet al. 2006.

Anaemia also remains a major public health problem, not least in malaria endemic areas, where it is primarily seen in young children in areas with stable malaria, but also in adults in malaria unstable areas [4-5].

In tropical regions, there are multiple aetiologies to anaemia and intestinal parasites as well as malnutrition can play a major role in anaemia occurrence [6], particularly when malaria prevalence is declining. Thus, exploring the relationship between malaria, intestinal parasites, malnutrition, and anaemia can provide useful information in order to guide public health programs aiming at reduction of child health problems in tropical regions.

This study was aiming (i) to assess the prevalence of malaria parasitaemia, intestinal parasites (IP), anaemia, among children less than 6 month and (ii) to explore the relationship between malaria, intestinal parasites, and anaemia.

2. Methodology

2.1. Population and Methods

2.1.1. Study Area and Population

The study was conducted at the Lamarame health post located in the Keur Socé, a rural community, at 17 Km from the city of Kaolack in central Senegal, and 200 Km from Dakar. The Lamarame health post is in the Ndoffane health district. It covers 73 villages located within a radius of 8 Km from the post, with a total population of about 30,000 inhabitants.

Malaria is endemic in this area with a seasonal pattern of transmission peaking during the rainy season (July–November).

Many programs aiming to reduce child health problems are being promoted in this area. These programs included two yearly vitamin A supplementation, and deworming with Mebendazole, in December and June of each year for children aged 1 to 5 years.

A Cross sectional survey household survey was carried out at the end of the malaria transmission season, in January 2010. one month after the second round of Mebendazole mass administration campaign.

2.1.2. Data Collection

(i). Structured Questionnaire

A code was given to every child after parents' informed consent. Each eligible child was examined by a physician prior to a biological assessment which included blood and stool samples. The mother was interviewed directly concerning the child's symptoms as well as well as sociodemographic characteristics using a standard questionnaire. Data obtained from physical examination and parents interview were assigned on a case report form (CRF).

(ii). Biological Assessment

Blood samples were collected using finger prick blood. The first drop was used to prepare thick and thin smears for the diagnosis of malaria. Thick and thin smears were stained with Giemsa and read by a laboratory technician. Malaria was defined as any asexual parasitemia detected on a thick or thin blood smear. Parasitemia was numbered and expressed by number of trophozoites/μL using the following formula: numbered parasites ×8000/200 assuming a white blood cell count of 8000 cells per μL. Absence of malaria parasite in 200 high power ocular fields of the thick film was considered as negative.

The second drop of finger prick blood was drawn into a microcuvette for Hb determination (g/dL) using HemoCue machine (HemoCue Hb 301). Moderate and severe anaemia were defined as hb concentration below 11 g/dL and 8 g/dL, respectively.

Fresh stools samples were collected into wide mouth screw-cap clean containers. Fecal samples were examined for the detection of intestinal parasite using the Ritchie technique. Intestinal parasite was recorded positive by the presence of helminthes and/or protozoans in the faces.

2.2. Sample Size

Taking into account an estimated prevalence of malaria of the order of 20% and a prevalence of anemia at the study area of 35%, a confidence level of 95% (&=5%), a statistical power of 80% (beta=20%) for an accuracy of 5%, the number of children aged between 4 and 6 weeks to be recruited during this survey was estimated at 110; considering a percentage of 10% non-response or defective sampling, this size was increased to 125 children.

3. Data Analysis and Management

The data was entered on Excel software and the analysis was done with STATA IC 10 software. Quantitative variables were described in terms of average, standard gap. Inter-group comparisons were made using an ANNOVA test or the Student t test after verification of the conditions of application of these tests. Where these tests were not applicable, nonparametric tests (Man withney, Kruskall Wallis) were chosen.

For qualitative variables, a description in terms of numbers, percentage of data filled in was made.

The analysis of contingency tables was done using Pearson's Chi Square or Fisher's exact test according to the conditions of applicability.

A bivariate analysis was used to see the existence of the association between malaria, anemia and intestinal parasitosis.

The significance threshold of the tests was set at 5%.

