Community-based prevalence of typhoid fever, typhus, brucellosis and malaria among symptomatic individuals in Afar Region, Ethiopia

Biruk Zerfu1, Girmay Medhin2, Gezahegne Mamo3, Gezahegn Getahun4, Rea Tschopp5,6, Mengistu Legesse2,∗

1 Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia, 2 Microbiology Research Unit, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia, 3 Department of Veterinary Microbiology, Immunology and Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia, 4 Melka Werer Health Center, Amibara district, Melka Were, Ethiopia, 5 Armauer Hansen Research Institute, Addis Ababa, Ethiopia, 6 Swiss TPH, Basel, Switzerland

Abstract

Background
In sub-Saharan Africa, where there is the scarcity of proper diagnostic tools, febrile illness related symptoms are often misdiagnosed as malaria. Information on causative agents of febrile illness related symptoms among pastoral communities in Ethiopia have rarely been described.

Methods
In this a community based cross-sectional survey, we assessed the prevalence of typhoid fever, typhus, brucellosis and malaria among individuals with a set of given symptoms in Amibara district, Afar Region, Ethiopia. Blood samples were collected from 650 study participants, and examined by Widal and Weil-Felix direct card agglutination test (DCAT) as well as test tube based titration test for Salmonella enterica serotype Typhi (S. Typhi) and Rickettsia infections. Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) were used to screen Brucella infection. Thin and thick blood smears were used to diagnosis malaria.

Results
Out of 630 sera screened by DCAT, 83 (13.2%) were reactive to H and/or O antigens for S. Typhi infection. Among these, 46 (55.4%) were reactive by the titration test at the cut off value ≥ 1:80. The combined sero-prevalence for S. Typhi by the two tests was 7.3% (46/630). The seroprevalence for Rickettsia infection was 26.2% (165/630) by DCAT and 53.3% (88/165) by the titration test at the cut off value ≥ 1:80. The combined sero-prevalence for Rickettsia infection by the two tests was 14.0% (88/630). The sero-prevalence for Brucella infection was 12.7% (80/630) by RBPT, of which 28/80 (35%) were positive by CFT.

* mengistu.legesse@aau.edu.et
combined sero-prevalence for *Brucella* infection by the two tests was 4.4% (28/630). Out of 650 suspected individuals for malaria, 16 (2.5%) were found positive for *P. falciparum* infection.

**Conclusion**

In this study, typhoid fever, typhus, brucellosis and malaria were observed among symptomatic individuals. The study also highlighted that brucellosis cases can be misdiagnosed as malaria or other disease based solely on clinical diagnosis. Therefore, efforts are needed to improve disease awareness and laboratory services for the diagnosis of brucellosis and other zoonotic diseases to identify other causes of febrile illness in this pastoral setting.

**Author summary**

Many diseases such as typhoid fever, typhus, brucellosis and malaria show common symptoms such as fever, headache, joint pain and back pain. Hence, in countries where there is a problem of appropriate laboratory based diagnostic tools, health workers cannot properly diagnose these diseases and provide appropriate treatment. A community-based study of the causative agents of the above mentioned illness would provide important information for health workers about some of the common causative agents in that particular area. In this study, we assessed the prevalence of typhoid fever, typhus, brucellosis and malaria among individuals who were complaining illnesses such fever, headache, joint pain and back pain in the pastoral of the Amibara district, Afar Region, Ethiopia. Among 650 individuals who were complaining symptoms, 46 (7.3%), 88 (14.0%), 28 (4.4%) and 16 (2.5%) were diagnosed for typhoid fever, typhus, brucellosis and malaria in that order. However, for the majority of the participants (75.4%), the cause of their illness remained unknown, and further investigations on the causative agents of febrile illness related symptoms is important in the present study area.

**Introduction**

Sub-Saharan Africa is plagued by a myriad of infectious diseases posing significant public health and economic challenges. In addition, the often non-specific clinical signs of these diseases and the scarcity of proper diagnostic tools are the major challenges for health professionals in properly diagnosing and treating adequately patients [1, 2]. Studies showed that symptoms such as fever, headache, joint pain and back pain are often misdiagnosed as malaria, especially until the introduction of rapid diagnostics for malaria though these symptoms are not only specific to malaria [3–5].

Many studies have shown that diseases such as typhoid fever, rickettsioses, brucellosis, Q fever, and leptospirosis are the leading causes for febrile illness with symptoms such as fever, headache, joint pain and back pain [6–9]. For instance, typhoid fever due to *S. Typhi* has been reported as the leading cause of over 21 million febrile cases and over 200,000 deaths each year in many low- and middle-income countries [7–9].

Brucellosis has been considered as an important zoonotic disease worldwide and is responsible for big economic losses as it causes abortion in livestock [10, 11]. It also causes a
considerable human morbidity and spontaneous abortion among pregnant women in endemic areas [12–15].

Although many malarious countries including Ethiopia are scaling up malaria intervention programs towards elimination, the disease remains one of the worst health problems with an estimated 216 million cases and 445 000 deaths globally in 2016, while most of the cases and deaths occurred in African [16]. Hence, in low- and middle-income countries where there is a shortage of effective routine diagnostic tools to identify a wide range of infectious diseases that manifest similar symptoms and where there is also a low awareness among community members and health professionals about the common causative agents of such illnesses, a community-based approach epidemiological survey would help health professionals to improve clinical diagnosis and provide appropriate treatment [1, 2].

In Ethiopia, there have been few health facilities based studies to determine the prevalence of typhoid fever, typhus, malaria and brucellosis among individuals presented with febrile illness related symptoms [17–19]. Communities based epidemiological data on the causative agents of common febrile illness related symptoms is generally lacking in the pastoralists areas due to the remoteness of sites and pastoralists way of life. Moreover, in the present study area, health professionals had no clear information on the magnitude of brucellosis, and its clinical based diagnosis might not be even considered. Hence, we assessed the prevalence of typhoid fever, typhus, brucellosis and malaria in individuals who were complaining of symptoms such as fever, headache, joint pain and back pain in the pastoral community of the Amibara district, Afar Region, Ethiopia.

Material and methods

Study area and population

The study was conducted in the pastoral community of Amibara district in the Afar Region of Ethiopia, around 260 km from Addis Ababa. The majority of the study population are pastoralists, depending on livestock for their livelihoods, while some started to practice agro-pastoralism and growing crops along river Awash. The study area and the population has been previously described in detail [20, 21].

