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# BURDEN OF MALARIA INFECTION AMONG NEONATES IN HIGHLY EPIDEMIC REGION OF KHYBER PAKHTUNKHWA, PAKISTAN

Shahid Niaz Khan*[1], Sultan Ayaz[1], Ijaz Ali[2], Sobia Attaullah[3], Sumaira Shams[1], Shehzad Zareen[1], Muhammad Asim Khan[1], Farzana Rashid[4] and Sanaullah Khan[1]

1. Department of Zoology, Kohat University of Science and Technology Kohat 26000, Khyber Pakhtunkhwa, Pakistan
2. Institute of Biotechnology and Genetic Engineering, Agriculture University Peshawar, Khyber Pakhtunkhwa, Pakistan
3. Department of Zoology, Islamia College (A Public Sector University) Peshawar, Khyber Pakhtunkhwa, Pakistan
4. Department of Zoology, Lahore College for Women University Lahore, Pakistan

***Corresponding Author:***
*Shahid Niaz Khan
Department of Zoology, Kohat University of Science and Technology, Kohat 26000, Khyber Pakhtunkhwa, Pakistan
Tel: +92-922-554440
Fax: +92-922-554556
Email: shahid_kust@yahoo.com, shahid@kust.edu.pk

## ABSTRACT

Malaria is a major public health burden worldwide causing low birth weight, premature birth and stillbirths during pregnancy, particularly in areas with high malaria transmission.Malaria severely affects pregnant women especially during their first pregnancy while information on the burden of malaria in early infancy is scarce. This study was aimed at assessing the prevalence of malaria among neonates of both rural and urban areas of district Kohat Khyber Pakhtunkhwa, Pakistan, which was characterized by unstable transmission of malaria. A total 615 blood samples of neonates; Male = 357 (58.04%) and Female = 258 (41.95%) were collected to investigate the various risk factors of malarial parasites. The result showed that malaria infection was prevalent in the neonates and during pregnancy. Only 12 (1.95%) male neonates were found malaria positive, while no female was found positive for the malarial infection. Only 9 (1.46%) neonates of age group 1-7 days old were positive for *Plasmodium falciparum* with symptoms of severe temperature and neonatal sepsis, while only 3 (0.48%) neonates of age group (23-30) days were positive for *Plasmodium vivax* with the symptoms of severe temperature and low birth weight.

**Keywords:** Malaria; Neonates; *Plasmodium vivax; Plasmodium falciparum*

## 1. INTRODUCTION

MALARIA is a disease of global importance that results in 300-660 million cases annually and an estimated 2.2 billion people at risk of infection [1]. Malaria is a serious problem and every 30 seconds a child dies from malaria [2]. An estimated one million annual deaths occur from malaria of which approximately 80% occur in infants and young African children [3]. Similarly Approximately 2.5 million malaria cases are reported annually from South Asia, of which 76% are reported in India [4]. Malaria in pregnancy (MIP) poses substantial risk to the mother, foetus and neonate. Both *Plasmodium falciparum* and *Plasmodium vivax* infections can cause adverse pregnancy outcomes, including maternal anemia, low birth-weight and stillbirths due to preterm delivery and foetal growth restriction [5].

Pregnant women are more susceptible than non-pregnant women to malaria, especially in first and second pregnancy [6]. On the contrary, congenital malaria remains extremely rare both in endemic and non-endemic areas [7]. In endemic countries congenital malaria is mainly caused by *P. falciparum*. In European countries most cases are due to P. vivax [8]. Pregnancy-Associated Malaria (PAM) occurs when red blood cells infected with malaria parasites gather in the placenta resulting in

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damage to both mother and developing infant. First-time mothers are particularly susceptible to PAM whereas women in subsequent pregnancies become protected against PAM. For the unborn child, maternal malaria increases the risk of spontaneous abortion, stillbirth, premature delivery and low birth weight, a leading cause of child mortality [9].

In Pakistan, *Plasmodium vivax* and *Plasmodium falciparum* are widely distributed species, which are responsible for Malaria [10].In Pakistan each year an estimated ¼ million cases of malaria infection occur [11]. The incidence of malaria has strikingly increased during the last ten years and the relative rate of occurrence of *P. falciparum* has increased from 45% 1995 to 68% in 2006 amongst malaria infection [12]. Malaria transmission in Pakistan is markedly seasonal mostly occurs after the July-August monsoon [13] and prone to epidemic outbreaks in particular geographical areas, especially the Khyber Pakhtunkhwa (NWFP) [14].