4. Results

4.1. Positioning of the Children of the Study

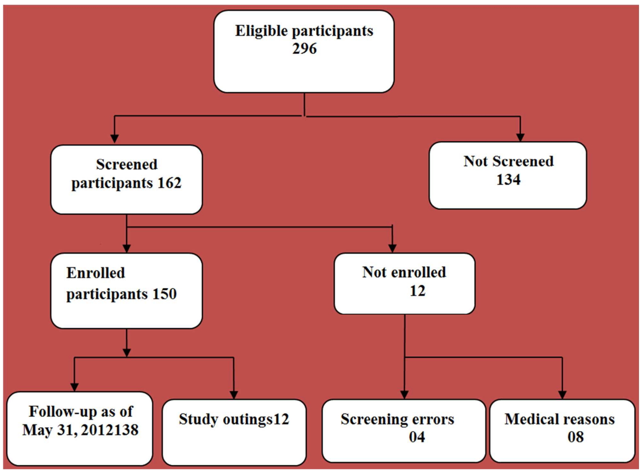

Figure 1. *Positioning of the children of the study.*

The recruitment of children took place over five months from December 2010 to May 2011 from the health centres of Keur Socé and Lamarame, all belonging to the rural community of Keur Socé. During this period, on a list of 296 eligible infants aged between 4 and 6 weeks, 162 were screened. 12 infants were not selected for non-compliance with the inclusion criteria. Of these 12, 8 were not selected for medical reasons and the other 4 for screening error on age. Thus, 150 children were enrolled in our study cohort.

Of the 150 included, there were 12 study outings including 2 deaths, 4 withdrawals of consent, 4 lost-of-sight and 2 excluded for change of residence outside our intervention area.

4.2. General Characteristics of the Survey Population

In our study population the age at inclusion varied between 28 and 48 days with an average of 36.3 days and 5.6 standard deviation. Male and female children accounted for 50% (75/150) each with a sex ratio of 1. The inclusion weight ranged from 2.2 kgs to 6.2 kgs. The average weight was 4.0+/-0.6. Children with low weight (between 2 and minus 4 kgs) accounted for 46.7% against 53.3% for those with a normal weight (between 4 and minus 7 kgs).

The size at inclusion ranged from 47 to 57 cm. The average size was 52.3 +/-2.1.

The number of children living with their mothers ranged from 1 to 8. The average number of children per mother in our study cohort was 3.4 +/-1.95. 54.8% of the mothers surveyed had between 1 and 3 living children against 28, 1% and 17.1% who respectively had between 4 and 5 children and more than 5 children.

The minimum age for mothers of children in the study was 17 years and 40 years for the maximum. The average age was 25.3 +/-5.6.

The majority of children included in this study 76.7% (115/150) were from villages polarized by the Keur Socé health post compared to 23.3% from the Lamarame health post polarized area with no statistically significant difference (p=0.666).

Table 1. *General characteristics of the survey population.*

Variables	Number	Pourcentage	IC 95%
Age Categories at Inclusion (days)			
[28-38]	97	64,67	[56,8-72,0]
[39-48]	53	35,33	[28,0-43,2]
Gender			
Male	75	50	[42,0-58,0]
Female	75	50	[42,0-58,0]
Weight at inclusion (kgs)			
[2-4]	70	46,7	[38,8-54,7]
[4-7]	80	53,3	[45,3-61,2]
Size at inclusion (cm)			
[45-50]	12	8	[4,4-13,2]
[50-60]	138	92	[86,8-95,6]
Number of living children of the mother			
[1-3]	80	53,3	[45,3-61,2]
[4-5]	41	27,3	[20,7-34,9]
More than 5	25	16,7	[11,3-23,3]
Not determined	4	2,7	[0,9-6,3]
Mother's age (years)			
Less than 20 years	23	15, 3	[10,2-21,8]
[20-35]	121	80,7	[73,8-86,4]
More than 35 years	6	4	[1,6-8,1]
Area of residence			
Villages pollarized by the HP of Keur Soce	115	76,7	[69,4-82,9]
Villages pollarized by the HP of Lamarame	35	23,3	[17,1-30,6]

P= 0.666 between residents of Keur Socé and Lamarame.