Study design and sample size estimation

A community based cross-sectional survey was carried out between September and December 2016 to determine the prevalence of typhoid fever, typhus, brucellosis and malaria among individuals who were complaining of a range of symptoms. The result of previous health facilities based sero-prevalence of brucellosis (34%) in other pastoral community areas of Ethiopia was used to estimate the sample size [17] for the study on brucellosis. Using this information, a sample size of 380 individuals (95% confidence level, 5% degree of accuracy and 10% compensation for refusal of blood sample) was initially considered. According to the information from Melka Worer health center 50% of patients with symptoms like fever, headache and joint pain often diagnosed as positive for typhoid, typhus or malaria. Based on this supplementary information, sample size was increased to 422 individuals (95% confidence level, 5% degree of accuracy and 10% compensation for refusal of blood sample). However, during the survey period all eligible individuals who came for diagnosis were considered and the sample size was increased to 650.
Study procedure and study participants

In this study, six accessible pastoral kebeles of the district were conveniently selected, and a house-to-house survey of all households in the selected kebeles was conducted by community health workers under the supervision of the research team. The heads of the households (husband or wife) or individuals over 18 years were asked if there was any family member (age ≥ 2 years) who manifested symptoms such as fever, fatigue, headache, joint pain, and back pain for short or long periods of time. The individuals were asked to come to the nearest health post, and they were interviewed in their local language (Afar language) using a structured questionnaire that captured common signs/symptoms they felt, onset of illness, treatment sought, and information on socio-demographic characteristics of the individuals. Body temperature was also recorded using a digital thermometer. All individuals equal or older than 2 years, who reported the above symptoms, came for examination and willing to provide blood, and gave informed consent and/or assents were included in the study.

Blood sample collection and examination

Three ml venous blood sample was collected into plain vacutainer test tube and transported to Melka Werer Health Center. Serum was separated and tested for typhoid fever, typhus and malaria on the same days. The remaining serum was stored at -20˚C until transported to the laboratory of Aklilu Lemma Institute of Pathobiology, Addis Ababa University and tested for brucellosis and also tested for typhoid and typhus by test tube based titration method. Anemic individuals and pregnant women were included in the malaria study and provided only finger prick blood sample.

Widal and Weilfelix direct card agglutination tests (DCAT) were used for the serological screening of S. Typhi and Rickettsia infections, respectively following the manufacturer’s instructions (Rapid Labs Ltd, Hall Farm Business Centre, UK), and as previously described [22, 23]. A test tube based titration test was performed for all samples that were found to be reactive by the DCAT and for other 25 randomly selected samples which were found non-reactive as previously described [24]. Rose Bengal Plate Test (RBPT) was used to screen for Brucella infection as previously described [25]. All sera which tested positive by the RBPT and other randomly selected 68 negative samples were further tested using Complement Fixation Test (CFT) following the guidelines of OIE 2008 [26]. The guideline of Ministry of Health (MOH) was followed for the diagnosis of malaria and identification of Plasmodium species at the Health center [27].

Data entry and analysis

Data was entered into EpiData 3.1 and analyzed with Stata/SE 11.0. Descriptive analysis was used to summarize the data in the form of frequencies and percentages of variables. Pearson chi-square test was used to evaluate the statistically significant difference in the level of prevalence of typhoid fever, typhus, brucellosis and malaria between male and female study participants and according to the reported clinical features. Bivariable and multivariable logistic regression analyses were performed to explore associations of socio-demographic characteristics of the study participants with increased odds of having higher prevalence of typhoid fever, typhus, brucellosis and malaria. P-value below 5% was considered as indicator of statistical significance.
Ethical consideration

This study received ethical clearance from the Institutional Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University (ALIPB/IRB/005/2015/16)). Permission was obtained from Amibara Health Office. Participants’ information sheet which contains the objective of the study, inclusion/exclusion criteria, the required data and methods of data collection as well as informed consent document were prepared in Amharic the national language of the country. Then, the elements of participants’ information sheet initially were orally translated to the local language and described to community leaders and to each of the study participant or parent in case of children under 18 years by trained local health personnel. Informed written consent was obtained from illiterate participants and/or assent in children aged between 12 and 18 years by signing with their finger. Blood sample was collected under aseptic condition by experienced laboratory technicians. Study participants who were found positive for the investigated diseases were treated accordingly as per physician recommendation.

Results

Socio-demographic characteristics of the study participants

A total of 657 individuals who were complaining various symptoms such as headache, joint pain fever and back pain appeared for clinical examination. However, seven individuals were not volunteers to consent to provide blood sample and they were excluded. Out of the 650 study participants, 630 provided venous blood and 20 provided finger prick blood sample due to anemia and/or pregnancy. The participants’ age ranged from 2 to 80 years with mean age 34.25 ±17.38 years. The majority were illiterate (75.2%) and pastoralists (78.8%) (Table 1).

Table 1. Socio-demographic characteristics of the study participants.

| Variables            | Number (%) |
|----------------------|------------|
| **Sex**              |            |
| Male                 | 253 (38.9) |
| Female               | 397 (61.1) |
| **Age (years)**      |            |
| 2–14                 | 114 (17.5) |
| 15–24                | 59 (9.1)   |
| 25–34                | 146 (22.5) |
| 35–44                | 120 (18.5) |
| ≥45                  | 211 (32.5) |
| **Religion**         |            |
| Muslim               | 612 (94.2) |
| Others               | 38 (5.8)   |
| **Ethnicity**        |            |
| Afar                 | 592 (91.1) |
| Others               | 58 (8.9)   |
| **Education status** |            |
| Illiterate           | 489 (75.2) |
| 1–12 grade attended  | 61 (9.4)   |
| Non-schooled children| 100 (15.4) |
| **Occupation**       |            |
| Pastoralist          | 514 (79.1) |
| agro pastoralist     | 67 (10.3)  |
| Others               | 69 (10.6)  |
| **Marital status**   |            |
| Married              | 474 (72.9) |
| Single/children      | 133 (20.5) |
| Other                | 43 (6.6)   |

https://doi.org/10.1371/journal.pntd.0006749.t001
Clinical signs/symptoms reported by the study participants

Table 2 shows the clinical signs, duration of the illness and treatment history as reported by the study participants. Headache (74.8%), joint pain (74.8%), and general malaise (24.9%) were the frequently reported symptoms. The duration of the illness reported by the participants ranged between 2 and 7300 days, with a median duration of 257 days. A total of 159 individuals (24.5%) reported that they sought treatment at various health facilities for their or their family member current illness. Among them, 68/159 (42.8%) were examined and treated for malaria, typhus or typhoid fever, while others (91 participants) reported that they and their families received a treatment though they did not get adequate information for which disease they were treated. The remaining 491 (75.5%) individuals did not seek treatment because of various reasons like distance from health facility, the intermittent nature of the illness or lack of money. History of abortion was reported by 116/325 (35.7%) women and the majority (70.7%) of them didn’t know the cause of the abortion.

