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Original papers

Serological screening of HIV and viral hepatitis revealed low prevalence among visceral leishmaniosis patients in Sudan

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ABSTRACT. There is an increasing concern about the co-infection of visceral leishmaniosis (VL) with human immunodeficiency virus (HIV) and/or viral hepatitis B/C. The aim of this study was to determine the prevalence of HIV and viral hepatitis co-infections among VL patients in a hyperendemic area in Eastern Sudan and to assess antibody levels in co-infected patients. This is a retrospective study where the sera of confirmed VL cases and non-VL individuals were analysed. The sera were screened for co-infections using immunochromatographic tests and ELISA for anti-HIV 1+2 antibodies, hepatitis B surface antigen (HBsAg), and anti-hepatitis C virus (HCV). Anti-*Leishmania donovani* antibodies in the sera of VL alone were assessed and compared to the sera of co-infected patients. Of the 100 screened VL sera, 6 (6%), 0 (0%), and 1 (1%) were positive for HBsAg, anti-HCV, and anti-HIV, respectively. These values were 5 (5%), 0 (0%), and 1 (1%) in the control group. Of note, the HCV screening test (Biorex, UK) showed positive reactivity in 32 (32%) and 17 (17%) sera of VL and control groups, respectively. All reactive sera tested negative in HCV ELISA. Of the 93 VL sera, 75 (80.6%) had strong DAT titers (1:≥102400), 2 (2.1%) demonstrated the lowest DAT titers (1:≤800), and 5 (5.4%) had marginal DAT titers (1:1600). Interestingly, the VL/HIV co-infected serum had a negative antibody titer (1:1600). Of the 6 VL/HBV co-infected sera, 1 (16.7%) and 5 (83.3%) demonstrated moderate (1:12800–1:51600) and strong (1:≥102400) DAT titers, respectively. The strong DAT titers observed in the VL/HBV co-infected sera were comparable to the DAT titers of the VL sera. The VL co-infection with HIV and hepatitis B/C is low in endemic areas in Eastern Sudan but may create a diagnostic difficulty. VL/HIV co-infected patients can have low *Leishmania* antibodies, thus alternative methodologies (e.g., antigen tests) may help the diagnosis.

Keywords: *Leishmania donovani*, hepatitis, HIV, Eastern Sudan

Introduction

Visceral leishmaniosis (VL) is a tropical disease that represents a public health problem in East Africa with increasing incidence in new areas [1–3]. The disease is associated with high mortality rates, and Sudan is among the six countries with more than 90% of global VL cases, and thousands of cases and deaths occur annually. The reported VL cases in Sudan are likely lower than the true number of cases [4].

VL can appear as a co-infection with other diseases (e.g., human immunodeficiency virus HIV and viral hepatitis) that are highly prevalent in many endemic areas and are associated with high mortality from VL [5]. *Leishmania* invades the body through the lymphovascular system and infects many cells of the body including the liver. In addition, some anti-*Leishmania* drugs affect liver cells and cause hepatocellular damage. This can facilitate infection by pathogens such as hepatitis B virus (HBV) and hepatitis C virus (HCV) [6]. The co-infection of VL with HIV and viral hepatitis is now recognized as a major problem and it attracting considerable attention. VL is an opportunistic infection associated with HIV-infected subjects. HIV infection increases the risk of developing symptomatic VL, and *Leishmania* infection speeds up the progression of HIV by promoting viral persistence and replication, which leads to less efficient viral control and immunodeficiency [7]. In addition, VL/HIV co-infected patients may have

atypical clinical presentation, which complicates the clinical diagnosis of the disease.

Co-infections of VL with HIV and/or viral hepatitis vary from country to country based on predisposing factors and endemicity of pathogens. In Sudan, studies have shown that VL co-infections with HIV and viral hepatitis are an emerging health problem, which requires the routine testing of VL patients for possible co-infections [8]. To develop better strategy for VL control, it is important to assess the occurrence of co-infections among VL patients. In Eastern Sudan, the Gedaref State is the main endemic area of VL. The Gedaref State is located hundreds of kilometers to the south-east of the capital. The endemic areas with high incidence of VL are situated between Atbarah and Rahad rivers [4]. This area lacks studies on the prevalence of co-infections with VL. The aim of this study was to determine VL co-infections with HIV and hepatitis B/C in the Gadaref State and to investigate the effect of co-infections on antibody levels to *Leishmania*.

Materials and Methods

Ethical consideration. Analysis was performed on archived and stored sera at the Laboratory for Biomedical Research (LBR). The laboratory performs routine investigations of VL. The sera were previously collected from patients referred to LBR and used in previous studies [9,10]. All procedures were performed in accordance with local and international ethical standards on human experimentation and the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/17-c_e.html). The study was approved by the Research Ethical Committee of the Federal Ministry of Health to be carried out in Sudan (23-06-2005). The patients were informed, and consent was obtained before sample collection.

Study design. This is a retrospective study where the sera of confirmed VL cases and non-VL cases were analysed. Sera were screened for co-infections using immunochromatographic rapid test kits to detect antibodies against HIV-1+2 and HCV. Screening for HBV was performed using ICT kits, which are based on the detection of HBsAg. All serological reactive sera were confirmed by ELISA. Anti-*Leishmania donovani* antibodies in the sera of VL alone were assessed and compared to the sera of co-infected cases using DAT.