In this study, only one clinical malaria case was noted to be 59 days of life with a parasitic density of 1640 parasites per microlitre of blood. A thick drop made after three days of treatment with ACTs returned negative. The prevalence of malaria in our cohort was 0.7% (1/138).

No cases of malaria infection were noted between six months and 12 months.

4.3. Prevalence of Anemia

The average hemoglobin level in children in the inclusion study was 12.5 +/-6. The minimum hemoglobin level noted was 9.4 g/dl and the maximum was 17.2 g/dl. The majority of children (83.3%) at inclusion had a normal hemoglobin level. The incidence of moderate anemia which was 16.7% at inclusion increased significantly to 55.3% at 6 months and 51.9% at 12 months compared to severe anemia which was 0% at inclusion, 0% at 6 months and 1.5% at 12 months. (Table 2)

Table 2. *Prevalence of anemia in the survey population.*

Hemoglobin level in g/dl At inclusion			At 6 months		At 12 months	
	%	[IC]	%	[IC]	%	[IC]
Normal (11-17)	83,3	[76,6-88,7]	44,7	[36,8-52,7]	46,6	[38,8-54,7]
Moderated anemia (inf à 11)	16,7	[11,3-23,3]	55,3	[47,3-63,2]	51,9	[44,0-59,9]
Severe anemia Hb inf à 8	0		0		1,5	[0,2-4,3]

P= 0.022 between anemia at birth and at six months.

4.4. Prevalence of Intestinal Parasites

The prevalence of carriage for the main intestinal parasitic species found in our study was: *Giardia intestinalis* (15.4%), *Ascaris lumbricoides* (8.5%), *Entamoeba coli* (1.5%), Yeast (1.5%), Trichuris trichiura (0.8%). (Table 3)

Table 3. *Prevalence of intestinal parasite carriage at the survey population level.*

Intestinal parasites	Number	Pourcentage	Confidence interval
Stool examinations performed	*130*	100	
Children with at least one parasitic type	33	25,4	[18,5- 33, 4]
Giardia intestinalis	20	15,4	[9,9-22,4]
Entameaba coli	2	1,5	[0,3-5,1]
Ascaris lumbricoides	*11*	8,5	[4,5-14,2]
Yeasts	2	1,5	[0,3 -5,1]
Trichuris trichiura	1	0,8	[0, 1-3,7]

It was demonstrated in this study that there was an association between age and parasitic carriage. Children aged 12 months had a 10% higher risk of carrying intestinal parasites (0R=1.1 [0.3-3.4], p=0.845) than children aged 9-10 months. In children aged 11 months there was no association between age and parasitic carriage.

An association between sex and parasitic carriage was noted. Girls had more than 10% risk of carrying intestinal parasites (OR=1.1 [0.5-2.4], p=0.761) than boys.

Children in the mother's [4-5] number group were shown to be 40% more likely to be carrying intestinal parasites (OR= 1.4 [0.5-3.4], p=0.460) than children in the [1-3] group.

It was shown that there was an association between the area of residence and the parasitic portage. Children from the Lamarame area had more than 10% risk of carrying intestinal parasites (OR=1.1 [0.4-2.7], p=0.773) than those from the Keur Socé area.

4.5. Factors Associated with Intestinal Parasitic Carriage

Table 4. *Factos associated with intestinal parasite carriage.*

Variables	Intestinal Parasitic Carriage				
	Number	*(%)*	ORa*	[95% IC]	p value
Age					
9-10 months	5	(25%)	1		
11 months	5	(22, 73%)	0,8	[0,2-3,6]	0,863
12 months	23	(26, 13%)	1,1	[0,3-3,4]	0,845
Gender					
Boy	16	(24,24%)	1		
Girl	17	(26,56%)	1,1	[0,5-2,4]	0,761
Number of children of mother					
[1-3]	17	*(25,37%)*	1		
[4-5]	11	(32,35%)	1,4	[0,5-3,4]	0,460
Plus de 5	5	(20,00%)	0,7	[0,2-2,2]	0,592
area of residence					
Keur Socé	24	(24,74%)	1		
Lamarame	9	(27,27%)	1,1	[0,4-2,7]	0,773

This study showed that there was an association between parasitic carriage and anemia. Children with parasites were more than 40% at risk of anemia (OR=1.4 [0.6-3.4], p=0.395) than negative children.