Sero prevalence of S. Typhi infection

Out of 630 sera screened by the DCAT, 83 (13.2%) were reactive for S. Typhi infection either against flagella (H) antigen (18/83, 21.7%) or against somatic (O) antigen (41/83, 49.4%) or against both H and O antigens (24/83, 28.9%).

Among the reactive sera to O antigen, 17 (19.54%), 8 (9.20%), 1 (1.2%) and 1 (1.2%) were reactive at the titration of 1:80, 1:160, 1:320 and 1:640, respectively. Among the reactive sera to H antigen, 15 (22.73%), 9 (13.64%) and 1 (1.52%) were reactive at the titration of 1:80, 1:160 and 1:320, respectively. Thus, the overall sero-prevalence of current infection with S. Typhi as indicated either by H and/or O antigen was considered as 7.3% (46/630) at the cut off value ≥ 1:80. The cases were more common among females than among males (17.2% vs

| Clinical features                        | Number (%) |
|-----------------------------------------|------------|
| Body temperature ≥ 37.5˚c                | 32 (5.4)   |
| Clinical symptoms                       |            |
| Headache                                | 486 (74.8) |
| Joint pain                              | 486 (74.8) |
| Malaise                                 | 162 (24.9) |
| Back pain                               | 145 (22.3) |
| Weakness                                | 92 (14.1)  |
| Vomiting                                | 10 (1.5)   |
| Illness duration                         |            |
| Less than 8 days                        | 249 (38.9) |
| 8–30 days                               | 162 (25.3) |
| Greater than 30 days                    | 229 (35.9) |
| Health facility visited                 |            |
| No                                      | 491 (75.5) |
| Yes                                     | 159 (24.5) |
| Reason for not visited health facility   |            |
| Lack of money                           | 101 (20.7) |
| Distance from health facility            | 59 (12.1)  |
| intermittent nature of the illness      | 118 (24.1) |
| Other reasons like work load            | 211 (43.1) |
| Abortion history in women                |            |
| No                                      | 209 (64.3) |
| Yes                                     | 116 (35.7) |
| Cause of the abortion                    |            |
| Disease                                 | 8 (6.9)    |
| Accident                                | 26 (22.4)  |
| Unknown                                 | 82 (70.7)  |

https://doi.org/10.1371/journal.pntd.0006749.t002
6.9%, $X^2 = 14.06, P < 0.001$) as detected by DCAT and by the titration test (9.4% vs. 4.1%, $X^2 = 6.35, P = 0.012$). All the randomly selected 25 samples which were non-reactive by DCAT were also found non-reactive by the titration test.

In multivariable regression analyses, being female (AOR = 2.21, 95%CI: 1.01–4.83, $P = 0.047$) and duration of illness above a month (AOR = 2.70, 95%CI: 1.02–7.18, $P = 0.046$) were found to be associated with a high sero-positivity for S. Typhi infection (Table 3).

### Sero prevalence of Rickettsia infection

Of the 630 sera screened for Rickettsia infection by DCAT, 165 (26.2%) were reactive. Out of these sera, 41 (21.8%), 33 (17.6%), 9 (4.8%) and 5 (2.7%) were reactive at the titration of 1:80, 1:160, 1:320 and 1:640, respectively. Hence, 88 (53.3%) samples were reactive by the titration test at the cut off value $\geq 1:80$. The combined sero-prevalence for Rickettsia infection by the two tests was 14.0% (88/630). The sero-prevalence was frequent among females compared to males (32.9% vs 15.8%, $X^2 = 22.74, P < 0.001$) by DCAT, as well as by titration test (18.5% vs. 6.5%, $X^2 = 9.81, P = 0.002$) by titration test. All the 25 samples which were non-reactive by DCAT were also non-reactive by titration test.

### Table 3. Socio-demographic of the study participants and prevalence of typhoid fever.

| characters       | Number tested | Number (%) positive by titration | AOR(95%CI)   | $P$ value |
|------------------|---------------|----------------------------------|--------------|-----------|
| Sex              |               |                                  |              |           |
| Male             | 247           | 10 (4.1)                         | 1            |           |
| Female           | 383           | 36 (9.4)                         | 2.21(1.01–4.83) | 0.047    |
| Age (years)      |               |                                  |              |           |
| 2–14             | 104           | 4 (3.9)                          | 1            |           |
| 15–24            | 58            | 5 (8.6)                          | 1.30(0.12–13.47) | 0.828    |
| 25–34            | 144           | 11 (7.6)                         | 1.11(0.11–11.30) | 0.930    |
| 35–44            | 117           | 10 (8.6)                         | 1.03(0.10–10.62) | 0.977    |
| $\geq 45$        | 207           | 16 (7.7)                         | 1.15(0.11–11.60) | 0.906    |
| Education        |               |                                  |              |           |
| Illiterate       | 479           | 38 (7.9)                         | 1            |           |
| 1–12 grade attended | 59     | 5 (8.5)                          | 1.94(0.54–6.97) | 0.308    |
| Children         | 92            | 3 (3.3)                          | 0.59(0.09–3.92) | 0.585    |
| Occupation       |               |                                  |              |           |
| Pastoralist      | 497           | 38 (7.7)                         | 1            |           |
| Agro-pastoral    | 67            | 1 (1.5)                          | 0.24(0.03–1.98) | 0.187    |
| Others           | 66            | 7 (10.6)                         | 1.34(0.43–4.13) | 0.616    |
| Marital status   |               |                                  |              |           |
| Married          | 464           | 38 (8.2)                         | 1            |           |
| Children         | 123           | 5 (4.1)                          | 0.66(0.11–4.10) | 0.657    |
| Other            | 43            | 3 (7.0)                          | 1.10(0.29–4.18) | 0.893    |
| Body temperature |               |                                  |              |           |
| $<37.5^\circ C$  | 545           | 41 (7.5)                         | 1            |           |
| $\geq 37.5^\circ C$ | 26     | 1 (3.9)                          | 1.02(0.11–9.31) | 0.985    |
| Headache         |               |                                  |              |           |
| No               | 155           | 13 (9.4)                         | 1            |           |
| Yes              | 473           | 32 (6.8)                         | 0.99(0.45–2.17) | 0.976    |
| Joint pain       |               |                                  |              |           |
| No               | 145           | 8 (5.5)                          | 1            |           |
| Yes              | 483           | 37 (7.7)                         | 0.91(0.37–2.22) | 0.839    |
| Back pain        |               |                                  |              |           |
| No               | 487           | 31 (6.4)                         | 1            |           |
| Yes              | 141           | 15 (10.6)                        | 1.39(0.66–2.94) | 0.386    |
| Illness duration |               |                                  |              |           |
| $<8$ days        | 239           | 10 (4.2)                         | 1            |           |
| 9–30 days        | 158           | 14 (8.9)                         | 2.31(0.88–6.06) | 0.090    |
| $>30$ days       | 223           | 21 (9.4)                         | 2.70(1.02–7.18) | 0.046    |

