# Prevalence of *Plasmodium Vivax Using PCR Method in* Afghan Refugee Ghamkol Camp District Kohat, Pakistan

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**Abstract:** Malaria is a disease caused by protozoan parasites belongs to the genus *Plasmodium*, which is transmitted by female Anopheles mosquito. It causes more than one million deaths per year. Afghan Refugees in Pakistan are at higher risk of malarial infection. Blood samples were collected from Afghan refugees in Ghamkol camp district Kohat. The samples were analyzed by Hemometer and Polymerase Chain Reaction. A total of 220 blood samples were examined by poly merease chain reaction (PCR) from susceptible peoples resides in Afghan refugee Ghamkol camp district Kohat. The DNA were extracted and amplified through PCR which confirmed the P.vivax detection. Overall prevalence of malaria 50.00% (110/220), among these Males were 50.00% (78/156) and females were 46.87% (30/64). It was observed an average low level of Hb(9.98gm/dl) in *P. vivax infection patients.* It was concluded that children at the age 1-20 years were more susceptible for malarial infection than above ages. *Plasmodium vivax* was more prevalent in Afghan refugee Ghamkol camp Kohat.
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**Keywords:** Prevalence, Plasmodium vivax and PCR

## 1. Introduction

Malaria is a prehistoric disease caused by protozoan parasites belongs to the genus *Plasmodium*, which is transmitted by female Anopheles mosquito (Cox, 2010). Malaria is the one of the 5 harmful diseases that causes casualties in pediatric populations (Jamal *et al.*, 2005). Amongst blood infections it is the most common communal health crisis of the tropics particularly in emergent countries with its morbidity and mortality at deplorable elevated levels (Cabe, 2001). The yearly death rate of malaria is about 1.5 to 2.7 million public while about 300 to 500 million individuals are at risk of malaria (Upadhyay *et al*., 2011). Malaria caused more than one million deaths per year globally (Bhalli & Samiullah, 2001). The probable occurrence of malaria worldwide has compacted by 17 percent since 2000 and malaria-specific death rates by 26 percent. These rates of turn downs are lower than globally agreed targets for 2010 (reductions of 50 percent) but nevertheless, they symbolize a chief accomplishment (World Malaria Report, 2011).

There are about four species of Plasmodium i.e. *Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale* (Upadhyay *et al.*, 2011).Additional species that chiefly transmit disease to primates (*Plasmodium simiovale and P.knowlesi*) may infects humans mainly those working in the jungle habitats near contaminated infected primates (Washington state dept of health, 2011). The most hazardous and most problematical malaria is caused by *P. falciparum* that may cause severe renal failure, low blood sugar, non cardiopulmonary edema and black water fever, while than highest manifestation of the malaria is the cerebral malaria (Bhalli & Samiullah, 2001; Mohapatra *et al*., 2006).

Majority of countries affected by the Malaria are amongst the poorest countries in the globe. Malaria is prevalent in Pakistan (Murtaza *et al.*, 2004). In Pakistan, an probable 500,000 cases of malaria arise annually (Ali *et al.*, 2008) while in Afghanistan occurrence of malaria was estimated to be 3 million cases annually, but study from 2001 to 2005 reviled that the frequency of *P. falciparum* and *P. vivax*, is increased from 3 million cases annually (Abdur rab *et al*., 2003). In Pakistan there are two species of Plasmodium which are prevailing i.e. *P. vivax* and *P. falciparum* (Khan *et al.*, 2004). Afghan Refugees in Pakistan are at elevated risk of malarial infectivity as compare to the local population, it is suggested that Afghan public have brought the malarial infection by them from Afghanistan (Suleman, 1988). Clinical complications are directly proportional to the Parasite count up at the erythrocytic stage, upper the parasitic density, high will be the complications (Murthy *et al*., 2000).

There are lots of diagnostic techniques for the recognition of malaria, still broadly used may perhaps be only 50 % precise (Chiodini, 1998). Test

like microscopy (thin and thick smears), judgment of thin and thick blood slides under the microscope is well thought-out a gold standard in the finding of malaria. Microscopy is very sensitive, revealing, low-cost but labor-intensive technique, requiring at least 25 minutes to one hour from Sample assortment to end outcome (Bain *et al*., 1997). The rapid antigen detection test (histidine-rich protein-2 Plasmodium aldolase and Plasmodium lactate dehydrogenase) and Polymerase Chain Reaction (PCR) which is very sensitive, precise, accurate and expensive test (Durand *et al*, 2005). Looking to above hazards and importance of malarial parasites the current study is designed to carry out the prevalence and molecular detection of malarial parasites in Afghan refugee camp district Kohat.

