

Incidence of *Babesia* infections causing pyrexia of unknown origin (PUO) amongst HIV/AIDS patients in Cameroon

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Abstract

Diagnosis and treatment of pyrexia of unknown origin (PUO) in HIV/AIDS patients in Cameroon hospitals is poor. In this study, HIV/AIDS patients on antiretroviral therapy but still presenting with pyrexia of unknown origin after undergoing treatment for malaria and typhoid were analyzed for babesiosis. An enzyme linked immunosorbent assay [Elisa] kit was employed to detect antibodies to *Babesia microti* and *Babesia bovis*. Out of 155 patients who consulted at Phytobiotechnology Research Foundation Clinics (PRF) in Bamenda and Bachua-Mamfe between June 2012 and December 2013, 60 patients presented with pyrexia of unknown origin. Clinical examination indicated that they had not defaulted on highly active antiretroviral therapy (HAART), while serological test and stool culture proved negative for *Salmonella typhi*. The patients were noted to have previously been on antibiotic medication and with an average CD4 count in the range of 200 to 600 cells per mm³. The patients were noted to have been on antibiotics including septrim, clotrimazole (bactrim) amoxicillin as well as pyrimethamine sulphadoxine and artemisinin based antimalarials (ACTs). Malarial tests using blood stained films in other health centers where the patients had consulted, suggested traceable positive malarial film while rapid diagnostic tests (RDTs) were recorded negative. Out of these 60 positive patients with PUO, 20 patients tested positive for *Babesia microti* and 28 for *B. bovis*. All patients with reported traceable malarial film (Malaria +) from the other hospitals and medical centres observed positive for Babesia infections.

No significant difference ($p < 0.05$) was observed between blood film of patients testing positive for traceable malarial or malarial 1+ with the positive serological test for Babesia antibodies for the same specimens. 50% of the patients responded that they were living or exposed to rodents, and birds and other animals at one point in their lives. It is concluded that Babesiosis constitutes one of the causes of pyrexia of unknown origin in HIV/AIDS patients and potential opportunistic zoonotic infection amongst HIV/AIDS patients and misdiagnosed as malaria in Cameroon.

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Introduction

Babesiosis which has not been previously reported in Cameroon is an infection caused by an intraerythrocytic parasite of the protozoa genus *Babesia*. Klaus et al (2002) reported that *Babesia* is one of the more common diseases of free living animals worldwide and an emerging tick-borne zoonotic infection in humans. Historically, the first described *Babesia* epidemic was reported in cattle with high mortality dates back to the biblical text of Exodus (Homer et al, 2000). The first human case of babesiosis was reported in 1957 in Yugoslavia.

Since the early 1950s, two species of the *Babesia*, the cattle species *Babesia divergens* and the rodent species, *Babesia microti* has been reported to cause a significant number of infections in humans in Europe and North America respectively. Ganstraow (1997) and Kjentrup and Conrad (2000). The parasites are named after the Romanian scientist Victor Babes, who in 1888 first identified Pear-shaped plasmodium-like parasites as a cause of febrile hemoglobinuria in cattle (Babes, 1888).

Levine in 1988 reported that more than 100 species of *Babesia* have been described globally based on intraerythrocytic stages detected in mammals. However, Klaus Peter et al (2002) noted that *Babesia* exhibits pleomorphism in different species of mammalian hosts coupled with insights from molecular systematic studies, and on account of this doubted the findings of Levine in 1988.

The strength of Levine's research is further challenged on the basis that some species of *Babesia* are less host specific and babesiosis is poorly diagnosed (Skotarczak, 2008). The Clinical manifestation of *Babesia* is unspecific and resembles that of malaria making the diagnosis of PUO challenging. The amoeba-like shape of *Babesia* has been previously classified as bacteria (Klaus –Peter et al 2002). The most common diagnosis of babesiosis involves staining blood smears with Giemsa like in the diagnosis of plasmodiasis.