https://doi.org/10.1371/journal.pntd.0006749.t003
In multivariable regression analyses, being female (AOR = 3.10, 95%CI: 1.67–5.77, \( P < 0.001 \)) and reporting headache (AOR = 2.80, 95%CI: 1.26–6.22, \( P = 0.011 \)) were significantly associated with sero-positivity for \textit{Rickettsia} infection (Table 4).

### Sero-prevalence of \textit{Brucella} infection

The sero-prevalence for \textit{Brucella} infection among the study participants was 12.7% (80/630) by RBPT and 35% (28/80) by CFT. The combined sero-prevalence for \textit{Brucella} infection by the two tests was 4.4% (28/630). The sero-prevalence for \textit{Brucella} infection was relatively high in the age group between 2–14 and 15–24 (Table 5). The sero-prevalence was also relatively high among individuals who reported drinking raw milk from aborted animals (13.0% vs. 6.9%) by RBPT and (20.6% vs 6.7%) by CFT. Among the study participants, 569 (90.7%) reported drinking raw milk from aborted animals, 566 (90.3%) touched aborted fetus/discharges from aborted animals without protection and 562 (90.1%) responded that they had no clear information about a disease that causes abortion in their animals.

In the univariable logistic regression analysis; being children (COR = 3.43, 95%CI:1.40–8.40, \( P = 0.007 \)) was found to be associated with high seropositivity for \textit{Brucella} infection. On the other hand, age 45 and above was found to be associated with a low risk for \textit{Brucella} infection (COR = 0.22, 95%CI: 0.06–0.75, \( P = 0.015 \)). In multivariable logistic regression analysis,
agropastoralism by occupation was associated with a high risk (AOR = 9.51, 95% CI: 2.30–39.34, P = 0.002) for Brucella infection. None of the 68 samples which were negative by RBPT was found positive by CFT.

The sero-prevalence of Brucella infection was not significantly associated with clinical symptoms reported by the study participants (Table 6).

Prevalence of *P. falciparum* infection

Of the 650 suspected individuals for malaria, 16 (2.5%) were found positive for *P. falciparum* malaria infection microscopically, and *P. falciparum* was the only species detected.

*P. falciparum* malaria cases were more common among males than among females (4.4% vs 1.3%, $X^2 = 6.14, p = 0.013$). The case was also high in the age group between 2–14 years (8.8%, $X^2 = 25.13, p < 0.001$) and among individuals with body temperature $\geq 37.5°C$ (18.8% vs 1.6%, $X^2 = 35.80, p < 0.001$). It was also high among individuals felt the illness for a week or less (4.4%, $X^2 = 6.59, p = 0.037$). Multivariable regression analysis showed that being a male (AOR = 4.47, 95% CI:1.24–16.14, P = 0.022) and having fever $\geq 37.5 °C$ (AOR = 9.17, 95% CI: 1.96–42.84, P = 0.005) were independently associated with increased odds of having *P. falciparum* malaria infection (Table 7).

Concurrent infections

Among the total 650 study participants who were tested for *S. Typhi, Rickettsial, Brucella and/or Plasmodium* infections, 344 (52.9%) were found to be positive for one or more of the infectious agents by the screening tests (Widal and Weilfelix direct card agglutination, Rose Bengal Plate Test and blood films). However, only 24.6% (160/650) were found to be positive for one or more of the infectious agents by the confirmatory tests (titration test for *S. Typhi and Rickettsia* infections, and Complement Fixation Test for *Brucella* infection).

---

Table 5. Socio-demographic characteristics of the study participants and prevalence of brucellosis.

| Characters         | Number tested | Number (%) positive by CFT | AOR (95% CI) | P value |
|--------------------|---------------|----------------------------|--------------|---------|
| Sex                |               |                            |              |         |
| Male               | 247           | 11 (4.5)                   | 1            |         |
| Female             | 383           | 17 (4.4)                   | 0.70(0.25–1.99) | 0.509   |
| Age (years)        |               |                            |              |         |
| 2–14               | 104           | 9 (8.7)                    | 1            |         |
| 15–24              | 58            | 4 (6.9)                    | 0.25(0.03–2.07) | 0.197   |
| 25–34              | 144           | 5 (3.5)                    | 0.29(0.02–3.43) | 0.323   |
| 35–44              | 117           | 5 (4.3)                    | 0.28(0.02–3.21) | 0.310   |
| $\geq$45           | 207           | 5 (2.4)                    | 0.09(0.01–1.06) | 0.056   |
| Education status   |               |                            |              |         |
| Illiterate         | 479           | 18 (3.8)                   | 1            |         |
| 1–12 grade attended| 59            | 3 (5.1)                    | 0.38(0.06–2.45) | 0.312   |
| Childern           | 92            | 7 (7.6)                    | 0.23(0.03–1.63) | 0.141   |
| Occupation         |               |                            |              |         |
| Pastoral           | 497           | 18 (3.6)                   | 1            |         |
| agro pastoral      | 67            | 6 (8.9)                    | 9.51(2.30–39.34) | 0.002   |
| Others             | 66            | 4 (6.1)                    | 3.43(0.55–21.50) | 0.189   |
| Marital status     |               |                            |              |         |
| Married            | 464           | 14 (3.0)                   | 1            |         |
| Children           | 123           | 12 (9.8)                   | 4.15(0.62–27.66) | 0.141   |
| Other              | 43            | 2 (4.7)                    | 2.63(0.46–4.88) | 0.275   |
| Drinking raw milk  |               |                            |              |         |
| No                 | 58            | 1 (1.7)                    | 1            |         |
| Yes                | 569           | 27 (4.7)                   | 3.51(0.30–40.61) | 0.315   |
| Touching aborted materials |   |                            |              |         |
| No                 | 61            | 3 (4.9)                    | 1            |         |
| Yes                | 566           | 25 (4.4)                   | 1.87 (0.28–12.26) | 0.514   |

https://doi.org/10.1371/journal.pntd.0006749.t005
Table 6. Sero-prevalence of *Brucella* infection and clinical features reported by the study participants.