The existence of an association between sex and anemia has also been demonstrated. Girls were more than 20% at risk of anemia (OR=1.2 [0.5-2.6], p=0.545) than boys.

Children in the mother's number group [4-5] were less than 30% at risk of anemia than those in the group [1-3].

Children living in the Lamarame area were twice as likely to be anemic (OR=2 [0.8-4.8], p=0.105) as those living in the Keur Socé area.

Children with parasitic infections at 12 months were less than 30% at risk of anemia compared to children at 9-10 months.

There was no association between anemia and malaria (p=0, 366).

4.6. Factors Associated with Anemia

Table 5. *Factors associated with anemia.*

Variables	Number	pourcentage	OR*	(95% IC)	p value
Parasit Carriage					
No	97	74,6	1		
Yes	33	25,4	1,4	[0,6-3,4]	0,395
Gender					
Boy	75	50	1		
Number of children of mother	75	50	1,2	[0,5-2,6]	0,545
[1-3]	80	53,3	1		
[4-5]	41	27,3	0,7	[0,2-1,7]	0,444
More than 5	25	16,7	0,6	[0,2-1,8]	0,435
Area of residence					
Keur Socé	115	76,7	1		
Lamarame	35	23,3	2,0	[0,8-4,8]	0,105
Age portage					
9-10 months	5	25	1		
11 months	5	22,73	0,25	[0,06-0,9]	0,042
12 months	23	26,13	0,7	[0,2-2,1]	0,533

5. Discussion

Malaria, intestinal parasitosis and anemia are one of the most common diseases affecting young children and especially infants with significant morbidity. The results of this study confirm here the very low prevalence of malaria before one year, but also and especially the still high prevalence of anemia and intestinal parasitic carriage in this age group.

The results of this study confirm the hypothesis that children during their first months of life are protected with relative immunity against malaria mainly due to a passive transfer of maternal antibodies, but also because of the protective role of Fetal hemoglobin (HbF). Indeed in our study cohort of 150 newborns a single case of malaria disease was detected after one year of follow-up.

Only one child was diagnosed with malaria with a relatively low parasite density of 1640 parasites per microlitre of blood. A very low prevalence in the study of 0.75% (1/133) was observed outside the 12 study withdrawals.

Several studies carried out in this direction support thus the results of our study.

In a retrospective review of records of children under the age of 15 admitted to hospital in Blantyre, Malawi, in 1998 and between 2005 and 2008, confirmed malaria cases among those under 6 months of age accounted for 4.8% 95% CI [2.8-6.7%] [7].

A study in Tori Bossito in Benin in 2007 showed that the median age for the first malaria infection was 4.88 months (0.53-11.86) in infants born of infected placentas, while it was 6.11 months (0.53-12.15) in those born of uninfected placentas.

She also showed that malaria cases are graded by age, from 4.7% to 1 month, 20.3% to 3 months and 54.7% to 6 months in children in this study [8].

In Uruba, Colombia, in an endemic area with an annual incidence of more than 40 cases per 1000 inhabitants, had conducted a descriptive and prospective study in mothers who suffered from malaria during pregnancy and their newborns. 116 newborns were included in the study and 80 umbilical cord samples were obtained. 5 cases of congenital infections representing a prevalence of 4.3% were diagnosed with one at birth and the others during the 21-day follow-up on average [9].

A study between January 2002 and December 2009 on congenital and neonatal malaria at a rural hospital in Kenya showed that out of 4,790 thick drip slides (93.7%) of all neonates in the study: 3,187 (92.6%) during the first week of life and 1606 (96%) after, only 18 (0.35%) of the newborns were carriers of *Plasmodium falciparum* and no newborn was carrier of another species of plasmodium. 11 of the 18 were admitted in the first week of life and were classified as having congenital malaria. Additional parasitic densities were very low [10].

