Research Article

Prevalence and distribution of human malarial infection in district Multan, Punjab, Pakistan

Muhammad Zafar1*, Iram Mushtaq2, Samrah Masud3, Muhammad Zahid Khan4, Muhammad Khalil Ahmad Khan5 and Asmatullah6
1. Postgraduate Department of Zoology, Govt. Emerson College, Multan-Pakistan
2. Department of Pahtology, University of Veterinary and Animal Sciences, Lahore-Pakistan
3. Department of Zoology, Institute of Pure & Applied Biology, Bahauddin Zakariya University, Multan-Pakistan
4. House Officer, Medical Unit 04, Bahawal Victoria Hospital and Quid-e-Azam Medical College, Bahawalpur-Pakistan
5. Department of Zoology, Govt. College of Science, Wahdat Road, Lahore-Pakistan
6. Department of Zoology, University of The Punjab, Quid-e-Azam Campus, Lahore-Pakistan
*Corresponding author’s email: m.zafar1214@gmail.com

Citation
Muhammad Zafar, Iram Mushtaq, Samrah Masud, Muhammad Zahid Khan, Muhammad Khalil Ahmad Khan and Asmatullah. Prevalence and distribution of human malarial infection in district Multan, Punjab, Pakistan. Pure and Applied Biology. Vol. 8, Issue 1, pp873-881. http://dx.doi.org/10.19045/bspab.2019.80029

Received: 16/11/2018 Revised: 21/02/2019 Accepted: 06/03/2019 Online First: 14/03/2019

Abstract
The present study was undertaken during 2015-17 with the objective to provide data of malarial infections in human population based on incidences in twenty different localities of district Multan, Punjab, Pakistan. The blood slides of suspected patients were studied to identify the malarial parasites. Slide positivity was found to be 1478 (39.20%) out of 3770 and Plasmodium vivax infection was 905 (61.23%), Plasmodium falciparum 425 (28.75%) and mixed species (P. vivax and P. falciparum) 148 (10.01%) were identified. In the month of December, 150 cases (73.25%) of P. vivax infection counted to be the highest, whereas, 42 cases (58.33%) of P. vivax in the month of September were recorded. The P. falciparum infection remained highest in the month of August with 58 cases (25.66%) and lowest in the month of May with 24 (23.76%). Similarly infection of mixed species remained highest 30 (13.27%) in August and lowest 7 (7.60%) in the month of February. Infection in male was highest 850 (57.51%) and female was lowest 628 (42.48%). No incidence of Plasmodium ovale and Plasmodium malariae infection was to be observed in this study. The results of present research work concluded that P. vivax and P. falciparum are serious health hazards which can ultimately lead to serious cerebral malaria problems. However, no association was found between age group, sex and type of infection. Therefore, it can be concluded that the incidence of any type of infection may happened from person to person of any age group and in any sex.

Keywords: Incidence; Infection; Multan; Mixed species; Plasmodium falciparum; Plasmodium vivax

Introduction
Malaria is known as a major parasitic diseases in all over the world. Over 225 million malarial cases were reported in 2011. Species Plasmodium falciparum is the most severe form of malaria parasite. Mostly it is transmitted through the bite of an infected female mosquito known as Anopheles when it feeds on human beings [1]. Malaria is known as one of the most devastating disease throughout in the world and 4 billion people live under the threat of malaria in 25 endemic countries [2] and each
year it kills over a million mostly children [3]. Malaria is considered as one of the major poverty disease and needs to be eradicated. Due to consequences of malaria, children below five years and pregnant women are felt hardest. With the lack of hygiene and proper nutrition, a child dies from malaria after every thirty seconds [4].

At CMH, Multan [5] 125 cases of *Plasmodium falciparum* malaria were studied to evaluate seasonal variations and modes of transmission. The incidence of *Plasmodium falciparum* remained high among troops during the months of August to November. In general population of district Buner, [6] studied prevalence of malaria and recorded highest rate of infection (11.71%) in the month of August while in March the lowest rate of infection (3.8%) was noted. In pediatric age group 250 cases of malaria were investigated by [7] who found high rate of infection of *Plasmodium vivax* (63.2%) as compared to infection of *Plasmodium falciparum* (35%). In Karachi including other areas of province Sindh, Malaria was studied by [8] it was observed that infection of *Plasmodium vivax* were more than two times higher than the infection of *Plasmodium falciparum*. In many districts of the province Sindh [9] fond that *Plasmodium falciparum* ratio was increasing day by day. In North West Frontier Province (NWFP) malaria was studied by [10] who observed cerebral malaria as more common infection in males and for pregnant females it was highly vulnerable. In another study [7] among the 165 cases of malarial parasite in children, 145 cases were diagnosed as *Plasmodium vivax* and 20 cases of *Plasmodium falciparum*. At Ayub Teaching Hospital Abbottabad the study regarding the pattern of malarial infection by [11] 1998 patients were screened and 150 (7.5%) were found to be infected by *Plasmodium vivax* in 72.2% and *Plasmodium falciparum* in 24.3% cases.