| Clinical Features | Test, RBPT | Test, CFT |
|-------------------|------------|-----------|
|                   | Number tested | Number positive(%) | Number tested | Number positive(%) | P value |
| Body temperature |              | | | | |
| <37.5˚c           | 545         | 65(11.9)   | 126          | 22(17.5)          | 0.960 |
| ≥37.5˚c           | 26          | 5(19.2)    | 6            | 1(16.7)           |       |
| Headache          |              | | | | |
| No                | 155         | 15(9.7)    | 36           | 5(13.9)           | 0.376 |
| Yes               | 473         | 65(13.7)   | 112          | 23(20.5)          |       |
| Back pain         |              | | | | |
| No                | 487         | 62(12.7)   | 114          | 24(21.1)          | 0.225 |
| Yes               | 141         | 18(12.8)   | 34           | 4(11.8)           |       |
| Malaise           |              | | | | |
| No                | 469         | 57(12.2)   | 109          | 20(18.4)          | 0.767 |
| Yes               | 160         | 23(14.4)   | 39           | 8(20.5)           |       |
| Joint pain        |              | | | | |
| No                | 145         | 22(15.2)   | 37           | 8(21.6)           | 0.628 |
| Yes               | 483         | 58(12.0)   | 111          | 20(18.0)          |       |
| Weakness          |              | | | | |
| No                | 539         | 65(12.1)   | 129          | 25(19.4)          | 0.709 |
| Yes               | 90          | 15(16.7)   | 19           | 3(15.8)           |       |
| Onset of the illness |            | | | | |
| <8 days           | 239         | 35(14.6)   | 63           | 13(20.6)          | 0.711 |
| 9–30 days         | 158         | 19(12.0)   | 35           | 5(14.3)           |       |
| >30 days          | 223         | 24(10.8)   | 48           | 8(16.7)           |       |

https://doi.org/10.1371/journal.pntd.0006749.t006

Table 7. Socio demographic of the study participants and prevalence of *P. falciparum* infection among the study participants.

| Variables                          | Number diagnosed | Number (%) positive | AOR (95% CI) | P value |
|------------------------------------|------------------|---------------------|-------------|--------|
| Sex                                |                  |                     |             |        |
| Female                             | 397              | 5 (1.3)             | 1           |        |
| Male                               | 253              | 11(4.4)             | 4.47 (1.24–16.14) | 0.022 |
| Age (years)                        |                  |                     |             |        |
| 2–14                               | 114              | 10 (8.8)            | 1           |        |
| 15–24                              | 59               | 2 (3.4)             | 0.04 (0.04–7.74) | 0.649 |
| 25–34                              | 146              | 0 (0.0)             | Empty       |        |
| 35–44                              | 120              | 2 (1.7)             | 0.63 (0.03–11.67) | 0.755 |
| ≥45                                | 211              | 2 (1.0)             | 0.25 (0.01–5.41) | 0.377 |
| Occupation                         |                  |                     |             |        |
| Pastoralist                        | 514              | 13 (2.5)            | 1           |        |
| Agro pastoral                      | 67               | 1 (1.5)             | 4.76 (0.38–16.15) | 0.227 |
| Others                             | 69               | 2 (2.9)             | 1.98 (0.28–14.24) | 0.496 |
| Education                          |                  |                     |             |        |
| Illiterate                         | 489              | 6 (1.2)             | 1           |        |
| 1–12 grade attended                | 61               | 2 (3.3)             | 0.40 (0.04–4.23) | 0.444 |
| Children                           | 100              | 8 (8.0)             | 1.71 (0.27–10.74) | 0.569 |
| Marital status                     |                  |                     |             |        |
| Married                            | 474              | 4 (0.8)             | 1           |        |
| Children                           | 133              | 11(8.3)             | 2.34(0.21–25.84) | 0.488 |
| Other                              | 43               | 1(2.3)              | 1.68 (0.12–24.16) | 0.700 |
| Body temperature                   |                  |                     |             |        |
| <37.5˚c                            | 557              | 9 (1.6)             | 1           |        |
| ≥37.5˚c                            | 32               | 6 (18.8)            | 9.17 (1.96–42.84) | 0.005 |
| Headache                           |                  |                     |             |        |
| No                                 | 160              | 2 (1.3)             | 1           |        |
| Yes                                | 486              | 14 (2.9)            | 3.64 (0.64–20.80) | 0.146 |
| Joint pain                         |                  |                     |             |        |
| No                                 | 160              | 7 (4.4)             | 1           |        |
| Yes                                | 486              | 9 (1.9)             | 2.04 (0.44–9.40) | 0.360 |
| Onset of the illness               |                  |                     |             |        |
| <8 days                            | 249              | 11(4.4)             | 1           |        |
| 9–30 days                          | 161              | 1(0.6)              | 0.21(0.02–1.92) | 0.167 |
| >30 days                           | 228              | 4 (1.8)             | 2.25 (0.47–10.80) | 0.312 |

https://doi.org/10.1371/journal.pntd.0006749.t007
Discussion

We investigated the prevalence of typhoid fever, typhus, brucellosis and malaria among individuals reported signs of fever, headache, joint pain and back pain in Amibara district, Afar Region, Ethiopia, through a community-based cross-sectional study. A quarter of the individuals (24.6%) were sero-positive for *S*. *Typhi*, *Rickettsia*, *Brucella* infection by confirmatory tests, and/or positive for *P. falciparum* infection by microscopy. This result is in line with previous health facility based studies in other parts of Ethiopia on causes of febrile illnesses [18, 19], and the highest disease prevalence was found for typhus (14.0%) followed by typhoid fever (7.3%).