In our study 85.33% of children slept under a treated net at the time of the survey, 99.3% were exclusively breastfeeding and 84.86% of mothers of children received at least one dose of TPI during pregnancy.

Intestinal parasitosis and anemia have always been important factors in morbidity in children under 5 years of age and especially those under 2 years of age. The results of our study confirmed this with, among other things, a carry rate of 25.4% intestinal parasites and a 55.3% and 51.9% anemia prevalence at 6 and 12 months of life respectively in our study cohort. This was confirmed by several studies carried out among children in Senegal and throughout Africa and elsewhere in the world.

In 2010, a study evaluating intestinal parasitosis and anemia in children under the age of ten in the same area of intervention as ours had similar results with an overall prevalence of 25.4% intestinal parasitoses. The species most frequently encountered were *Giardia intestinalis* (14.8%), Entamoeba coli (11%), *Hymenolepis nana* (1.6%), *Ascaris lumbricoides* (0.5%) [11].

All the studies carried out in Senegal confirm, like ours, the predominance of the species *Giardia intestinalis, Entamoeba coli and Ascaris lumbricoides* [11-14].

Other studies in Africa and elsewhere in the world have confirmed the endemicity of intestinal parasitoses with varying frequencies for different species.

In Côte d'Ivoire, for example, in 1997, the overall prevalence of portage was 38.9% with frequency *Entamoeba coli* (22.4%), Trichuris trichiura (9.5%), *Giardia intestinalis* (1.4%), *Hymenolepis nana* (0.8%) [15].

In Chad in 2006, a study had obtained a 51% prevalence of intestinal parasitic carriage. The most common species were Entamoeba histolytica (30%), *Hymenolepis nana* (13%); *Ascaris lumbricoides* (10%), *Trichomonas* (6%) and *Giardia inestinalis* (3%) [16].

In Thailand in 2002, in a study assessing the prevalence of intestinal parasites in children between the ages of 3 and 5 years, a carry rate of 22.7% was found. *Giardia lamblia* was the most common species [17].

Concerning anemia, in the same study in 2010, had found a prevalence of anemia at 48.5%, with no association found between carrying intestinal parasites and anemia [11].

In a 2011 malaria prevalence survey at Keur Socé, 81.7% and 52.1% of children aged 6 to 2 years and 3 to 9 years had anemia prevalence in the dry season, respectively.

In Morocco in 2009, a study of school-aged children found a prevalence of moderate anemia (hemoglobin levels below 11g/dl) at 76.6% [18].

6. Conclusion

Malaria and intestinal parasitoses constitute in rural Africa in addition to nutritional deficiencies, the predominant factors or often associated with anemia. According to the World Health Organization, infants between six and 24 months of age are among the most vulnerable to anemia. In Senegal, the national malaria survey in 2008-2009 showed a higher prevalence among rural children at 81%.

Given the importance of malaria, intestinal parasitosis and anemia in young children, we proposed to conduct this survey to better study them.

The limits of our study:

1) The low prevalence of malaria at the time of our study in the rural community of Keur Socé;
2) The lack of evaluation of protective factors against malaria, including the determination of fetal hemoglobin, could have produced a lot of information;
3) Non-analysis of maternal antibody kinetics;
4) Not using the stool concentration method for a better diagnostic tool for stool exams;
5) Failure to use the Kato method to determine the pest load of each species.

Ethical Approval and Consent for Participation

This study was conducted according to the declaration of Helsinki and existing national legal and regulatory requirements. Informed consent of parent or legal representative was required prior the participation to the study. To respect the confidentiality, an identification code was given to each participant.

Availability of Data and Material

All data generated or analysed during this study are included in this manuscript and are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no competing interest.

Author Contributions

AG, SL conceived and designed the study. AG, MS, SL supervised the data collection. AG analysed the data. SL wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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