In Balochistan, cerebral malaria is declared as a major community problem. In a study [12] to observed epidemiology of cerebral malaria and its mortality in patients in Quetta City, 63% cases of cerebral malaria in children while 37% cases in adults were investigated. Furthermore, in a study of urban and rural areas of Quetta district [13, 14] in 2003 and 2004 in the age group of 22 and above, higher rate of incidence of *Plasmodium falciparum* (17.8%, 16.4%, respectively) were noted. A recent report about malaria from Pakistan, which was published in 2017, evidence sustained decrease in the number of cases, but however we are unable to achieve the target in all over the world about malarial eradication [15].

Many studies have been conducted to investigate the knowledge, attitudes and practices of people with respect to malaria but only few to know the cause of malaria. Investigating the rate of prevalence of malarial parasites in human population of the Multan district was the objective of present study.

**Materials and methods**

Multan is located in the southern part of the province Punjab, Pakistan. Multan is known as an arid area with very hot summer and cold winters. In winter the average lowest temperature is 4.4°C (40.1°F) while in summer the highest average temperature recorded is 42.2°C (108.1°F). Different localities of Multan district were studied during August 2015 to July 2017 and blood of human patients suffering from malaria was screened to record malarial parasite species. The patients were categorized into 5 different age groups: 1-10 years of age, 11-20 years, 21-30 years, 31-40 years and 41 years and above. Two different methods were adopted to detect malarial cases. [16] A) PCD (Passive Case Detection) technique where blood films were taken from health stations from the patients with symptoms leading to malaria. B) ACD
(Active Case Detection) where blood films are prepared and collected from the persons with symptoms and signs of malaria during home visits. The blood slides collected using the PCD and ACD [16] techniques were carried to laboratory where these slides were stained with the help of Giemsa’s stain. From the keys furnished by [17] identification of species of parasites was made.

**Results**

A total 3770 blood smears were prepared from 5 age groups ranging from 1 year to 41 years and above from different localities of Multan District of Southern Punjab during August 2015 to July 2017. However, due to different localities with different hygienic conditions variations were observed throughout the district Multan. Out of 3770 cases studied, 1478 cases were found to be of Plasmodium positive counting to 39.02% of total population studied. Regarding species, *Plasmodium vivax* was observed to be the highest 905 (61.23%) as compared to that of *Plasmodium falciparum* 425 (28.75%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 148 (10.01%) (Table 1 & 2).