However, the sero-prevalence of *Rickettsia* infection in this study was lower than the one reported from other parts of Ethiopia [19, 28], but higher than the findings of the studies by Tadesse and Tadesse [18] and Birhane et al. [29]. The variation might be linked to the type of environment in the study area. Studies also showed that the occurrence of typhus in Ethiopia is linked to poor hygienic/crowded living condition, where it can cause high mortality rates [30]. The sero-prevalence of *Rickettsia* infection was high among individuals reported headache. Headache has been shown to be one of the main clinical symptoms of endemic typhus [31]. The study also showed that the seroprevalence for *Rickettsia* infection was more common among females than among males. This high sero-prevalence for *Rickettsia* infection among females could be due to the large number of female study participants involved in this study. A previous retrospective sero-prevalence study of typhus among prisoners in Ethiopia also showed a higher seroprevalence among males than among females which could be due to a higher proportion of male study participants (86%) compared to that of female study participants (14%) [28]. However, further well designed community-based study is needed to investigate the reason including differences in treatment-seeking behaviour among adult females and males in the present pastoral area.

Nevertheless, our study showed that typhus is one of the major public health concerns in the study area. Hence, emphasis should be given to appropriate diagnosis/treatment and prevention of *Rickettsia* infection like through increasing community’s awareness in the present study area.

The second most common disease found was typhoid fever (7.3%). Our result is comparable with other health facility based studies in different parts of Ethiopia [18, 24]. The observed sero-prevalence is lower than the results of health facility based studies in Ziway area [19] and Northwest Ethiopia [29]. Various factors could explain these differences: seasons, environmental hygiene, geographical location and the nature of the study population [32]. The study also showed that the seroprevalence for *S*. *Typhi* infection was slightly high among females which is similar to the results of other previous study in Ethiopia [18]. In the present study, the proportion (61%) of female participants was higher than the proportion (39%) of male participants, and this might contribute to the observed high sero prevalence for *S*. *Typhi* infection among females. However, a previous health facility-based study in Ethiopia revealed a slightly higher seroprevalence for *S*. *Typhi* infection among female study participants (22.5%) compared to that of males (16.7%) despite a higher proportion of male participants (60%) compared to that of females (40%) [29]. In present study area, pastoralists share stagnant and open natural water sources with their livestock, which increases the risk for getting *S*. *Typhi* infection and favors the spread of the disease. Moreover, the high seroprevalence for *S*. *Typhi* infection among females could be associated with the daily living habits of females like frequency of exposure to contaminated water during fetching water from river and wells or washing clothes as most of these activities are usually performed by females.

In addition, health facilities in the study area had limited laboratory facilities to accurately diagnosis and treat the disease. Thus, increasing access to safe water, strengthening health
facility/system for the diagnosis of typhoid fever and treatment as well as increasing community awareness are very important in order to reduce the mortality and morbidity due to this disease.

Several studies have shown the occurrence of brucellosis in livestock in different parts of Ethiopia [33–35]. However, there was no health facilities/community based information on the status of brucellosis in humans in the present study area. The overall sero-prevalence of brucellosis in the study participants was 4.4% by RBPT and CFT. The result is higher than the finding of health facilities based previous studies in individuals with febrile illness in other part of Ethiopia [19,36], but lower than the results of health facility based study from Borena area, South Ethiopia and Metema area, north Ethiopia [17]. Another recent study from Jimma area (south Ethiopia) also revealed a low sero-prevalence of Brucella infection as detected by RBPT (2.1%) and CFT (0.0%) [37].

Brucellosis has an overlap of clinical symptoms with many other febrile diseases, and can be misdiagnosed with malaria or other diseases due to lack of awareness of medical staff and lack of diagnostic capabilities in the present study area. In this study, 4.4% of symptomatic study participants who were found sero-positive for Brucella infection, would not have been diagnosed for brucellosis if the physician would have based the diagnosis solely on clinical signs. Therefore, efforts need to be made to improving laboratory services for the diagnosis of brucellosis in the present study area.

In the present study, significant difference was not found in the prevalence of brucellosis between males and females, which is similar with other studies done in Ethiopia [36], Tanzania [38] and Kenya [39]. However, a hospital based study in Uganda showed a higher sero-prevalence of Brucella infection among males than in females [40]. This can be explained by the fact that among pastoralists, both women and men are equally exposed to risk factors for Brucella infection. Unlike results from Uganda [40] and Bangladesh [41], where elders were more affected by brucellosis, our study showed relatively high prevalence of Brucella infection in the younger age group and children. Children can be exposed to Brucella infection by regularly drinking raw milk, contaminated soil with the bacteria and having regular close contact with livestock, particularly goat and sheep [35].

This study, was undertaken during a high malaria season though it showed a low prevalence of undiagnosed and untreated malaria (2.5%) among the study participants compared to results of previous community-based prevalence study of malaria among non-febrile individuals in Gondar town, North Ethiopia [42], and in the pastoral community of the Bena-Tsemay district, South Ethiopia [43]. Although this low prevalence of malaria might be due to the result of the prevention and control measures employed by the Ministry of Health to eliminate malaria in the country [44], the present observed prevalence of undiagnosed and untreated malaria cases should not be considered insignificant since these undiagnosed and untreated individuals would contribute to the transmission of the disease among the community. Moreover, in the present study area, P. falciparum which causes the most severe form of malaria is the widely distributed species as previously reported [45]. Hence, strengthening community based malaria case detection in this area, for example through community health extension workers is very important in order to achieve the plan for malaria elimination.

In this study, among other clinical features, body temperature ≥ 37.5 °C was found to be strong indicator for infection with falciparum malaria. Previous studies also suggested that increased body temperature could be helpful in diagnosing and treating children with febrile illness [46, 47].

In Ethiopia, serological tests (Widal and Weil-Felix) using the DCAT are commonly used to diagnose typhoid fever and typhus. A number of subsequent studies indicated that these
tests are extremely valuable in the absence of adequate laboratory facilities and culture methods like in resources limited countries [48–51].

On the other hand, several previous studies have shown a high seroprevalence of S. Typhi infection using serological based screened tests, but revealed absence or very low prevalence using blood culture or fecal samples which are considered as gold standard tests for the diagnosis of a current infection with S. Typhi [24, 29, 52, 53]. Bacteriological isolation is the gold standard for the diagnosis of current infection with Brucella. RBPT was found to be simple and useful for the screening for Brucella infection in health institutions where bacterial culturing facilities are not available [25], though it is not a useful test to distinguish between acute and chronic Brucella infection [54]. Whereas, CFT can be used as a confirmatory test.

In this study, we have used Widal, Weil-Felix, and RBPT/CFT tests to report the seroprevalence for S.Typhi, Rickettsia and Brucella infections, respectively despite the fact that these serological based tests are not convincing tests for the diagnosis of current infection because of short comings such as false positivity due to previous exposure or false negativity in an endemic setting [24], and this could be one of the major limitations of the present study.

