FIRST IMMUNOLOGICAL RECORD OF *BABESIA MICROTI* HOBET SULINEAGE ANTIBODIES AMONG HUMAN BLOOD DONORS IN QENA GOVERNORATE, UPPER EGYPT, EGYPT
By

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Abstract

The zoonotic babesiosis is a growing public health concern. This parasite mainly occurs in U.S. (*B. microti*) and Europe (*B. divergens* & *B. venatorum*). Emerging cases have been increasingly reported worldwide especially in immunosuppressed individuals. Other than tick bite, possible modes of infection include perinatal transmission and blood transfusion. To date, in Egypt, three cases of human babesiosis were reported. This study evaluated *Babesia* spp. infection in man in Qena City. Forty three persons were examined. All sera were subjected to serological examination using either indirect fluorescent antibody test (IFA) or western blot (WB) analysis or both tests. Using IFA 13/43 patients were positive for *Babesia* at different titers. The five out of the 13 positive IFAT samples were positive by using WB test (11.6%). This could be the first study in Qena that screened Egyptian patients using well-defined *Babesia* strain for human babesiosis. Blood transfusion is a possible babesiosis mode of infection to man and thus patients in need of blood transfusion may undergo immunosuppressive status so they may develop babesiosis, so screening of blood donors for human babesiosis is needed to exclude transfusion induced babesiosis.

Keywords: Egypt, Blood donors, *Babesia microti*, Hoetsu, Western Blot, IFA

Introduction

The human babesiosis, a zoonotic infection transmitted by *Ixodid* ticks, is an emerging public health problem worldwide. *Babesia microti* infection mainly occurred in the United States while *B. divergens and B. venatorum* were mainly in Europe. However, emerging cases caused by novel zoonotic *Babesia* spp. as well as parasites closely related to but well-known distinct from the pathogenic *Babesia* were increasingly reported worldwide (Ord and Lobo, 2015). The clinical manifestations of human babesiosis ranged from clinically asymptomatic infection to severe fulminant malaria-like illness, which led to death without proper treatment (Onyiche *et al*, 2019). It is clinically manifested when the carrier undergoes immunosuppression (Homer *et al,* 2000). Other than tick bite, possible modes of transmission include perinatal (Yabsley and Shock, 2012) and blood transfusion or needle-stick injury (Abdel-Motagaly *et al*, 2017).

In Africa, human babesiosis has been rarely reported due to widespread of the risky malaria particularly the fatal malignant form (Saleh *et al,* 2019). But, babesiosis is an emerging tick borne zoonotic disease caused by intraerythrocytic parasites of the genus *Babesia*. Babesiosis is one of the commonest infections of free-living animals worldwide and most prevalent in rodents, carnivores, sheep, and cattle, a fact increased the concern about the emerging zoonosis. Like malaria agent *Plasmodium*, the parasite B*abesia* attacks and damages host's red cells (Dvoraková and Dvorácková, 2007).

In South Africa, two cases were identified as *Babesia* intraerythrocytic characteristic morphology. One patient recovered following treatment with quinine and tetracycline but, second died despite of the specific treatment (Bush *et al,* 1990). In Nigeria, *Babesia* spp. antibodies were detected in normal pop-

ulation by using immunoflurescence antibody test (IFA). Nigerian strains *B. bigemina* and *B. bovis* in bovine and *Babesia ratite* in the semi-domestic rat *Arvicanthis niloticas* were used as antigens, positivity of 94,173 sera, which reacted with at least one of three antigens (Leeflang *et al,* 1976).

In Egypt, only four human babesiosis cases were reported (El Bahnasawy *et al,* 2011). The bovine babesiosis was caused mainly by *Babesia bovis* and *B. bigemina. B. bovis* parasites were known to be transmitted to the Egyptian animals by *Rhipicephalus microplus* but *B. bigemina* was known to be transmitted by both *R. microplus* and *R. annulatus* (Abdel-Shafy and Mahmoud, 2020; Adham *et al,* 2009) as well as from rodents and domestic animal.

This study aimed to screen the Egyptian human *Babesia* carriers by using a known *Babesia* strain among blood donors in Qena Governorate, Upper Egypt, with respect to all clinical requirements and regulations.