**Table 1. Area-wise prevalence of Malaria infection in Southern Punjab (District Multan)**

| Sr. No. | Area Name                  | No. of Slides Examined | Total No. of Positivity | Plasmodium vivax No. and % | Plasmodium falciparum No. and % | Mixed species (P. vivax and P. falciparum No. and %) |
|---------|----------------------------|------------------------|--------------------------|-----------------------------|--------------------------------|---------------------------------------------------|
| 1       | Shah Rukn-e-Alam           | 230                    | 42                       | 30                          | 10                             | 2                                                 |
| 2       | Chowk Kumharan Wala        | 280                    | 106                      | 77                          | 20                             | 9                                                 |
| 3       | Delhi Gate                 | 125                    | 45                       | 25                          | 15                             | 5                                                 |
| 4       | Pak Gate                   | 160                    | 62                       | 40                          | 14                             | 8                                                 |
| 5       | Haram Gate                 | 100                    | 35                       | 22                          | 8                              | 5                                                 |
| 6       | Bohar Gate                 | 74                     | 20                       | 10                          | 7                              | 3                                                 |
| 7       | Daulat Gate                | 82                     | 22                       | 11                          | 8                              | 3                                                 |
| 8       | Shuja Abad City            | 618                    | 290                      | 145                         | 100                            | 45                                                |
| 9       | Jalal Pur Pirwala          | 504                    | 118                      | 79                          | 26                             | 13                                                |
| 10      | Multan Cantt.              | 172                    | 65                       | 34                          | 25                             | 6                                                 |
| 11      | Dera Adda                  | 110                    | 50                       | 31                          | 15                             | 4                                                 |
| 12      | Old Shuja Abad Road        | 82                     | 36                       | 23                          | 10                             | 3                                                 |
| 13      | Manzoor Abad Chowk         | 168                    | 90                       | 62                          | 20                             | 8                                                 |
| 14      | Gui Zaib Colony            | 124                    | 65                       | 40                          | 20                             | 5                                                 |
| 15      | Nawan Shehr                | 116                    | 58                       | 35                          | 19                             | 4                                                 |
| 16      | Hassan Abad                | 60                     | 17                       | 9                           | 6                              | 2                                                 |
| 17      | Jahangir Abad              | 240                    | 95                       | 65                          | 22                             | 8                                                 |
| 18      | Ghanta Ghar                | 180                    | 82                       | 45                          | 32                             | 5                                                 |
| 19      | Pul Shawala                | 130                    | 70                       | 47                          | 20                             | 3                                                 |
| 20      | Jalil Abad                 | 215                    | 110                      | 75                          | 28                             | 7                                                 |
| Total   | 3770                       | 1478 (39.20%)           | 905 (61.23%)              | 425 (28.75%)                 | 148 (10.01%)                   |                                                   |
Table 2. Month-wise prevalence of Malarial infection in Southern Punjab (District Multan)

| Month         | No. of Slides Examined | Total No. of Positivity | Plasmodium vivax No. and % | Plasmodium falciparum No. and % | Mixed Species (P. vivax and P. falciparum) No. and % |
|---------------|------------------------|-------------------------|-----------------------------|---------------------------------|-----------------------------------------------------|
| August 2015   | 210                    | 85                      | 48                          | 25                              | 12                                                  |
| September 2015| 235                    | 40                      | 22                          | 14                              | 4                                                   |
| October 2015  | 122                    | 52                      | 30                          | 17                              | 2                                                   |
| November 2015 | 205                    | 63                      | 40                          | 17                              | 6                                                   |
| December 2015 | 214                    | 105                     | 70                          | 25                              | 10                                                  |
| January 2016  | 274                    | 46                      | 30                          | 12                              | 4                                                   |
| February 2016 | 198                    | 55                      | 33                          | 17                              | 5                                                   |
| March 2016    | 174                    | 61                      | 35                          | 18                              | 8                                                   |
| April 2016    | 78                     | 45                      | 26                          | 16                              | 3                                                   |
| May 2016      | 114                    | 66                      | 47                          | 12                              | 7                                                   |
| June 2016     | 110                    | 71                      | 49                          | 16                              | 6                                                   |
| July 2016     | 220                    | 97                      | 62                          | 25                              | 10                                                  |
| August 2016   | 278                    | 141                     | 90                          | 33                              | 18                                                  |
| September 2016| 87                     | 32                      | 20                          | 10                              | 2                                                   |
| October 2016  | 90                     | 34                      | 19                          | 10                              | 5                                                   |
| November 2016 | 78                     | 40                      | 25                          | 11                              | 4                                                   |
| December 2016 | 222                    | 99                      | 80                          | 13                              | 6                                                   |
| January 2017  | 108                    | 58                      | 19                          | 34                              | 5                                                   |
| February 2017 | 98                     | 37                      | 23                          | 12                              | 2                                                   |
| March 2017    | 97                     | 42                      | 25                          | 14                              | 3                                                   |
| April 2017    | 104                    | 40                      | 23                          | 13                              | 4                                                   |
| May 2017      | 111                    | 35                      | 20                          | 12                              | 3                                                   |
| June 2017     | 128                    | 56                      | 31                          | 19                              | 6                                                   |
| July 2017     | 215                    | 78                      | 38                          | 30                              | 10                                                  |
| Total         | 3770                   | 1478                    | 905 (61.23%)                 | 425 (28.75%)                    | 148 (10.01%)                                        |