The study participants were recruited based on the clinical signs/symptoms reported by the study participants that may not necessarily have been caused by an infectious agents that cause acute or chronic illness and this might result in a selection bias. The primary objective of this study was to identify the prevalence of brucellosis, malaria, typhoid and typhus among symptomatic individuals with febrile illness related symptoms. However, in an endemic area, these diseases could be prevalent among asymptomatic individuals. Hence, the findings of this study cannot be generalized to the entire population in the study area.

In pastoralists like in the present study area who are living in close contact with their animals on a daily basis, many neglected zoonotic diseases such as campylobacteriosis, Q fever, and leptospirosis can cause a significant health problems both in humans and animals [55]. In the present study, among 650 individuals who complained various illnesses, only 160 (24.6%) were diagnosed for one or more of the above mentioned diseases, and in the majority (75.4%) of the symptomatic individuals the cause of their illness remained unknown, because diagnosis of other related diseases was not considered due to lack of diagnostic tools/reagents and this could also be considered as one of the limitations of this study.

**Conclusion**

In this study, typhoid fever, typhus, brucellosis and malaria were observed among symptomatic individuals. The study also highlighted that brucellosis cases can be misdiagnosed as malaria or other disease based solely on clinical diagnosis. Therefore, efforts are needed to improve disease awareness and laboratory services for the diagnosis of brucellosis, which should be considered in the routine differential clinical diagnosis of febrile illness in the study area. Only a quarter of the study participants (24.6%) were diagnosed for one or more of the above mentioned diseases. In the majority (75.4%) of the symptomatic individuals, the cause of their illness remained unknown. In addition, a high prevalence of unexplained abortions in women (35.7%) was observed. Hence, further community based studies on other zoonotic diseases like leptospirosis and Q fever are warranted to identify other causes of febrile illness in this pastoral setting.

**Supporting information**

S1 Checklist. STROBE checklist.

(DOC)
Acknowledgments
We are grateful to study participants, data collectors and Amibara district Health Bureau and Community Leaders.

Author Contributions
Conceptualization: Biruk Zerfu, Girmay Medhin, Gezahegne Mamo, Gezahegn Getahun, Mengistu Legesse.

Formal analysis: Biruk Zerfu, Girmay Medhin, Rea Tschopp, Mengistu Legesse.

Writing – original draft: Biruk Zerfu, Mengistu Legesse.

Writing – review & editing: Biruk Zerfu, Girmay Medhin, Gezahegne Mamo, Gezahegn Getahun, Rea Tschopp, Mengistu Legesse.

References
1. Kasper MR, Blair PJ, Touch S, Sokhal B, Yasuda CY, Williams M, et al. Infectious Etiologies of Acute Febrile Illness among Patients Seeking Health Care in South-Central Cambodia. Am J Trop Med Hyg. 2012; 86: 246–253. https://doi.org/10.4269/ajtmh.2012.11-0409 PMID: 22302857
2. Tam PYI, Obaro SK, Storch G. Challenges in the Etiology and Diagnosis of Acute Febrile Illness in Children in Low- and Middle-Income Countries. J Pediatric Infect Dis Society. 2016: 5: 190–205.
3. WHO Guidelines for the Diagnosis and Treatment of Malaria in Africa. AFRO Technical Papers. Vol. 22. Geneva: World Health Organization; 1992: pp 1–46.
4. Hertz JT, Munishi OM, Sharp JP, Reddy EA, Crump JA. Comparing actual and perceived causes of fever among community members in a low malaria transmission setting in northern Tanzania. Trop Med Internat Health 2013; 18: 1406–1415.
5. Prasad N, Murdoch DR, Reyburn H, Crump JA. Etiology of Severe Febrile Illness in Low and Middle-Income Countries: A Systematic Review. PLoS ONE 2015; 10: e0127962. https://doi.org/10.1371/journal.pone.0127962 PMID: 25621200
6. Iheukwumere I, Nwachukwu, Chuks N, Kanu, Mercy A. Manifestations, Mismanagement and Diagnostic Challenges of Malaria and Typhoid Fever. Malar Chemoth Cont Elimination, 2013; 2: 109.
7. Mogasale V, Maskery B, Ochial RL, Lee JS, Mogasale VV, Ramani E, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health 2014; 2: e570–80. https://doi.org/10.1016/S2214-109X(14)70301-8 PMID: 25304633
8. Eng SK, Pusparajah P, Matalib NSA, Ser HL, Chan KG Lee LH. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 2015; 8: 284–293.
9. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev 2015; 28: 901–936. https://doi.org/10.1128/CMR.00022-15 PMID: 26180063
10. WHO 2006. The Control of Neglected Zoonotic Diseases. A route to poverty alleviation Report of a Joint WHO/DFID-AHP Meeting with the participation of FAO and OIE Geneva, 20 and 21 September 2005.
11. Ducrotay MJ, Bertu WJ, Ocholi RA, Gusi AM, Bryssinckx W, Welburn S, Moriyon I. Brucellosis as an emerging threat in developing economies: lessons from Nigeria. PLoS Negl Trop Dis 2014; 8(7):e3008. https://doi.org/10.1371/journal.pntd.0003008 PMID: 25058178
12. Elshamy M, Ahmed AI. The effects of maternal brucellosis on pregnancy outcome. J Infect Dev Ctries 2008; 2(3):230–4. PMID: 19738356
13. Dondev V, Karadzovski Z, Kaspinov B, Lazarevik V. Epidemiological and public health aspects of brucellosis in the Republic of Macedonia. Biolog Med Sci 2010; 1: 133–54.
14. Rujeni N, Mbanzamihigo L. Prevalence of Brucellosis among Women Presenting with Abortion/Stillbirth in Huye, Rwanda. J Trop Med 2014; 10:1155.
15. Liu Q, Cao L, Zhu XO. Major emerging and re-emerging zoonoses in China: a matter of global health and socioeconomic development for 1.3 billion. Inter J Infect Dis 2014; 25:65–72.
16. WHO. World Malaria Report 2017: Summary. Geneva: World Health Organization (WHO/HTM/GMP/ 2017.4). Licence: CC BY-NC-SA3.0 IGO.
17. Regassa G, Mekonnen D, Yamuah L, Tilahun H et al. Human Brucellosis in Traditional Pastoral Communities in Ethiopia. Inter J Trop Med 2009; 4: 59–64.
18. Tadesse H, Tadesse K. The etiology of febrile illnesses among febrile patients attending Felege Selam Health Center, Northwest Ethiopia. Amer J Biomed Life Sci 2013; 1: 58–63.
19. Feleke SM, Animut A, Belay M. Prevalence of Malaria among Acute Febrile Patients Clinically Suspected of Having Malaria in the Zeway Health Center, Ethiopia. Jpn J Infect Dis 2015; 68, 55–59. https://doi.org/10.7883/yoken.JJID.2013.062 PMID: 25420658
20. Legesse M, Ameni G, Mamo G, Medhin G, Shawel D, Bjune G, Abebe F. Knowledge and perception of pulmonary tuberculosis in pastoral communities in the middle and Lower Awash Valley of Afar region, Ethiopia. BMC Public Health. 2010; 10:187. https://doi.org/10.1186/1471-2458-10-187 PMID: 20380747
21. Mamo G, Abebe F, Worku Y, Hussein N, Legesse M, Tilahun G, Medhin G, Bjune G, Ameni G. Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Ethiopia. J Vet Med Ani Health. 2013; 5(6):171–179.
22. Shanthi J, Usha Rani R, Balagurunathan R. A brief study of diagnosis and frequency of typhoid fever incidence by Widal test. Annals Biologi Reseach 2012; 3 (4):1847–1851.
23. Danave D, Kothadia SN. Role of Weil Felix Test for Rickettsial Infections. IOSR-JDMS, 2015; 14: 52–54
24. Wasihun AG, Wlekidan LN, Gebremariam SA, Welderufael AL, Muthupandian S, Halie TD et al. Diagnosis and Treatment of Typhoid Fever and Associated Prevailing Drug Resistance in Northern Ethiopia. Int J Infect Dis 2015; 35:96–102. https://doi.org/10.1016/j.ijid.2015.04.014 PMID: 25931197
25. Diaz R, Casanova A, Ariza J, Moriyón I. The Rose Bengal Test in human brucellosis: a neglected test for the diagnosis of a neglected disease. PLoS Negl Trop Dis. 2011; 5(4):e950. https://doi.org/10.1371/journal.pntd.0000950 PMID: 21526218
26. MOH. Federal Democratic Republic of Ethiopia Ministry of Health2012 National Malaria Guidelines. Addis Ababa: Ethiopia www.moh.gov.et/National/malaria/guidelines
27. Michael GDB, Nasinsky G, Benti DG. Sero-prevalence of human brucellosis community awareness and practices on its zoonotic importance in Jimma town and Chora Botor district, Ethiopia. J Z D 2016; 1:1:58–64.
28. Swai ES, Schoonman L. Human brucellosis: sero-prevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. Zoon Publ Health 2009; 56:183–7.
29. Maiyo G, Obey J.K. Distribution and prevalence of human brucellosis among patients reporting at chemundu dispensary, Nandi County, Kenya. Baraton Interdis Res J 2016; 6:73–82
40. Turnwine G, Enock M., John David K, David OO, Samuel M. Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. BMC Pub Health 2015; 15:900.