Materials and Methods

Study area: Qena Governorate is one of the Upper Egyptian Governorates those stretches along Nile Valley. It is an agricultural governorate so that activities of the majority of the inhabitants are farmers and animal husbandry mainly cattle, camel sheep and goats.

Study design: Aseptic sera were randomly collected from 43 Egyptian blood donors attended South Valley University Hospitals from November 2016 to April 2017. All sera were serological examination by indirect fluorescent antibody test (IFA). Positive and negative IFA-cases were evaluated by western blot (WB) analysis, as well as the routine serological examination for the HBV, HCV and HIV (Hu *et al,* 2019).

Laboratory workup: Specific pathogen free golden hamsters (Std: Syrian) bat 4 weeks old were purchased from SLC Inc. (Shizuoka, Japan). Animal experimentation was carried out after the Laboratory Animal Control Guidelines of National Institute of Infectious Diseases (With permission Nos. 213126 and

114014). Erythrocytes infected with *B. microti* Hobetsu lineage, IoSG2003 strain, which was isolated from *Ixodes ovatus* tick in Japan, was used to prepare the antigens for indirect fluorescent antibody test and western blot analysis (Zamoto-Niikura *et al,* 2015).

Indirect fluorescent antibody test: Antigen slide was prepared (Zamoto-Niikura *et al,* 2016). The patient's serum was diluted 1:32 with Tris buffered saline containing 5% immunoblock (DS Pharma) and Tween 20. The slides were placed in a humidified chamber and 20µl of the diluted serum was added to each well. After 1hr of incubation at 37°C, the slides were washed in PBS & 20µl of Alexa Fluor488 conjugated goat anti-human IgG (H+L) diluted 1:500 (Life Technologies). Then, the slides were incubated at room temperature for 1hr, washed in PBS, and then examined with a fluorescence microscope (Olympus IX71) x200. Hamster antiserum raised against *B. microti* IoSG2003 was used as control.

Western blot analysis: antigens were separated by 10% SDS-PAGE. The separated proteins were blotted onto Immobilon-P PVDF membrane (Millipore) that was blocked with 5% skim milk in Tris buffered saline with 0.1% Tween 20. The membranes were incubated with patient's serum (1:50) and then with goat polyclonal antibody to human IgG (alkaline phosphatase, Sigma). The immunoreactive antigens were detected by using 5-bromo-4-chloro-3-indolylphosphatenitroblue tetrazolium alkaline phosphatase (NBT/BCIP) liquid substrate system (Sigma). Hamster antiserum raised against the *B. microti* IoSG 2003 was diluted 1:200 as control.

Anti-HCV, HBsAg & HIV antibody testing were performed by using commercially available assays (Abbott Laboratories, Abbot Park, IL, USA). Data were expressed in numbers and numerically percentage.

Results

The present study was conducted on 43 participants of whom 20 (46.5%) were males and 23 (53.5%) were females with ages ranged from 17 to 40 years old. A total of 17/

43 (39.5%) patients were suffered from viral hepatitis (11 patients were positive to HCV antibodies and six were positive to HbsAg), but none of them were HIV positive.

Using IFA test, it was found that 13/43 patients were *Babesia* positive (30.2%), five of them were females and eight were males. Positive samples were reactive at different titers more than 1:32, which was the recommended positive value. General examination showed that six patients out of 13 positive suffered from viral hepatitis (two HBV & four HCV) but the remaining seven patients were negative for viral hepatitis (Tab. 1; Fig. 1).

By using Western blot analysis to confirm positive results obtained by IFA tests, out of the 13 positive IFAT samples, five patients only were positive, one sample reacted non-specifically to more than one lineage of *B. microti* and the seven IFAT positive samples were WB negative.

To confirm the IFA negative results, WB analyzed three randomly selected IFA negative samples was performed. Two out of the three samples reacted to more than one lineage of *B. microti* which was considered as non-specific reaction and only one showed weak positive reaction to *B. microti hobetsu* type (Tab. 2; Fig. 2). The combined use of IFA & WB proved five patients (one female and four males) to be babesiosis positive (11.6 %), three out of these five positive patients were HCV antibodies positive.