Among children from 1-10 years, 431 slides were examined, out of which 195 (45.24%) slide positivity of *Plasmodium* was to be found and the most prevalence species was observed to be *Plasmodium vivax* 140 (71.79%) as compared to with that of *Plasmodium falciparum* 39 (20%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 16 (8.20%). In the age group of 11-20 years 736 slides were examined and
260 (35.32%) slide positivity was to be found and the commonest species of malarial parasites observed was *Plasmodium vivax* with a highest incidence of 172 (66.15%) as compared to other species of plasmodium such as *Plasmodium falciparum* 68 (26.15%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 20 (7.69%). Similarly in the age group of 21-30 years, 820 slides were examined out of which 302 (36.82%) positivity was to be found and the highest incidence of *Plasmodium vivax* 190 (62.91%) was observed as compared to that of *Plasmodium falciparum* 90 (29.80%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 22 (7.28%). In the age group of 31-40 years 863 slides were examined and only 306 (35.45%) slides were found to be positive, out of which the highest incidence of *Plasmodium vivax* 197 (64.37%) was the commonest species of malarial parasites as compared to *Plasmodium falciparum* 81 (26.47%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 28 (9.15%), while in the age group of 41 years and above 920 slides were observed and 415 (45.10%) slides were found to be positive, out of which 206 (49.63%) the most common species of malarial parasites was *Plasmodium vivax* as compared to that of *Plasmodium falciparum* 147 (35.42%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 62 (14.93%) (Table 3).

It was also observed that there is seasonal variations in infection of different plasmodium species, as the highest rate of infection of *Plasmodium vivax* 150 (73.52%) was noted in December while lowest in September 42 (58.33%) while infection of *Plasmodium falciparum* was found to be highest in August 58 (25.66%) and lowest in May 24 (23.76%) as compared to the infection of mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) highest in the month of August 30 (13.27%) and lowest in the month of February 7 (7.60%) (Table 4).

Infection in males was 850 (57.51%), out of which *Plasmodium vivax* was highest 508 (59.76%) as compared to that *Plasmodium falciparum* 248 (29.17%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 94 (11.05%) while in females 628 (42.48%) positivity was to be found, out of which *Plasmodium vivax* was highest 397 (63.21%) as compared to that of *Plasmodium falciparum* 177 (28.18%) and mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) 54 (8.59%) (Table 5). The comparison of these results with those of other studies which have been done in Pakistan is in discussion.

### Table 3. Age-wise overall prevalence of Malarial infection in accordance to Total Number and Percentage in Southern Punjab (District Multan) from August 2015 July 2017

| Sr. No. | Age (Years) | Total Number of Slides Examined | Total Number of Positivity | Overall % Infection | Infection by *Plasmodium vivax* number and %age | Infection by *Plasmodium falciparum* number and %age | Mixed species (*Plasmodium vivax* and *Plasmodium falciparum*) number and %age |
|---------|-------------|--------------------------------|---------------------------|---------------------|-----------------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|
| 1       | 1-10        | 431                            | 195                       | 45.24%              | 140 (71.79%)                                  | 39 (20%)                                       | 16 (8.20%)                                      |
| 2       | 11-20       | 736                            | 260                       | 35.32%              | 172 (66.15%)                                  | 68 (26.15%)                                    | 20 (7.69%)                                      |
| 3       | 21-30       | 820                            | 302                       | 36.82%              | 190 (62.91%)                                  | 90 (29.80%)                                    | 22 (7.28%)                                      |
| 4       | 31-40       | 863                            | 306                       | 35.45%              | 197 (64.37%)                                  | 81 (26.47%)                                    | 28 (9.15%)                                      |
| 5       | 41-above    | 920                            | 415                       | 45.10%              | 206 (49.63%)                                  | 147 (35.42%)                                   | 62 (14.93%)                                     |
| Total   |              | 3770                           | 1478                      | 39.20%              | 905 (61.23%)                                  | 425 (28.75%)                                   | 148 (10.01%)                                    |
Table 4. Month-wise prevalence of Malarial infection in accordance to Total Number and percentage in Southern Punjab (District Multan) from August 2015 to July 2017