41. Rahman AK, Dirk B, Fretin D, Saegerman C, Ahmed MU, Muhammad N, et al. Seroprevalence and risk factors for brucellosis in a high-risk group of individuals in Bangladesh. Foodborne Pathog Dis 2012; 9:190–7. https://doi.org/10.1089/fpd.2011.1029 PMID: 22300225

42. Tiliaye T, Deressa W. Prevalence of urban malaria and associated factors in Gondar Town, Northwest Ethiopia. Ethiop Med J 2007; 45:151–8. PMID: 17642171

43. Debo GW, Kassa DH. Prevalence of malaria and associated factors in Benna-Tsemay district of pastoralist community, Southern Ethiopia. Trop Dis Tra Med & Vac 2016; 2:16.

44. Deribew A, Dejene T, Kebede B, Assefa GT, Melaku YA, Misanaw A. Incidence, prevalence and mortality rates of malaria in Ethiopia from 1990 to 2015: analysis of the global burden of diseases. Malar J 2017; 16:271. https://doi.org/10.1186/s12936-017-1919-4 PMID: 28676108

45. Chanie M, Erko B, Animut A, Legesse M. Performance of carestart malaria pf/pv combo tests for the diagnosis of Plasmodium falciparum and Plasmodium vivax infections in the Afar Region, north east Ethiopia. Ethiop J Health Dev 2011; 25: 206–11.

46. Ejezie GC, Ezedinachi EN. Malaria parasite density and body temperature in children under 10 years of age in Calabar, Nigeria. Trop Geogr Med 1992; 44:97–101. PMID: 1496732

47. Stauffer W, Fischer PR. Diagnosis and Treatment of Malaria in Children. Clin Infec Dis 2003; 37: 1340–1348.

48. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H, et al. Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. J Clin Microbiol. 1999; 37(9):2882–6. PMID: 10449489

49. Wilke A, Ergonul O, Bayar B. Widal test in diagnosis of typhoid fever in Turkey. Clin Vaccine Immunol 2002; 9: 936–941.

50. Azizi T, Haque SS. Role of Widal Test in the Diagnosis of Typhoid Fever in Context to Other Test. Amer J Biochemist 2012; 2(1): 16–18.

51. Chowdhury MAY, Haque MG, Karim AMMR. Value of Widal Test in the Diagnosis of Typhoid Fever. Medicine Today 2015; 27: 28–32.

52. Gupta A, My Thanh NT, Olsen SJ, Sivapalasingam S, My Trinh TT, Phuong Lan NT, et al. Evaluation of community-based serologic screening for identification of chronic Salmonella typhi carriers in Vietnam. Int J Infect Dis. 2006; 10(4):309–14. https://doi.org/10.1016/j.ijid.2005.06.005 PMID: 16412678

53. Anthony Obinna E, Maryjude Chiamaka I, Oliver Olugbue N. Co-infection of malaria and typhoid fever in a tropical community. Animal Res Intern 2008; 5(3): 888–891.

54. El-Fekhfakh EA, Hassanain NA, El-Folly RF, El-Harrir H. Assessment of Rose Bengal test in diagnosing Egyptian human brucellosis. J Egypt Soc Parasitol. 2011; 41(2):497–512. PMID: 21980786

55. WHO. Research Priorities for Zoonoses and Marginalized Infections. Technical report of the TDR Disease Reference Group on Zoonoses and Marginalized Infectious Diseases of Poverty. WHO, 2012; Technical report series; no. 971