## 2. Material and Methods

### Study Area

District Kohat is an area of the 2,545 square kilometers (983 sq mile) located at 33°35'13N 71°26'29E with 489 meters (1607 feet) latitude with 1,250,000 population (1998 cencus) in Khyber Pakhtunkhwa province of Pakistan (Location of Falling Rain Genomics, Inc. 1996-2010). Ghamkol camp is located about 5 kilometers at the North-East of Kohat city. Ghamkol Camp is the largest Afghan Refugee camp in Kohat; it is divided in to 3 camps i.e. Camp no.1, Camp no. 2 and Camp no. 3. Afghan Refugees migrated from Afghanistan during Soviet War in 1980s. According to Census Report 2009 there are about 1.7 million Afghan peoples registered in Pakistan. Most of them live in Khyber pukhtunkhwa. (Express Tribune, 2011) Patient selection Afghani population (who were suspected to have malarial infection) was included in this study. Blood samples were randomly collected from varying age groups (1-51 years old) from both the sexes during visit whom was clinical suspected.. The complete record of each subject was recorded on a separate Performa regarding to his/her clinical histories, hygienic condition and socio-economic status.

### Sample Collection and processing

3 ml blood was collected by disposable sterile syringe from each patient and poured into 5ml EDTA tube to avoid blood clotting. Each tube was labeled according to the patient specific ID code as per proforma record and the samples were processed in the Molecular Parasitology & Virology Laboratory, Department of Zoology Kohat University of Science and Technology Kohat for further experimental analysis by Hemometer, Microscopy, Rapid Diagnostic Test and PCR. The Microscopy and RDT was used for initial screening of the samples so that PCR could be performed with minimum expenditure for confirmation.

### DNA Extraction:

DNA was extracted by Vivantis **GF-1 Nucleic Acid Extraction Kits** with the manufacturer's protocol.

### Primers:

For the finding of *P. vivax* four published primers were designed to detect the *P.vivax* circumsporozoite (VCS) protein. Those four primers are VCS-OF (Outer Forward), VCS-OR (Outer Reverse), VCS-NF (Inner Forward) and VCS-NR (Inner Reverse).

### Sequence:

VCS-OF ATGTAGATCTGTCCAAGGCCATAAA
VCS-OR TAATTGAATAATGCTAGGACTAACAATATG
58 degree C Annealing Temperature for 25 Cycles (Mallika, *et al.*, 2005).

### Sequence:

VCS-NF GCAGAACCAAAAAATCCACGTGAAAATAAG
VCS-NR CCAACGGTAGCTCTAACTTTATCTAGGTAT
62 degree C Annealing Temperature for 30 Cycles (Mallika, *et al.*, 2005).

### DNA Amplification

PCR was performed by Nyx technic Thermal Cycler in two rounds.the PCR mix was prepared which included tag buffer$NH_3$ $(SO)_4$ 2.2ml, $MgCl_2$ 2.4ml, dNTPs (50mM) 1.1ul, VCS-OF 1.0ul, VCS-or 1.0ul tag polymerase 0.5ul, target DNA5.0ul and the distil water add upto 20ul. All the reagent were remain the same in 2nd round except the primers VCS-OF and VCS-OR which are replaced by VCS-NF and VCS-NR PCr was performed with the following programmed;PCR Conditions for Round 1; with initial denaturation cycle 1 at 95oc for 5minutes, 25 cycle including denaturation at 94ocfor 0.45minute, annealing at 58oc for 0.45minute , extension at 72oc for 0.50 minte and final extension at 72oc for 5.00 minutes and similarly the the programmed for 2nd round as , with initial denaturation cycle 1 at 95oc for 5minutes, 25 cycle including denaturation at 94ocfor 0.45minute, annealing at 61oc for 0.45minute , extension at 72oc for 0.50 minute and final extension at 72oc for 5.00 minutes.