| Month         | Total number of slides examined | Total number of positivity | Plasmodium vivax number and %age | Plasmodium falciparum number and %age | Mixed species (Plasmodium vivax and Plasmodium falciparum) number and %age |
|---------------|--------------------------------|----------------------------|----------------------------------|--------------------------------------|--------------------------------------------------------------------------------|
| August 2015 & 2016 | 210+278=488 | 85+141=226 | 48+90=138 (61.06%) | 25+33=58 (25.66%) | 12+18=30 (13.27%) |
| September 2015 & 2016 | 235+87=322 | 40+32=72 | 22+20=42 (58.33%) | 14+10=24 (33.33%) | 4+2=6 (8.33%) |
| October 2015 & 2016 | 122+90=212 | 52+34=86 | 30+19=49 (56.97%) | 17+10=27 (31.39%) | 5+5=10 (11.62%) |
| November 2015 & 2016 | 205+78=283 | 63+40=103 | 40+25=65 (63.10%) | 17+11=28 (27.18%) | 6+4=10 (9.70%) |
| December 2015 & 2016 | 214+222=436 | 105+99=204 | 70+80=150 (73.52%) | 25+13=38 (18.62%) | 10+6=16 (7.84%) |
| January 2016 & 2017 | 274+108=382 | 46+58=104 | 30+19=49 (47.11%) | 12+34=46 (44.23%) | 4+5=9 (8.65%) |
| February 2016 & 2017 | 198+98=296 | 55+37=92 | 33+23=56 (60.86%) | 17+12=29 (31.52%) | 5+2=7 (7.60%) |
| March 2016 & 2017 | 174+97=271 | 61+42=103 | 35+25=60 (58.25%) | 18+14=32 (31.06%) | 8+3=11 (10.67%) |
| April 2016 & 2017 | 78+104=182 | 45+40=85 | 26+23=49 (57.64%) | 16+13=29 (34.11%) | 3+4=7 (8.23%) |
| May 2016 & 2017 | 114+111=225 | 66+35=101 | 47+20=67 (66.33%) | 12+12=24 (23.76%) | 7+3=10 (9.90%) |
| June 2016 & 2017 | 110+128=238 | 71+56=127 | 49+31=80 (62.99%) | 16+19=35 (27.55%) | 6+6=12 (9.44%) |
| July 2016 & 2017 | 220+215=435 | 97+68=175 | 62+38=100 (57.14%) | 25+30=55 (31.42%) | 10+10=20 (11.42%) |
| Total | 2154+1616=3770 | 786+692=1478 (39.20%) | 492+413=905 (61.23%) | 214+211=425 (28.75%) | 80+68=148 (10.01%) |
Table 5. Sex-wise prevalence of Malarial Infection in accordance to Total Number and Percentage in Southern Punjab (District Multan) from August 2015 to July 2017

| Total Number of Slides Examine d | Total Number of Positivit y | Number of Male Positive | Mixed Species (Plasmodiu m vivax and Plasmodiu m falciparum) | Number of Female Positive | Mixed Species (Plasmodiu m vivax and Plasmodiu m falciparum) |
|----------------------------------|-----------------------------|-------------------------|-------------------------------------------------------------|---------------------------|-------------------------------------------------------------|
| 3770                             | 1478                        |                         |                                                             |                           |                                                             |

| Plasmodiu m vivax | Plasmodiu m falciparum | Mixed Species | Plasmodiu m vivax | Plasmodiu m falciparum |
|-------------------|------------------------|---------------|-------------------|------------------------|
| 508 (59.76%)      | 248 (29.17%)           | 94 (11.05%)   | 397 (63.21%)      | 177 (28.18%)           |
| 850 (57.51%)      |                        |               | 628 (42.48%)      |                        |

Discussion
In Pakistan, malaria is considered to be a continuous severe public health problem [18]. From the Eastern Mediterranean region in 2010, over one million malarial cases through microscopy confirmed were reported of which 25% came from Pakistan [1]. Approximately 325 million people affect through malaria and causes more than a million of deaths per year throughout the world. Plasmodium falciparum has high mortality as compared to other plasmodium and it causes several diseases such as algid malaria, renal failure and cerebral malaria [5].

This survey was conducted to provide up to date information about malarial prevalence throughout District Multan. In this analysis, samples were collected from patients suffering from malaria with symptoms and one limitation is the potential for district Multan region in accordance to treatment seeking behaviour and centres for access to treatment. But unfortunately prior to diagnosis information on treatments taken was not collected and could be included in this study. In addition to this, season variations were also to be noted in the district areas of Multan where the highest rate of infection of Plasmodium vivax in December, Plasmodium falciparum in August and mixed species (Plasmodium vivax and Plasmodium falciparum) in August. Despite this information, the data presented in this study is more comprehensive and shows current characterization of prevalence of malaria in Multan District than has previously been available.