Although malaria is quite common in Pakistan, but from different regions of Pakistan epidemiological data is insufficient to exactly asses the prevalence of malaria [15], particularly regarding the prevalence and its associated risk factors of this disease in neonates in the suburbs of the country where although transmission is unstable, but high as a result of topography, attitude, rainfall, poor drainage system and high human-vector contact to mention a few. This work was, therefore, aimed at assessing the prevalence of malaria in newly born babies of district Kohat.

## 2. METHODS

**2.1. Study Area:** District Kohat consist of the 2,545 square kilometers (983 sq mile) situated at 33°35'13N 71°26'29E with 489 meters (1607 feet) latitude with 1,25,000 population (1998 census) in Khyber Pakhtunkhwa province of Pakistan. Kohat is a medium sized town, with an altitude of 489 meters (1607 feet). Its topography is dominated by mountains and hills. Annual temperature fluctuates between 5ºC to 40ºC and annual rainfall ranges from 24 to 321mm. It is bounded by Tribal Areas in the northwest which share boundaries with Afghanistan. The free moment and migration of the people across the border appears to facilitate malaria transmission in Kohat. It is at high risk for malaria and the disease reaches its peak after heavy rainfalls during July and August.

**2.2. Patient Selection:** About 615 Blood Samples were randomly collected from 1-30 days old neonates in different hospitals, maternity centers and during field survey of urban rural areas of District Kohat. The complete record of each neonate was compiled on separate data sheet regarding to his/her clinical and maternal histories with the assistance of medical staff/ parents.

**2.3. Blood Collection:** Blood was collected from each susceptible neonate on a clean microscopic slide with the help of disposable pickers' under aseptic conditions and then brought to the Molecular Parasitology and Virology Laboratory of Department of Zoology Kohat University of Science and Technology Kohat for further analysis. Heel-stick was the preferred method to collect a blood sample from neonates and infants under 6 months of age. Prick was made on the flat underneath plane of the foot.

**2.4. Sample Preparation:** Thick and thin blood films of each blood sample were made on grease free slide and stained with Geimsa's stain as described in Cheesbrough,1998 [16] and Njoku *et al*; 2000[17].

**2.5. Thin blood film:** A drop of each blood sample was placed near one end of a grease free glass slide; the edge of a cover slip held at approximately angle 45 was used to smear the blood evenly on the slide to produce a uniform spread thin smear of the sample. It was labeled with diamond pencil at the margin and allowed to dry in a horizontal position at room temperature. Duplicate copies of thin film smear of each blood sample were made.

**2.6. Thick blood film:** A drop of each blood sample was place at the center of a clean grease-free glass slide and the edge of another glass slide was used to smear the sample to produce a circular thick film of about 23 cm in diameter. It was labeled with a diamond pencil at the margin and allowed to dry at room temperature. This was carried out in duplicates for each blood sample.

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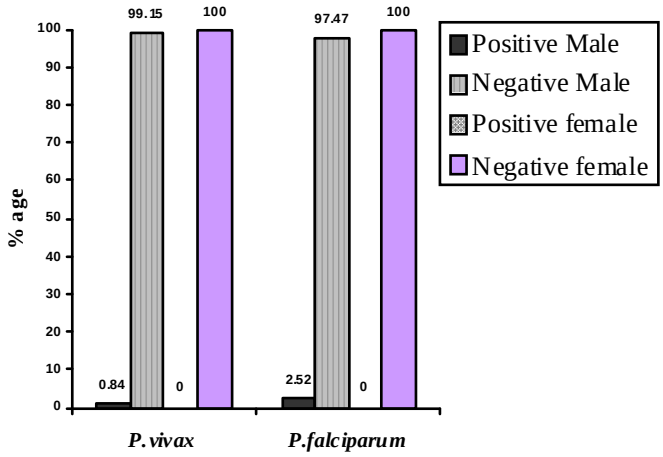
**2.7. Staining techniques & Sample Examination:** All the blood films were allowed to stay overnight before staining. The blood films were dehaemoglobinized by immersing in buffered solution. Thin blood films were fixed in absolute alcohol for 60 second and allowed to dry in the air. Both thin abd thick blood films were stained accordingly and examined microscopically (Binocular Olympus Japan; CX-31) using oil immersion objective as described in Cruickshank *et al*; 1985 [18] and Njoku *et al*; 2000 [17].

**2.8. Data Analysis:** Statistical analysis was performed by using SPSS. By applying Chi square t test the p-value less than 0.05 was considered statistically significant.