### GEL Electrophoresis:

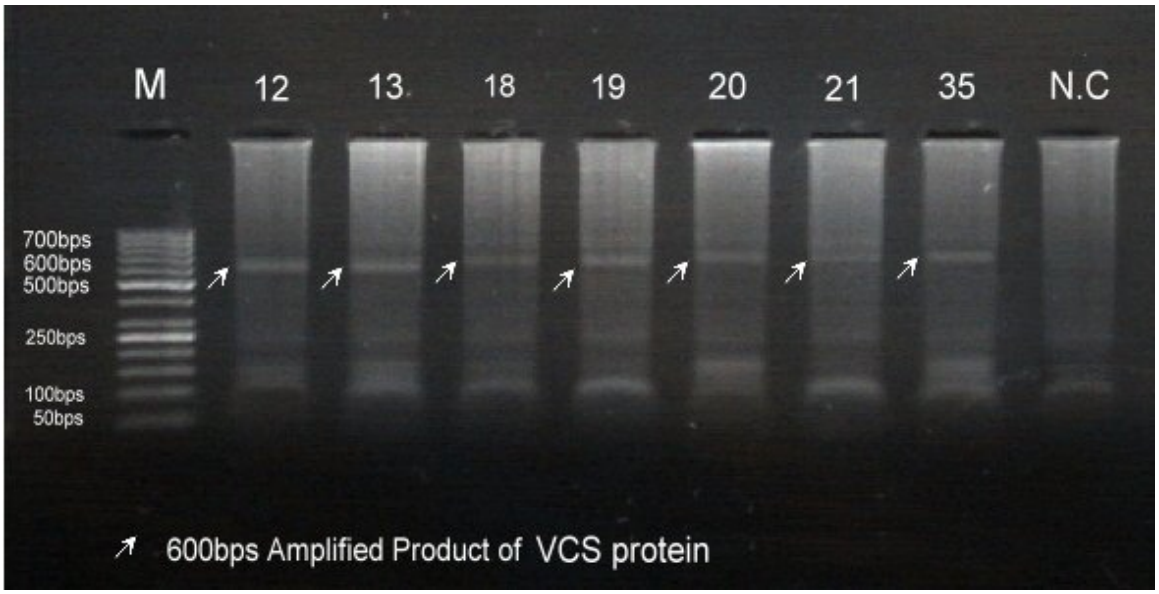
For the detection of PCR amplified VCS gene, gel electrophoresis was performed and visualized in UV Transilluminator.

**Data Analysis**

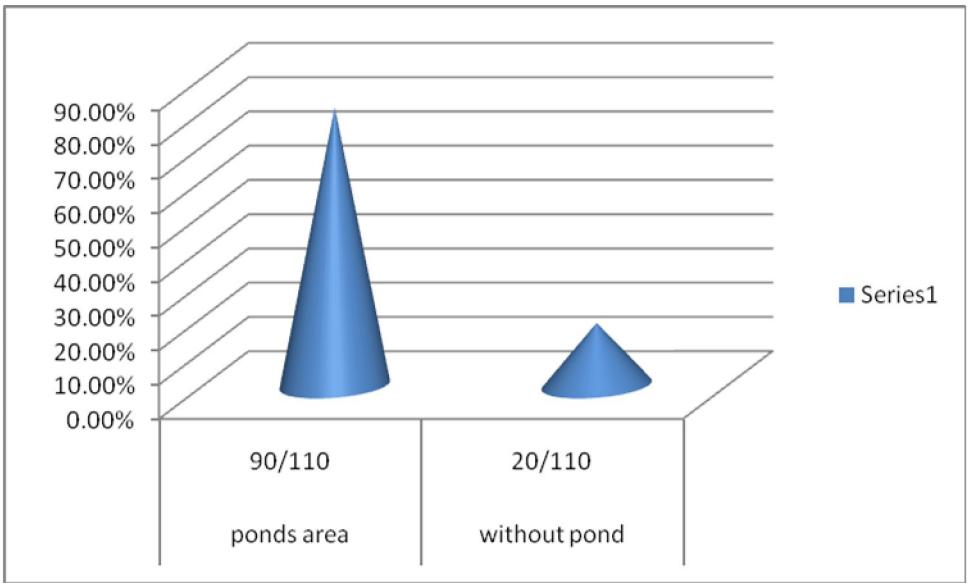
Statistical study was done by means of "STATISTIX", version 9.0, Korean made software. Variables included for evaluation were age, sex and area for parasitic infection.