Table 1: Positive and negative *Babesia* samples using indirect fluorescent antibody.

IFAT	No.	Percent	Hbs-Ag positivity	HCV antibody positivity
Negative	30	69.8%	4 (13.3%)	7 (23.3%)
Positive	13	30.2%	2 (15.3%)	4 (31%)
Total	43	100.0%	6 (14%)	11 (25.6%)

Table 2: Blood donors examined by IFAT and WB analysis

Sex	Age	Hepatitis markers	IFAT	WB
Male	35	HBV	±	Negative
Female	34	HBV	x64	Negative
Female	30	None	x64	Negative
Female	35	None	x64<	Positive (HOBETSU specific)
Male	40	None	x64	Positive (HOBETSU specific)
Male	32	None	x64	Negative
Female	26	None	x64	Negative
Male	40	Hepatitis C	x64	Positive (HOBETSU specific weak)
Female	30	Hepatitis B	Negative	Positive (HOBETSU specific weak)
Male	39	Hepatitis C	x64	Positive (HOBETSU specific)
Female	25	None	x64	Negative
Male	17	Hepatitis C	x64<	Positive (HOBETSU specific)
Male	31	None	Negative	Positive (non-specific)
Male	31	None	x32	Negative
Male	28	Hepatitis B	Negative	Positive (non-specific)
Male	40	Hepatitis C	x64	Positive (HOBETSU specific)

Discussion

Rodents are major reservoir for *B. microti* group worldwide and various species including species of *Apodemus, Myodes, Microtus, Ruttus* and *Sigmodon* were infected (Saleh *et al,* 2015). In Egypt, *B. microti* was identified in *R. rattus*, *R. norvegicus*, *Acomys c. dimidiatus* and *Dipodillus d. dasyullus* by microscopic examination of Giemsa stained erythrocytes (El-Bahrawy *et al,* 1993). Rodents were frequently occur in the farmland, such as sugar cane, and are supposed to be *Arvi-*

canthis niloticus and *R. norvegicus* (El-Kady *et al,* 1998; Farghal *et al,* 2000).

In the present study, three strains within *B. microti* group were used as antigens since human babesiosis in the world was generally caused by *B. microti* US lineage (Zamoto-Niikura *et al,* 2016). Strikingly five patients having antibodies reactive to one of the *B. microti* group, Hobetsu lineage was identified. This result led to confirm by the western blot analysis. The Hobetsu lineage was reported only in Japan where rodents were ma-

jor reservoir for this lineage throughout the country and human infection was evident by retrospective serological survey where spotted fever (tick-borne) was endemic (Tsuji et al, 2001). In the western blot analysis, some patients had specific antibodies reactive to approximately 35kDa Hobetsu lineage antigen, which was also recognized by convalescent serum of hamster.