Oval, pear or ring-shaped and an amoeba-like forms of parasite (trophozoites) are seen in erythrocytes just like in malaria (Plates 1 and 2). Although haemozoin are distinguished in the macrophages of blood smears with *Babesia* unlike plasmodiasis (Plates 1 and 2) most medium skilled health personnel are unaware.

In cases of low parasitaemia of both malarial and Babesiosis where infected cells are undetected clinical and empirical diagnosis remain daunting. Babesia species cannot be identified on the bases of morphology and it is difficult to distinguish between babesia and plasmodium parasites (Skotarczak, 2008)

It is for this reason that we noticed PUO in patients attending hospitals in Cameroon are poorly diagnosed. The most common subjective diagnosis is to screen for typhoid and malaria. Traceable (malaria +1) is widely reported as the etiology of PUO in most patients who have been on treatment and in some cases termed drug resistant plasmodiasis.

To add, Babesiosis is more commonly observed in elderly people and patients who have undergone one form of surgery such as splenectomy or those with immunodeficiency such as HIV/AIDS. This scenario is further exacerbated by the fact that most incidents of babesiosis remain undetected and undiagnosed especially with people with a correctly functioning immune system. Babesia piroplasms have not been well documented in Sub Saharan Africa especially in Cameroon. In this study, we report the incident of babesiosis amongst HIV/AIDS patients in Cameroon who are presenting with PUO and have been defaulted on HAART and other co-therapies.

Materials and methods

A case control study was employed. HIV/AIDS patients on anti-retroviral drugs from the designated health centers in Cameroon were considered in this study.

The patients were presenting with fevers of unknown origin and have been on a range of antibiotics including Bactrim, Amoxicillin, Maloxinine, Fansidar and Artemisin based drugs and analgesic.

PUO persisted despite being on HAART and the above therapies. The patients reported for integrative medical care at the Phytobiotechnology Research Foundation Clinic (PRF) and consented in an exit pool to be placed on phyto-nutriceuticals supplements plus plant extracts produced at PRF Clinics in Bamenda, Cameroon.

A structured open questionnaire was designed to gather clinical and anthropogenic information on the health status of each patient. The topical questions consisted of their HIV status, the last test for malaria and typhoid and the kind of animals they do live with or have been predisposed to. Each of the patients was retested for HIV using Determine and Bioline test kits following standard protocol (Yongabi et al, 2009). Three blood smears were collected according to methods of Yongabi et al (2009) and stained with Giemsa stain (Cheesbrough,1984) and

microscopically observed for trophozoites of plasmodium and Babesia species according to methods (Skotarczak, 2008)

Serum from each of the blood specimens from each patient each time they reported at PRF Clinic was tested for antibodies for *salmonella typhi* using (latex antibodies) (Cheesbrough 1984).

Each patient was assigned one of our health workers who went to collect blood specimen from patients living at home with the patients and healthy and uninfected. This was discussed freely and patients consented without coercion. Similarly, non HIV/AIDS patients attending PRF Clinics presenting with PUO were also analyzed as a control.

Serological Assay

The Elisa kit for *Babesia microti* and *Babesia bovis* antibody was used according to manufacturer's instruction (Davies diagnosis) and guidelines specified in the FAO/IAEA manual. The kit was commercially purchased. *Babesia mcroti* IFA test, a commercially available test kit (*B.microti* – IFA igm/ias Davies diagnostics) was used according to manufacturer's instructions to diagnose serum antibodies to *B. microti*.

Table I: total N° of patients diagnosed with PUO per symptoms and signs

Total N° of patients	PUO, Headache, General.malaise fevers
155	90 102 155 150
Prevalent frequent drugs taken	Bactrim, Septrim, Amoxicillin, Fansidar /maloxine

Table 2: Results for serum samples of HIV/AIDS patients and Non-patients

N° of patients	Seropositive for <i>B.microti</i>	Seropositive for <i>B.bovis</i>	Non-positive
155	25	85	5