## 3. RESULTS

The study was carried out to know the prevalence status of malarial parasites and their associated risk factors in 1-30 days old (mean age: 15.5 days) neonates from June 2011 to May 2012. A total 615 blood samples of neonates; male 357 (58.04%) and females 258 (41.95%) neonates (M: F = 2:1) were investigated for *Plasmodiasis* with consensus of their parents during the study period. Only male neonates were observed to have neonatal malaria (NM) 12/357 (3.36%), while no positive case was reported in the female neonates (Figure 1). None of these neonates had previously been transfused with blood. Infection of *P. falciparum* was found to be higher 2.52% than *P. vivax* 0.84%.

**Figure 1**
**Over All Epidemics of Malarial Parasites in Male and Female Neonates**




Among all the age groups, the highest infection of only *P. falciparum* 9/252 (3.6%) was found in the age group of 1-7 days old neonates (Table 1). In male neonates of age group 23-30 days, only 3/18 (16.66%) was *vivax* positive while no single case was found positive for malaria in the rest age groups. However, no significant difference was found between the age groups of babies and positive malaria ($p > 0.05$).

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**Table 1**
**Age wise analysis of malarial parasites in Male neonates**

| Age Groups (Days) | Data Analysis *n* (%) | | | | Total *n* (%) |
|---|---|---|---|---|---|
| | *P. falciparum n* (%) | | *P. vivax* n (%) | | |
| | Positive | Negative | Positive | Negative | |
| 1-7 | 9 (3.6) | 243 (96.40) | 0 (0.00) | 252 (100.00) | **9/252 (3.6)** |
| 8-15 | 0 (0.00) | 60 (100.00) | 0 (0.00) | 60 (100.00) | **0/60 (0.00)** |
| 16-22 | 0 (0.00) | 27 (100.00) | 0 (0.00) | 27 (100.00) | **0/27 (0.00)** |
| 23-30 | 0 (0.00) | 18 (100.00) | 3 (16.66) | 15 (83.33) | **1/18(16.7)** |
| **Total** | **9 (7.20)** | **348 (92.80)** | **3 (0.84)** | **354 (99.15)** | **12/357 (3.36)** |

Further analysis of the above data was statistically non-significant ($p$ = 0.2208)

The clinical features regarding the sign and symptoms of the malaria infection in neonates was recorded with the consensus of their parents and compared with the neonates having no malaria infection. Out of 12 positive neonates, in age group 1-7 days, 6 positive neonates were observed with severe temperature (mean:98.5±1.0 $^0$F; Range 101.3±2.5$^0$F) and 3 positive neonates were observed with neonatal sepsis, while 3 of the positive neonate from age group 23-30 days were observed with Low weight (Table 2). The mean birth weight of babies with positive *Plasmodiasis* was 2.93 (0.31) kg as compared to the mean of 3.15 (0.21) kg in those neonates who were without malaria infection.

**Table 2**
**Clinical Symptoms of Malarial Parasites in Different Age Groups**

| *Clinical Symptoms* | *Age Groups (days)* | | | | *Total* n (%) |
|---|---|---|---|---|---|
| | 1-7 | 8-15 | 16-22 | 23-30 | |
| Temperature/Fever ($^0$F) | 6 | 0 | 0 | 0 | 6/12 (50.00) |
| Neonatal sepsis | 3 | 0 | 0 | 0 | 3/12 (25.00) |
| Low weight | 0 | 0 | 0 | 3 | 3/12 (25.00) |
| **Total** | **9** | **0** | **0** | **3** | **12 /615 (1.95)** |

First Three positive neonates were *P. falciparum* positive found in hospital, having severe temperature with low birth weight and negative mother's previous malarial record while Five positive neonates were *falciparum* positive, found in Hospital, having Temperature with low weight and positive mother's previous malarial record, which was *P. falciparum* positive during pregnancy. Another one positive neonate was also *P. falciparum* positive, found in Hospital as well, having Neonatal sepsis with negative mother's previous malarial record. While remaining three positive neonates were *P. vivax* positive, found from field, having temperature & Low birth weight with negative mother's previous malarial record.

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In hospitals a total of 324 samples were examined out of which 9 (2.80%) were found positive for *P. falciparum* while rest of the 315 (97.22%) samples were negative. In field a total of 180 samples were examined, out of which 3 (1.67%) was found positive for *P. vivax* while rest of the 177 (98.33%) samples were negative. In Maternity centers 12 samples were examined with no positive case, in Private clinics 63 samples were examined with no positive case as well and in Private Labs 36 samples were examined where no positive case was found (Table 3).