**3. Results**

A total of 220 blood samples (Male = 156 and Female = 64) were randomly collected from susceptible peoples resides in Afghan refugee



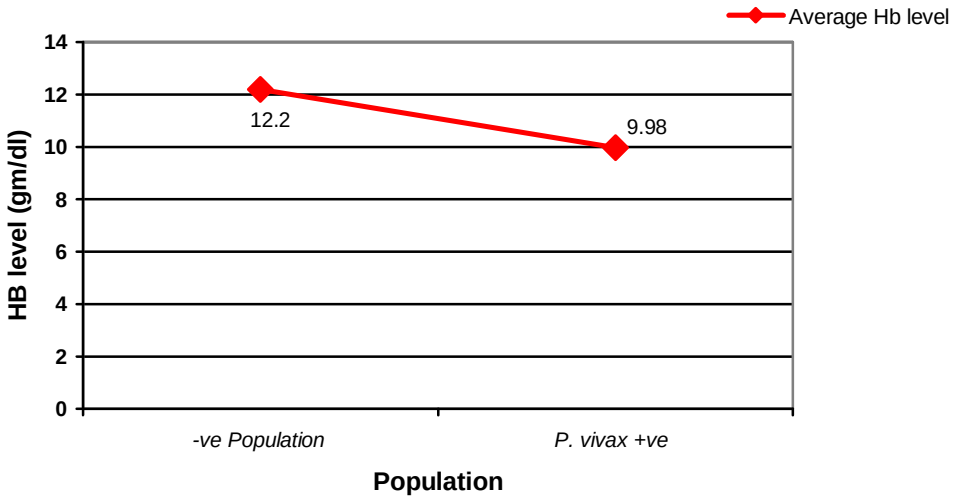

**Fig; 1** DNA Gel result, M; 50bp marker, Lane; 12, 13, 18,19, 29 positive Lane; 35 positive control, Lane; NC negative control




**Fig; 2** Showing the effect of ponds on the prevalence of malaria disease.

**Table-2: By Age Prevalence of *Plasmodium vivax* in Afghan Refugee Ghamkol Camp**

| Age Groups (in years) | Total Cases | PCR | |
|---|---|---|---|
| | | *P. vivax* | |
| | | +ve (%) | -ve(%) |
| **<1** | 4 | 2 (50.00) | 2(50.00) |
| **1-10** | 46 | 20 (43.47) | 26(56.52) |
| **11-20** | 70 | 36(51.42) | 34(48.57) |
| **21-30** | 56 | 34(60.71) | 22(39.28) |
| **31-40** | 34 | 14(41.17) | 20(58.82) |
| **41-50** | 4 | 2 (50.00) | 2 (50.00) |
| **51>** | 6 | 2(33.33) | 4(66.66) |
| **Total** | **220** | **110** | **110** |




**Fig; 3** Hemoglobin level analysis

**Table-1 Sex wise prevalence of *Plasmodium vivax***

| Diagnostic Technique | Total positive | *Plasmodium vivax* | | | |
|---|---|---|---|---|---|
| | | Male | | Female | |
| | | *+ve* n (%) | *-ve* n (%) | *+ve* n (%) | *-ve* n (%) |
| PCR | 110 | 78(50.00) | 78(50.00) | 30(46.87) | 34(50.00) |

Ghamkol camp district Kohat in the months September, 2011. Overall prevalence of malaria 50.00% (110/220), among these Males were found to be more infected with malaria i.e. 50.00% (78/156) as compared females i.e. 46.87% (30/64) as shown in table-1. All the samples were experimentally evaluated by a Polymerase Chain Reaction and 600bp bands were visualized in 2%agarose gel.(figure-1).

**By Age Prevalence**

In age group less than 1 year, only 2 positive cases were examined. In age group 1-10 years 20 positive cases were observed, in age group 11-20 years 36 positive cases were observed, in age group 21-30 years 34 positive cases were observed, in age group 41-50 years and 51> years 2 positive cases were observed in each. A large population of age group 11-20 years was positive in this study (Table-2).