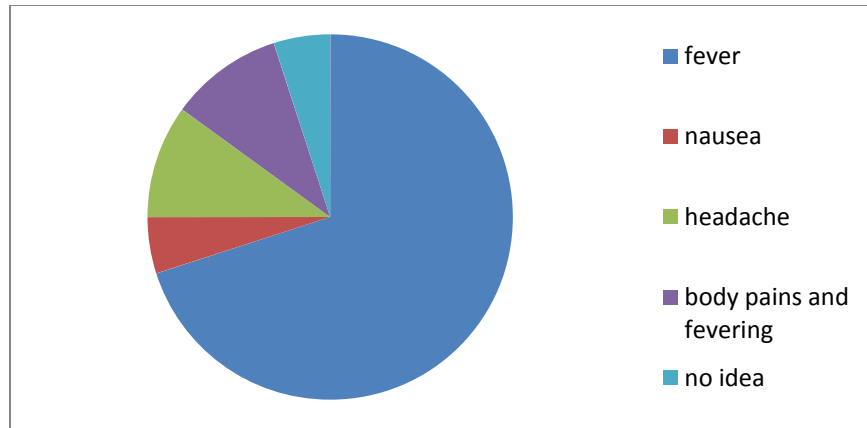


Fig 1. Respondents knowledge on the common symptoms of malaria which overlap for Babesia.

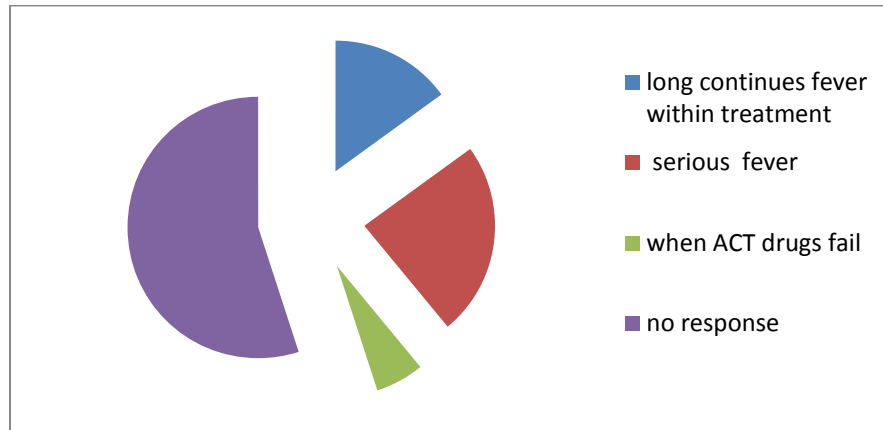


Fig 2. Definitions of resistance to Artemisin based drugs for malaria.

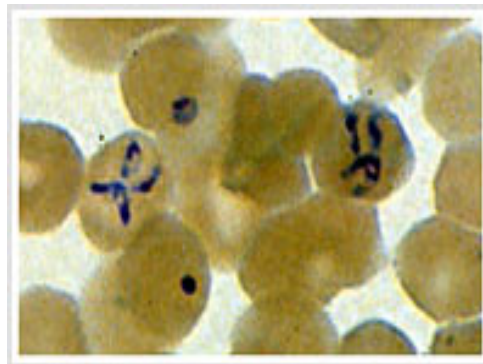


Plate 1. Trophozoites of Babesia sp in a Giemsa stained field.

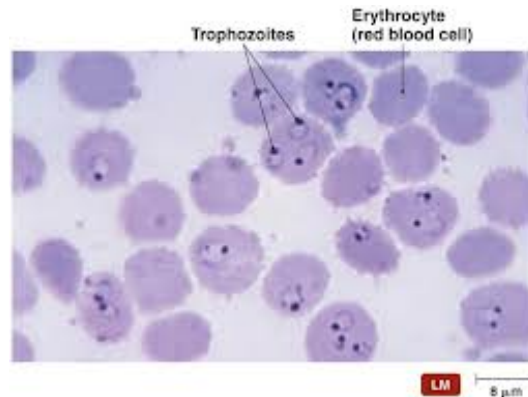


Plate 2. Trophozoites of *Plasmodium falciparum* in a Giemsa stained film.