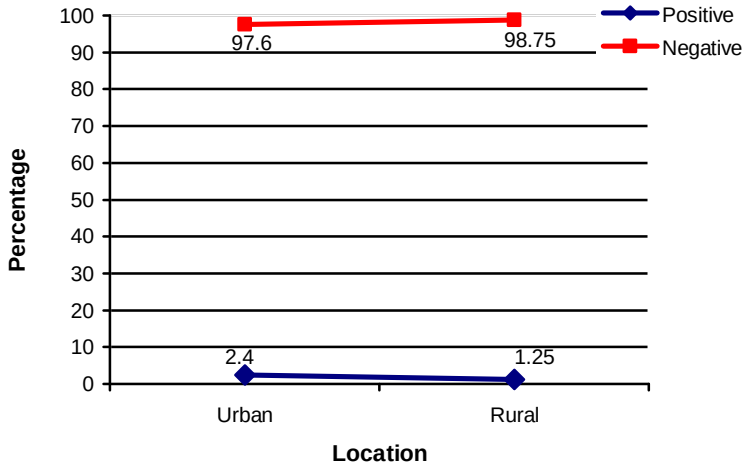
**Table 3**
**Location wise analysis of malarial parasites in Males neonates**

| Location | Data Analysis (%) | | | | Total n (%) |
|---|---|---|---|---|---|
| | *P. falciparum* n (%) | | *P. vivax* n (%) | | |
| | Positive | Negative | Positive | Negative | |
| Hospital | 9 (2.80) | 315 (97.22) | 0 (0.00) | 324 (100.00) | 9/324 (2.8) |
| Field | 0 (0.00) | 180 (100.00) | 3 (1.67) | 177 (98.33) | 3/180 (1.66) |
| Maternity Centers | 0 (0.00) | 12 (100.00) | 0 (0.00) | 12 (100.00) | 0/12 (0.00) |
| Private Clinics | 0 (0.00) | 63 (100.00) | 0 (0.00) | 63 (100.00) | 0/63 (0.00) |
| Private Labs | 0 (0.00) | 36 (100.00) | 0 (0.00) | 36 (100.00) | 0/36 (0.00) |
| **Grand Total** | **9 (1.47)** | **606 (98.50)** | **3 (0.48)** | **612 (99.50)** | **12/615 (1.95)** |

Further analysis of the above data by chi square t test was found statistically significant and the value of $p = 0.0630$

Out of 375 samples, in urban areas only 9 (2.4%) were malaria. In about 240 cases of urban areas only 3 (1.25%) were positive (Figurer 2).

**Figure 2**
**Area Wise Analysis of Malarial Parasites in Neonates**




In the months of June 2011 to Sep 2011, 168 cases were analyzed out of which 6 (3.60%) were found positive. In the months of Oct 2011 to Jan 2012, 348 cases were analyzed out of which 6 (1.72%) were found positive, while in the months of Feb 2012 – May 2012, 99 cases were analyzed and no positive case was reported (Figure 3).

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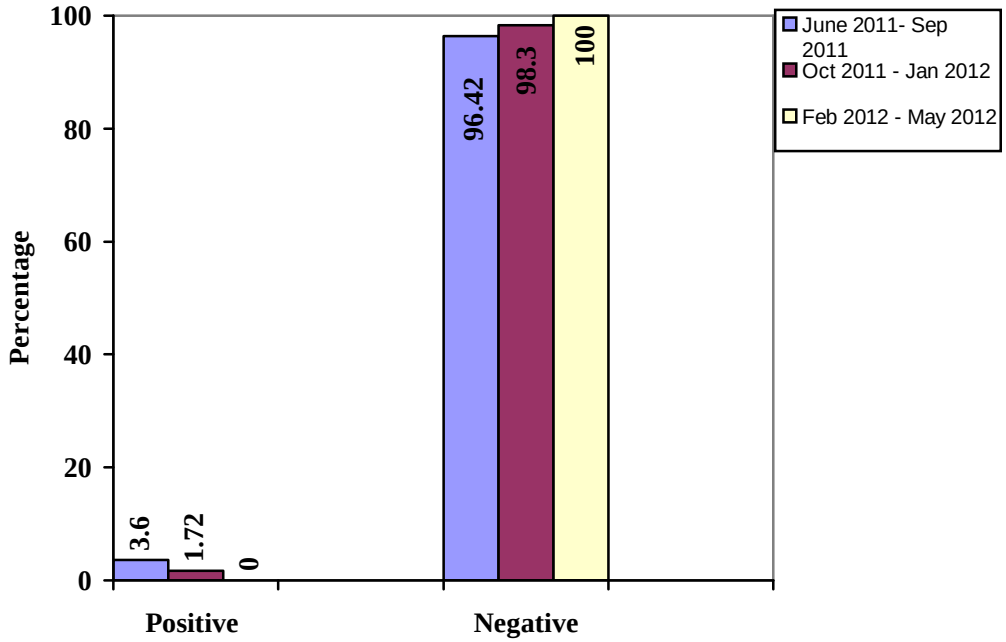
**Figure 3**
**Month Wise Analysis of Malarial Parasites in Neonates**