The prevalence of Plasmodium vivax was observed as highest (61.23%) as compared to that of Plasmodium falciparum (28.75%) and mixed species (Plasmodium vivax and Plasmodium falciparum) (10.01%) in the present study. Many researchers reported higher incidences of Plasmodium vivax (60.4%), Plasmodium falciparum (37.6%) in District of Multan, while in districts Quetta (66.7% Plasmodium vivax, 30.8% Plasmodium falciparum), Muzafarabad (90.3% Plasmodium vivax, 48.2% Plasmodium falciparum) and Kohlu (58.8% Plasmodium vivax and 41.2% Plasmodium falciparum) [13, 19-21] respectively.

In the present analysis, Plasmodium vivax 71.79%, 66.15%, 62.91%, 64.37% and 49.63% was observed in the patients of the age group 1-10, 11-20, 21-30, 31-40, and 41 years & above respectively. Similarly, Plasmodium falciparum 20%, 26.15%, 29.80%, 26.47% and 35.42% was observed in the age group 1-10, 11-20, 21-30, 31-40, and 41 years & above, while mixed species
(Plasmodium vivax and Plasmodium falciparum) 8.2%, 7.69%, 7.28%, 9.15% and 14.93% was observed in the age group 1-10, 11-20, 21-30, 31-40, and 41 years & above respectively. Similarly [22] found 8.5% and 73.8% in the patients of the age group of 11-20 years and 21 years & above respectively in the Kashmiri settled in Muzaffarabad and Plasmodium falciparum 18%, 22.4%, 26.1% and 33.5% in the age group of 1-10, 11-20, 21-30 and 31-40 years was observed in the refugees of Zhob respectively [23].

In the malarial patients of Iran, slide positivity rate was to be reported 9.7% [24]. 43.3, 33.3; 16.1, 15.5; 33.9, 11.5; 25.5, 28.5; 27.2, 41.7% slide positivity rate of Plasmodium vivax and Plasmodium falciparum in Kharan, Quetta, Qila Abdullah, Mastung and Khuzdar rural and urban areas respectively by [13, 14, 19-21, 27]. In another study in 33 different localities of Quetta (city) visited showed that P. vivax was found to be the most common species with the highest rate of frequency (84.53%) as compared to that of P. falciparum (6.02%) in children [28]. These results are similar with our findings.

In this study, mixed infection of 8.2%, 7.69%, 7.28%, 9.15% and 14.93% was observed. Mixed infection on 2.4% was observed in Multan district by [25]. However, in Quetta district, the same 2.4% was observed [26].

During this study no case of Plasmodium ovale or Plasmodium malariae infection was to be observed and the same were the observations of [25] in District Multan.

**Conclusion**

Pakistan is facing several challenges in the control and management of malaria including lack of diagnostic facilities, misdiagnosis and use of presumptive treatments. The results of this study indicate that Plasmodium infection in Pakistan are largely attributed to Plasmodium vivax but Plasmodium falciparum and mixed species of Plasmodium infections are also prevalent. In addition to this, regional variation in the prevalence and species composition of malaria is very high. So, malaria control efforts should be focused where malaria prevalence is highest with emphasis on improving species diagnosis.

**Authors’ contributions**

Conceived and designed the experiments: M Zafar, Laboratory Experiment: M Zafar & Asmatullah, Data Collection: MKA Khan & MZ Khan, Analysis of Data: IM & S Masud, Experiment Supervision: Asmatullah, Paper Writing: M Zafar.

**References**

1. Organization WH (2010). Report on the fourth malaria border coordination meeting between Afghanistan, Islamic Republic of Iran and Pakistan, Islamabad, Pakistan, 29 September–1 October 2009.
2. Organization WH (2005). The World Health Report 2005: Make every mother and child count, World Health Organization.
3. Korenromp E (2005). Malaria incidence estimates at country level for the year 2004. Proposed estimates and draft report. World Health Organization, Roll Back Malaria, MERG Task Force on Malaria Morbidity.
4. Crawley J, Chu C, Mtive G & Nosten F (2015). Malaria in children. The Lancet 375: 1468-1481.
5. Bhalli M (2001). Falciparum malaria-a review of 120 cases. J-College of Phy and Surgeons of Pak 11: 300-303.
6. Muhammad N & Hussain A (2011). Prevalence of malaria in general population of district buner. J of Postgraduate Medical Institute (Peshawar-Pakistan), pp 17.
7. Jalal-Ud-Din KS & Ally SH (2006). Malaria in children: study of 160 cases at a private clinic in Mansehra. J Ayub Med Coll Abbottabad 18: 44-45.
8. Mahmood KH (2005). Malaria in Karachi and other areas in Sindh. Pak Armed Forces Med J 55: 345-348.
9. Nizamani A, Kalar N & Khushk I (2006). Burden of malaria in Sindh, Pakistan: a two years surveillance report. *Jlumhs* 5: 76-83.

