Detection of Malaria Parasites in donated blood in Zimbabwe using the rapid SD Bioline test kit.

Danai Samakande[a], Arthur Mandisodza[a], Vasco Chikwasha[b], Rooyen Mavenyengwa[c], George Mavunganidze[d], Judith Parirewa[d]

a. Departments of Medical Laboratory Sciences, College of Health Sciences, University of Zimbabwe.
b. Department of Community Medicine, College of Health Sciences, University of Zimbabwe.
c. Department of Medical Microbiology, College of Health Sciences, University of Zimbabwe.
d. National Blood Service Zimbabwe

Correspondences: Prof Arthur Mandisodza, Department of Medical Laboratory Sciences, University of Zimbabwe College of Health Sciences,
PO Box A178, Avondale, HARARE. ZIMBABWE
Email: amandisodza@yahoo.com
Phone: +263 4 791631: Extn- 2397/2295
Mobile: +263 772 249 569

Abstract

The potential of transfusion transmitted malaria infection in Zimbabwe is high because blood donors are not screened for malaria infection. The study was carried out to assess possible malaria infection in blood donors from malaria endemic regions using the rapid and routine microscopy methods. Experts repeated the microscopy method on samples that were positive with the rapid SD Bioline immunochromatography method.

All blood donors were negative for malaria using the conventional thin and thick blood film methods. Eight (4.5%) blood donors were positive using the SD Bioline immunochromatography method. Repeats of the microscopy tests on eight positive SD Bioline results by experts were negative. Bulawayo, Zimbabwe's second largest city, had the highest malaria positive donor blood. There was an association between malaria infection and Rh D positive blood donors.

It can be concluded that screening blood donors for malaria using more sensitive techniques is necessary to avoid transfusion-transmitted malaria infection.

Keywords: ***blood donors, blood film, endemic, immunochrmatography, malaria, rapid test, sensitive, transfusion-transmitted***

1. Introduction

Malaria infection remains one of the major causes of death in the world despite efforts to control it at both national and international levels. Transmission of malaria parasites (sporozoites) is often through bites by the female mosquitoes of species

Anopheles during their mammalian blood meal. Malaria parasites of Plasmodium species include *P. falciparum, P. malariae, P. ovale* and *P. vivax.* After multiplication in the liver, the parasites may invade the red blood cells where they can survive as free *merozoites, gametocytes* and *ring forms* for days or weeks, even at refrigeration temperatures. This makes them transmissible through blood products. Malaria is very prevalent in Sub Saharan Africa. But, people from the malaria endemic areas of the region are slightly protected against malaria infection because their chronic exposure to the parasites has left them semi-immunized. This has made them asymptomatic carriers of malaria parasites (*Kinde-Gazard, 2000; Slinger et al., 2001; Basu and Kaur, 2005; Kitchen Chiodini., 2006; Uneke et al., 2006*).

Transfusion transmissible infections involving all the human Plasmodium species was first reported in the early twentieth century. Studies in many countries in Sub Saharan Africa and Asia have indicated that malaria is transmissible by transfusion. Due to the endemicity, many apparently healthy asymptomatic blood donors may potentially transmit malaria through transfusion (*Dodd, 2007; Lara, 2007; Hoque et al., 2008; Marcel and de Savigny, 2008*).

In Zimbabwe, the *P. falciparum* malaria parasite is responsible for most of the malaria infections. With the exception of malaria, all transfusion transmissible infectious (TTIs) agents such as immunodeficiency virus (HIV), Hepatitis C and B viruses, Cytomegalovirus (CMV) and Syphilis are tested in donated blood. Malaria is only screened through a counseling process which involves asking prospective donors whether they had contracted malaria in the past three weeks before donation or whether they have visited a malaria endemic area lately. This method may be very limited because it is affected by factors such as prospective donor behavior and his or her level of education. In Zimbabwe, a curative approach, in the absence of malaria testing, involves treatment with antimalarial drugs if a patient develops fever soon after transfusion.

In Sub Saharan Africa, malaria is mostly tested by a microscopy methods. Given the asymptomatic nature of donors from malaria endemic areas and the low parasite concentration, these methods may be limited in sensitivity and accuracy. They are also laborious, dependent on highly skilled personnel and not practical for large-scale donor screening procedures (*Rosenberg, 1990; Mungai et al., 2001; Chitiyo, 2012*).

This study was carried out to detect malaria parasites in donated blood using the conventional microscopy and the World Health Organization approved rapid SD Bioline testing kit. The SD Bioline test has been found to still detect malaria antigens 28 days after the patient has fully recovered (*Marcel and de Savigny, 2008*).

2.0 Materials and Methods

2.1 Ethical Approval

Permission to carry out this prospective cross sectional study was granted by the Joint Research and Ethics Committee (JREC-159/10) and the National Blood Service Zimbabwe (NBSZ). Blood donor anonymity by use of bar coding system and good laboratory practice were maintained.

2.2 Sample Collection

One hundred and seventy-six (176) ethylene diamine tetra-acetic acid (EDTA) blood samples (calculated sample size = 85) were collected from asymptomatic blood donors from known malaria endemic areas of the country and were sent to the five NBSZ collection centres (Bulawayo, Harare, Gweru, Masvingo and Mutare) during a seven months period, from November 2010 to May 2011. The blood samples were immediately transported to the NBSZ headquarters in Harare in cooler boxes for malaria testing. Bar codes were used to identify blood samples.