## 4. DISCUSSION

Malaria is a major health problem in Pakistan today [19]. It is a leading cause of preventable deaths in the country. The annual incidence of malaria in Pakistan was 62 per 100,000 populations in 2001[20]. Pregnant women are at a greater risk of acquiring malaria due to depression of cell-mediated immunity [21]. Malaria in pregnancy can lead to death of the mother, abortion of the foetus or a still birth. Low birth weight has also been described as one of the effects of malaria on the foetus [22].

In the current study it was found that risk of *Plasmodiasis i*n neonates was very rare not only in the studied area of district Kohat, but also in the malaria-endemic region as per study of Nweneka *et al.,* 2004 [23]. It was observed that 50% of the infected neonates were suffered from severe temperature /Fever similar to the study of Stauffer and Fischer, 2003 in non-immune neonates, malaria commonly presented with high temperature that might be followed by chills and headache [24]. Predominant clinical symptoms of the neonatal malaria were 25% Neonatal Sepsis (respiratory distress), while 25% of the neonates showed low birth weight. These findings are quite comparable to the study of Ibhanesebhor, 1995 [25], while a difference 41.3% was observed in neonatal sepsis from the study of Afolabi *et al.,* 2001 [26] in Nigeria. This difference might be due to the geographical/environmental conditions and variation in sample size.

The malaria in neonates has much affect on the birth weight as well as on the weight of the placentas. The birth weight of the non malarious neonates was significantly higher than the malarious neonates as observed by Anagnos *et al.,* 1986 [27], while in the current study a same significant difference was found as a low birth weight among the positive neonates as compared to

normal ones. It is necessary to mention that as placenta provide nourishment and nutrients to the fetus so any upset condition to the placenta leads to pathological and clinical abnormalities the to the fetus. We could find out to detect headache in neonates but chill or fever was easily observed to be major symptom of *Plasmodiasis*.

One of the major risk factor to neonatal malaria was the mother's previous record. Malaria severely affects pregnant women. In the present study it was found that 42% of the infected neonats were having congenital malaria. Pregnant women those were non-immunized were found to be at high risk of malarial infection. The *falciparum* positive neonates have severe temperature whose maternal malarial record was positive from 5 years. The same was also documented by Medical News *in* 2007 [7]. The congenital malaria is mostly caused by *Plasmodium falciparum* studied by Mwangoka *et al.,* 2008 [28].The mother may or may not show the symptoms of malaria while in our study 9/12 (75%) infected neonates had *falciparum* malaria, of those 5 showed the positive maternal record while 4 showed the negative maternal record (potentially positive & asymptomatic).

A meaningful difference was observed between urban and rural areas for the infection of malarial parasites. Neonatal malaria was found to be higher 75% in urban areas of district Kohat as compared less infection 25% in rural areas of the same district. Similar findings were observed in the general population of Quetta urban (22.89%) and rural (16.74%) by Tareen *etal*., 2012 [29]. The difference in percents in both studies might be due to the sample size and age groups of the subjects. The reasons behind the high ratio of malarial parasitemia in urban areas were poor hygienic conditions, lack of awareness, lack of expertise for delivery of neonates and severe loading shedding as might contribute to the neonatal malaria in the studied areas.

Malaria is associated with seasonal variations as most of the malarial infection occurs in September and November, following the monsoon season [30]. Furthermore, epidemic outbreaks in particular geographical areas like especially in the Khyber Pakhtunkhwa have the highest malaria burden [8].we found that the highest infection 6/168 (3.60%) was found in the months of June to September, while Tareen *etal*., [29] analyzed the highest malaria infection in April (55.33%) while least prevalence in the month of January (8 .88%). This difference in the studies might be due to seasonal variations in temperature of various months of both the provinces.

**Conclusion:**

Risk of malarial parasites among newly born babies in district Kohat was very low 1.95% along with its possible congenital transmission. An increased prevalence of *Plasmodium falciparum* was observed in neonates from rural areas rather than the urban, which was congenitally transmitted to neonates. Severe temperature, neonatal sepsis and low birth weight were the common clinical manifestations.

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