10. Saleem I, Pirzada A & Rahman R (2006). Noor-ul-Iman. Cerebral malaria: an experience in NWFP, Pakistan. *Pak J Med Sci* 14: 35-9.

11. Idris M, Sarwar J & Fareed J (2007). Pattern of malarial infection diagnosed at Ayub Teaching Hospital Abbottabad. *J Ayub Med Coll Abbottabad* 19: 35-6.

12. Durrani A, Durrani I, Abbas N & Jabeen M (1997). Epidemiology of cerebral malaria and its mortality. *JPMA; The J of the Pak Medical Assoc* 47: 213-215.

13. Yasinzai MI & Kakarsulemankhel JK (2003). Incidence of malaria infection in rural areas of District Quetta, Pakistan. *On Line J Med Sci* 3: 766-72.

14. Yasinzai MI & Kakarsulemankhel JK (2008). Incidence of human malaria infection in desert area of Pakistan: District Kharian. *J of Agri and Social Sci* (Pak).

15. Khan AR, Khan N & Khan H (2018). Frequency of slide positivity in clinically suspected malaria cases. *Gomal J of Medical Sci* 12(118-20). Malaria Control Program, Balochistan. Survey Report of 2016-2017, pp 1.

16. Manson-Bahr PH (1946). Manson's Tropical Diseases: A Manual of the Diseases of Warm Climates. *Academic Medicine* 21: 318.

17. Paniker CJ (2007). Textbook of medical parasitology, Jaypee Brothers Medical Publishers (P) Ltd.

18. Qayum M, Zahir H, Ahmad N, Ilyas M, Khan A & Khan S (2012). SPHERE-based assessment of knowledge and preventive measures related to malaria among the displaced population of Jalozai, Pakistan. *JPMA-J of the Pak Medical Assoc* 62: 344.

19. Yasinzai MI & Kakarsulemankhel JK (2008). Incidence of human malaria infection in northern hilly region of Balochistan, adjoining with NWFP, Pakistan: district Zhob. *Pak J Biol Sci* 11: 1620-1624.

20. Yasinzai MI & Kakarsulemankhel JK (2008). Incidence of human malaria infection in Barkhan and Kohlu, bordering areas of East Balochistan. *Pak J of Medical Sci* 24: 306.

21. Yasinzai MI & Kakarsulemankhel JK (2007b). Incidence of human malaria infection in central areas of Balochistan: Mastung and Khuzdar. *Rawal Medical J* 32: 176-178.

22. Jan A & Kiani T (2001). Haematozoan parasites in Kashmiri refugees. *Pak J Med Res* 40: 10-12.

23. Khadim M (2002). Malaria a menace at Zhob Garrison. *Pak Armed Forces Med J* 52: 203-207.

24. Karimi-Zarch A, Mahmoodzadeh A & Vatani H (2006). A survey on malaria and some related factors in south east of Caspian Sea. *Pak J Med Sci* 22: 489-92.

25. Yar H, Masood K, Maqbool A & Malik G (1998). Prevalence of malarial parasite species in Multan district. *The Professional* 5: 183-7.

26. Sheikh AS, Sheikh A, Sheikh N & Paracha S (2005). Endemicity of malaria in Quetta. *Pak J of Medical Res* 44: 7.

27. Yasinzai MI & Kakarsulemankhel JK (2007). Incidence of malaria infection in Pak-Afghan border area of Pakistan: District Qilla Abdullah-Chaman. *Hamard Medicus October* 62-66.

28. Sumbal A, Iqbal MY, Naseem M & Khan L (2017). Frequency of *Plasmodium vivax* and *Plasmodium falciparum* in school going children of Quetta (City), Balochistan 13(6): 43-50.