**By ponds prevalence:**

Among 110 PCR positive patients, 81.81% (90/110) lived in the area where there were ponds or dense greenery, while rest of the 18.18% (20/110) live in the area with no ponds or greenery. A large numbers of malaria infected population were those people who live near ponds, which are the mosquito breeding places. (Fig.2)

**Clinical Symptoms:**

Among 110 PCR positive patients 102 had Headache, 94 had back pain, and 106 had temperature while 50 had vomiting. Temperature and Headache were the most frequent clinical symptoms in malaria positive patients.

**Hemoglobin level analysis**

An average low Hb level i.e. 9.98gm/dL was observed in positive cases as compare to negative ones which was 12.2gm/dL (Figure-3)**.**

**4. Discussions**

Malaria is one of the vital community health troubles in Pakistan. Globally it affects about 300 million populations, and also causes more than one million mortalities per annum. Malaria attacks on all age groups and both of the sexes. Accurate identification of the malarial parasites and its treatment is very necessary for the anticipation of the Malaria (Jamal *et al.,* 2005). Malaria was a major health trouble during emergency in Afghanistan and still a health issue in the refugees in who came into Pakistan (Rowland, 2001). Zucker, 1996 suggested that the import of malaria by travelers, immigrants, and refugees is important and rising health problem.

Current study reveled that Afghan Refugees resides in Ghamkol camp District Kohat are at higher risk of *Plasmodium vivax* malarial infection. Housing situation of the Ghamkol is not good as it should be. There is no proper drainage system as a result of which water logged condition is so common that provide the best breeding place for mosquitoes. The finding of the present study coincides with the previous study conducted by Suleman, 1988.

Poor Afghani people are unable to protect themselves from malaria; same situations (that lead to spread malaria) were reported by W.H.O, 1990 and Zulueta, 1989.

It was reported that refugees can have a very elevated occurrence of malaria infections with or without showing any symptoms (Babiker in 1998; Franks 2001). The finding of the above study was correlated with the present study that Afghan refugees are at higher risk of malarial infection in Ghamkol camp district Kohat.

In current study a high percentage (50.00%) of the Afghani population (reside in Ghamkol Camp District Kohat) were confirmed positive for the malaria infection. Positive results for *P. vivax* of this study are somewhat matching (with a little bit increase) with the study of Idrees *et al*., 2007. increase may be due to managemental, hygienic and environmental condition of the area.

In present study, a total of 110 PCR positive patients 50.00% (78/156) male while 46.87% (30/46) female were positive for the malarial infection, a large number of male population was positive as compare to female, with male to female ratio of 1.15:0.5. Similar case was in the study of Idris *et al.,* 2007. This may be due to the activities of the male population in Afghan refugees where male are active for the outside activities and are more prone to biting of the mosquitoes and malarial infection while female are restricted to their homes and are comparatively safe for the bite mosquitoes.In the current study it is revealed that the infection of the *P. vivax* was concentrated the younger age groups as compare to older ages, similar findings were reported by Carneiro *et al.,* 2010.

In this study I used 61 degree Celsius annealing temperature at 25 cycles for VCS-NF and VCS-NR, and got positive amplified product of Pvcs while Aarti, *et al*, 2010 used for *Plasmodium Vivax* Circumsporozoite (Pvcs) primers for the amplification of *Plasmodium Vivax* DNA This contrast is may be due to voltage fluctuation of the electricity in the laboratory as protocol of Aarti, *et al*, 2010 was not giving proper results in current study.

It was proved in the current study as improper DNA extraction Hemoglobin remains intact in the extracted DNA, which adversely affect the PCR and do not give proper results. Same findings were concluded in the study of Al-Soud, 2001 and Radstrom *et al.,* 2004.

Malaria infection causes lower the hemoglobin level which was studied in all positive patients. A low Hemoglobin level was observed in malaria positive cases i.e. an average 9.98 gm/dl for the infection of *P. vivax.* this parameter was studied by DeMaeyer & Adiels-Tegman, 1985 and concluded that Hemoglobin concentration becomes lower due to RBC destruction and also due to the removal of parasitized and non-parasitized RBCs.

**5. Conclusion**

It was found that children at the age 1-20 years were more susceptible for malarial infection than above ages. *Plasmodium vivax* was sole aetiological agent for cause of malaria in Afghan refugee Ghamkol camp Kohat Pakistan**.**

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