Discussion

The serological data shows that Babesiosis could be regarded as the cause of PUO in HIV/AIDS patients in Cameroon (Table 2). The results showed that Pyrexia of unknown Origin is prevalent amongst HIV/AIDS patients in Cameroon (Table 1). Babesia infection is possibly one cause of PUO amongst HIV/AIDS in Cameroon Table 1 and 2). Babesiosis is pathogenic, invasive disease of humans and animals such as cats, dogs, and cattle of the patients were not analyzed. This serves as a limitation to the control of effectively establish a zoonotic connection. However, 90% of the patients in both Bamenda and Mamfe did acknowledge being exposed to these animals, fleas and ticks at one point it was observed that patients from a nomadic community who hail from Sabga in the North West Region of Cameroon were all positive for *B bovis* irrespective of whether they had HIV or not.

Further interviews with some of these respondents suggested they had none of the symptoms they consider as febrile illness which usually compelled them to seek hospital attention. Other group of respondents indicated the presence of these symptoms which they would normally consider right away as malaria (Figures 1 and 2). The potential of asymptomatic babesiosis cannot be ruled out. This poses a potential risk of transmission through blood especially as blood screening for transfusion do not at the moment consider screening for Babesia. *B microti* and *B bovis* have been considered as surrogate markers in a seroepidemiological study to detect antibodies in patients exposed to ticks and human control groups in mid-western Germany. The screening in this study, we checked *B microti* and *B bovis*. This strategy can be used to serological surveillance of Babesiosis in sub Saharan Africa

.Babesia prognosis and diagnosis is an uncommon routine in Cameroon hospitals. This may be the trend across Africa but in Europe, the diagnosis of human babesia has been mainly been on the detection of parasites in blood smears of patients with clinical symptoms of the diseases. *B microti* and *B bovis* have been isolated from ticks, rodents, cattle in Germany. It is likely that *B microti* and *B bovis* are found tropical ticks (*Boophilus ssp*) rodents and cattle in Cameroon. Our survey also suggests that most homes in Cameroon harbor rodents.

It is also suggested that seroepidemiological data on Babesiosis must consider interspecies reactivity of anti-genic components within the genus Babesia and possible cross reactivity with other bacteria or parasite agents. In this study, only *plasmodium falciparum* and *salmonella typhi* were tested alongside Babesia among the patients. The results obtained cannot conclusively rule out possibility of cross reactivity.

Symptoms of Babesia generally include: Fever, lack of appetite, apathy, haemoglobinuria, bilirubinuria, polycliromasia, progressive, haemolytic anemia, spleen and hepatomegalia, Jaundice, vomiting and death. These were commonly observed amongst the patients in this study (Fig. 1)

The possible overlap of these symptoms with malaria, syphilis, hepatitis, salmonellosis, is suggestive of the challenges in the differential diagnosis of Babesiosis. The study also revealed that resistance of clinical malaria to artemisin based combinational therapy is high (Fig. 2)

It is likely that babesia may be resistance to these drugs as well since from this study no clear diagnosis as to whether the febrile illness amongst the patients was exclusively malaria or babesia.

Conclusion

Although this is a preliminary study, the incidence of babesiosis amongst HIV/AIDS patients in Cameroon is real. The possibility of the incidence of babesia amongst other immunoincompetent patients in Cameroon needs to be studied. We also conclude that Babesia is also a cause of febrile illness and PUO amongst HIV/AIDS patients in Cameroon and that the etiology of febrile illness amongst patients in Cameroon is currently misdiagnosed. The results also demonstrated that not all traceable malaria as reported as malaria one plus is truly malaria. These cases could be babesia, this has been elucidated in this study as RDTs were negative for some of the cases reported by medical personnels as traceable malaria. This is the first report of Babesiosis in Cameroon. It is recommended that detail survey of the incidence of babesiosis in Cameroon be carried out and also included in routine tests for screening of blood donor persons.

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