2.3 Testing and Statistical Analysis

The blood samples were tested for malaria infection using both microscopic method and rapid SD Bioline Malaria Ag *P.f/P.v Pan* kit method. The rapid test kit was supplied by Standard Diagnostics, Inc. (156-68, Hagal-dong Korea 446-930). The microscopy test is based on the examination of both thin and thick blood films for the presence of malaria parasites, specifically ring forms for *P. falciparum* identification, after staining with Giemsa. The SD Bioline Antigen test is based on the principle of immunochromatography. The test line is precoated with monoclonal antibodies directed against HRP-2 (Histidine rich protein 2) which is specific to *P. falciparum*. Known positive and negative samples were used as controls.

The *Microsoft excel* was used to capture data and statistical analysis was done using the *STATA 10.1* statistical package.

3.0 Results

There were 90 (51.1%) female and 86 (49.9%) male blood donors between the ages of 16 - 63 years. No malaria parasites were detected on both thin and thick blood films. The prevalence of malaria in donated blood was 8 (4.5%) using the SD Bioline immunochromatography assay whereas it was 0(0%) by the standard microscopy method. Double checking by repeating microscopic testing by an expert on all the 8 positive SD Bioline results produced negative results. The sensitivity of the Bioline was 0% while the specificity was 95.5%. Five (5.8%) and 3 (3.3%) of males and females were positive respectively. Bulawayo had the highest malaria positive blood donors 3(1.7%). All the 8 (100%) SD Bioline positive blood donors were Rhesus D antigen positive.

Table I*: Cross checking of SD Bioline results with Microscopy*

		Microscopy		
		Positive	Negative	Total
SD Bioline Rapid Test	Positive	**0**	**8**	**8**
	Negative	0	168	168
	Total	**0**	**176**	**176**

4.0 Discussion

The transmission of malaria parasites through donated blood is real in Sub Saharan countries as long as blood is not tested for malaria before transfusion (*Basu and Kaur. 2005; Kitchen, 2006; Uneke et al., 2006*). A single infected unit of blood may transmit malaria to a minimum of 4 patients who receive different blood products.

The absence of malaria parasites using the microscopy method in our study could have been associated with its lack of sensitivity and accuracy. The semi-immunity and low concentration of the parasites in the red cells of donors from malaria endemic areas could be the reason why the microscopy method was negative (*Rosberg,1990;Mungai et al., 2001;Chitiyo,2011*). Although the application of the SD Bioline immunochromatographic assay enabled detection of malaria parasites, its sensitivity was very poor. It has been found that the test can cause false positives due to cross reactivity with Rheumatoid factor (*Garba*

et al., 2016). However, due to its good specificity, it may be quite useful in diagnosis of malaria as it is based on identification of HRP II which is specific for P. falciparum malaria parasite (*Lqbal, et al., 2000; Garba et al., 2016*). However, the immunochromatographic assay may be affected by cross reactivity with Rheumatoid factors resulting in false positive (*Lqbal, et al., 2000*). A recent Nigerian study has shown that the rapid diagnostic test correlated very well with microscopy test although its sensitivity is lower than the recommended WHO value >95% (*Garba et al., 2016*). This confirms the lack of accuracy of both methods in our Zimbabwean setting, maybe, due to poor technical skills.

Bulawayo processes blood from south western, west and northwestern regions of the country where malaria is very endemic. This could be the reason why there were more donors who tested positive.

The association of Rh D antigen and *P. falciparum* infection was not clearly understood. Recent studies have shown that more than 88% of people infected by malaria were Rhesus D positive individuals (*Ayele et al., 2014*). The interaction of blood group antigens such as Gerbich, MNSsU and Rhesus systems is generating research interests around the globe (*Rowe et al., 2004*). It is also interesting to note that antigens to these systems are largely protein in nature (Walker et al., 1990). The above observations seem to support the belief that red blood cell antigens have a protective role against disease burdens. Individuals who are Duffy positive (Fy a+b+) have been found to be more susceptible to *P. vivax* infection than their Duffy negative (Fy a-b-) counterparts while the five principle Rhesus antigens (D, C, c, E, and e) were associated with increased susceptibility to *P. falciparum* infection (*Walker et al., 1990; Montoya et al., 1994*). It has been suggested that the role of some blood group antigens is a result of genetic polymorphism to resist infection by allowing parasites to attach to antigenic co-receptors. As they sit on the red cell membrane, they are recognized by the spleen and are destroyed by the organ in the process (Saul, 1999).

5.0 Conclusion

It can be concluded that malaria can be detected using rapid SD Bioline immunochromatographic method because it is specific, reliable, easy to use, and simple to interpret and has a short turnaround time. However, its usefulness can be compromised by false positives associated with cross reactions. Failure by the microscopic methods to pick a single positive from malaria endemic areas of the country questions the reliability of

laboratory skills of those tasked to screen for malaria in blood donors. The county has suffered acute shortage skilled technical staff due to skills flight caused by the current economic climate.

Nevertheless, efforts should be made to test for malaria parasites in donor before it is transfused to prevent transfusion transmitted malaria infection. Theoretically, a single blood donation has the potential to infect at least four recipients. It is not enough to rely on counseling prospective blood donor to decide whether the donor should be accepted or deferred or accepted from donating blood. "*Prevention is always better than curing*".

6.0 References

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