In Egypt, four proven human babesiosis cases were reported. A splenectomized farmer in El-Minia Governorate who was suffered from fever, myalgia and anorexia, was the first case of human babesiosis. The patient serum showed positive (1:30) against *Babesia caballi*, though cross-immune reaction against *Plasmodium* spp. was not denied (Leeflang et al, 1976). The second case of human babesiosis was a farmer from El-Minia Governorate, referred to Ain-Shams University Hospitals with prolonged fever of unknown origin, anemia, hepatomegaly and splenomegaly, and previously underwent corticosteroid therapy and was sero-negative for malaria, but with typical ring forms of *Babesia* in thin and thick blood smears (Michael et al, 1987). The third case was a 21 years-old young manual worker in car repairing center from Alexandria was referred to the fever hospital as he suffered from three months fever of unknown origin. Before being referred, he was previously diagnosed as a case of an acute rheumatic fever and was kept under corticosteroid therapy for 10 weeks, with fluctuating fever without any response to either cortisone or broad spectrum antibiotic. On admission he was feverish, pulse 115/minute, & Bp. 110/60, cachetic, anemic and underweight. There was a reddish brown rash over the chest and abdomen which started as a small red macula on then gradually enlarged and occupied the whole chest and creped over the abdomen. A subcutaneous nodule was found over the dorsum of his left hand (3cm in diameter, soft, slightly tender, movable and not attached to the surrounding structures). There was a hepatomegaly (8cm mid-clavicular consistency firm with slight tenderness) and splenomegaly (4 fingers, firm and slightly tender), but without lymph node enlargement. The chest and heart were clinically free except for tachycardia and haemic murmurs all over his chest. He was proved to be babesiosis case by positive thin and thick blood films. The patient was given Quinine as 650 mg/8h & Clindamycinas 600mg/8h), also blood exchange improved the fever (El Bahnasawy and Morsy, 2008). The last proven human was a 12 years old boy was referred to the hospital with intermittent fever of unknown origin. On clinical, parasitological and serological bases the case proved to be babesiosis. The boy acquired the infection from his pet dog which was heavily infested with *Rhipicephalus sanguineus* and suffered a mild feature of animal babesiosis. The boy was successfully treated with Atovaquone plus Azithromycin without relapse for one month follow up. The pet dog was sent to Abbassia Governmental Veterinary Hospital to treat babesiosis and tick infestation (El Bahnasawy et al, 2011). Apart from patients, Adham et al. (2009) in Giza Governorate by PCR studied the prevalence of *B. bovis* and *B. bigemina* in 5,243 hard and soft ticks. Primers DNA verified from the sequence of Mexico strain of both species were used that generated two fragments of 275 & 175bp of *B. bovis* & *B. bigemina* respectively. Moreover, *B. behnkei* sp. nov, was found in Wagner's gerbil *Dipodillus dasyurus* from Sinai Mountains. Phylogenetically this novel species is included in the Duncani group, which included the emerging zoonotic parasites, *B. duncani* and *Babesia* sp. CA1 (Arai et al, 2003). Antigen-antibody cross reactivity between *B. microti* group and *B. duncani* was supposed to be low (Bajer et al, 2014).

Several studies reported various *Babesia* spp. in farm animals such as cattle and sheep in Qena Governorate. Fereig et al. (2017) reported that the seroprevalence of babesiosis in cattle was 9.0% & 33.2% for *B. bovis* and *B. bigemina* respectively. Moreover, *Babesia bigemina* and *B. bovis* in ruminants, incl-

uded cattle, sheep and water buffaloes, calves as well as horses and pets were reported all over Egypt with various infection rates (Nagaty, 1947; Maronpot and Guindy, 1970; Michael and El Refaii, 1982; Mazyad and Khalaf, 2002; Farah *et al,* 2003; Saleh, 2009: Nayel *et al,* 2012; Ibrahim *et al,* 2013; Mahmmod, 2014; Elsify *et al,* 2015; Mahmoud *et al,* 2015; Sa-lem *et al,* 2016; Hussein *et al,* 2017).

In the current study showed also the association between positivity for HCV antibodies and *Babesia* antibodies. Al-Agroudi *et al.* (2029) in retrospective study evaluated the malaria situation in the last 3 years in an Egyptian Fever Hospital. They reported a total of 100 malaria patients were youth returning back from Africa with mainly *P. falciparum* followed by *P. vivax*, mixed infection and only one case of *P. ovale* All patients were successfully treated except two whom was co-infected with HIV. This raised a question about association between babesiosis and/or malignant malaria and viral infection?

Conclusion

No doubt, nowadays, zoonotic diseases in spite of the causative agent top the medical health problems worldwide. Human babesiosis shared signs and symptoms with many arthropod-borne infectious diseases, especially malignant malaria, even in stained blood smears. No doubt, blood smears for *Babesia* species needs a well-trained technologist. This may pave the way to misdiagnosis and thus its underestimation not only in Egypt but also in other regional countries.

Screening of blood donors for human babesiosis is a must to exclude babesiosis. IFA test proved a preliminary test for screening but must be confirmed by highly specific and more accurate WB analysis.

Ethical statement: The study was approved by the Institutional Ethics Review Board of South Valley University, approval No. 59. Informed consent was obtained from each participant and all were dealt with the humanity rules.

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Explanation of figures

Fig. 1: B. IFA test of Egyptian patients' sera for anti-IgG against *B. microti hobestsu* lineage. A: Infected hamster positive control.
Fig. 2: Protein pattern analysis by western blot technique for sera of *B. microti hobetsu* type infected patients & infected control hamsters.