Testing algorithm and the collection of sera. Two hundred sera of VL (n=100) and non-VL (n=100) were obtained from the serum bank facility at LBR of Ahfad University. VL was diagnosed by detecting *Leishmania* parasites in lymph node aspirates. Testing was done as part of the routine diagnosis at the time of diagnosis. Lymph node aspirates were collected from enlarged inguinal lymph nodes; smears were made, fixed by methanol, stained with a Giemsa stain, and examined by two experienced laboratory technicians. Blood specimens were collected from VL and controls; sera were separated by centrifugation and kept at −20°C at LBR. Serology using DAT was performed according to the standard method that has been previously described by Harith et al. [11]. Briefly, a serum diluent containing 0.2% gelatin (Difco Laboratories) was dissolved by heating in physiological saline with 0.8% (v/v) 2-mercaptoethanol (Sigma, USA). All sera were tested using two-fold serial dilutions from 1:100 up to 1:102400 in V-shaped microtiter plates (Greiner, Frickenhausen, Germany). After the addition of antigen (50 µL/well), the plates were carefully shaken by hand on a level surface for 30 s and then covered and left for 18 h at room temperature. The test was read visually against a white background, and the end-point reaction was localized as a blue spot similar to the control well. A titer of 1:≥3200 was considered positive.

HIV testing algorithm. The detection of HIV infection was performed according to the guidelines of the Sudanese Ministry of Health. The VL and control sera were tested using a serial testing algorithm; specifically, the reactive sera from the first test were subsequently retested using a second test to confirm the positive result. The first test used was the rapid HIV test (Biorex diagnostic, United Kingdom). This is a rapid immunochromatographic test device for the qualitative detection of antibodies to HIV-1 and HIV-2 in human blood or sera. The test was used and interpreted as described by the manufacturer. Ten µL of serum specimens was added to the device using a plastic dropper. Then, few drops of serum diluents were added, and the results were read after 15 min as *reactive* if both red test and control bands appeared. Sera were considered *non-reactive* if only one band appeared. The test was considered invalid if no control band appeared, and the test was repeated with a new test device. All reactive sera from the first test were subsequently retested with an anti-HIV (1 and 2) ELISA kit (Enzygnost®, Germany). This test is an

Table 1. Human immunodeficiency virus and hepatitis B/C co-infections among the VL and control sera detected by the rapid immunochromatographic test

Study groups	Immunochromatographic Test (Biorex Diagnostics, UK)		
	HBsAg	HCV antibody	HIV antibody
VL (n=100)	11/100 (11%)	32/100 (32%)	7/100 (7%)
Non-VL (n=100)	8/100 (8%)	17/100 (17%)	6/100 (6%)

VL, visceral leishmaniosis; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus. The values are the number positive/total (%).

indirect ELISA assay for the quantitative detection of circulating antibodies to human immunodeficiency virus type 1 (HIV-1) and/or human immunodeficiency virus type 2 (HIV-2). The test uses synthetic peptides and recombinant antigens of the HIV-1 and HIV-2 envelope and core proteins. Sera were tested according to manufacturer's instruction, and reactive sera were retested twice.

Viral hepatitis testing protocol. The VL and control sera were tested for HCV and HBV via the serial testing algorithm; the reactive sera from the first test were subsequently retested using the second test to confirm the positive result. The first test used the rapid HCV antibody test (Biorex diagnostics, United Kingdom) and the rapid HBsAg test strips (Biorex diagnostics, United Kingdom), which are rapid immunochromatographic tests for the qualitative detection of antibodies to HCV and HBV surface antigens in human blood or sera. The tests were used and interpreted according to the description by the manufacturer. Sera were considered *non-reactive* if only one band appeared; the test was considered invalid if no control band appeared, and the test was repeated with a new test device. All reactive sera from the first test were subsequently retested with the anti-HCV ELISA kit (Biorex diagnostics, United Kingdom) and HBsAg ELISA kit (Biorex diagnostic, United Kingdom). The sera were tested according to manufacturer's instruction, and reactive sera were tested twice.

Data analysis. Descriptive data were presented as frequency, where variables were summarized in cross tables. Anti-*Leishmania* antibodies in sera were assessed by DAT and expressed as antibody titers, as previously reported [11].

Results

The sera used in this study were collected from the Algadaref State, which is a known VL-endemic area in Eastern Sudan. The sera included 100 confirmed VL patients (case) and 100 non-VL cases (control). The latter sera were collected from the same endemic area as that of VL. Most members of the study population were 1–15 years old; there were more males than females (62.9% and 37.1%, respectively). The following clinical features were observed: splenomegaly (25%), hepatomegaly (5%), lymphadenopathy (5%), splenomegaly and hepatomegaly (27.5%), splenomegaly and lymphadenopathy (17.5%), splenomegaly, and hepatomegaly and lymphadenopathy (20%).

All VL and non-VL sera were initially screened for co-infections with HBV, HCV, and HIV using rapid immunochromatographic tests (ICT) that are based on the detection of HBsAg, anti-HCV, and anti-HIV, respectively. Reactive sera were confirmed by ELISA tests. The occurrence of HBV,

Table 2. Human immunodeficiency virus and hepatitis B/C co-infections among the VL and control sera detected by ELISA

Patient group	ELISA		
	HBsAg	HCV antibody	HIV antibody
	Biorex diagnostic, UK	Biorex diagnostic, UK	Enzygnost, Germany
VL (case)	6/100 (6%)	0/100 (0%)	1/100 (1%)
Non-VL (control)	5/100 (5%)	0/100 (0%)	1/100 (1%)

VL, visceral leishmaniosis; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus. The values are the number positive/total (%).

Table 3. Comparison of antibody levels for *Leishmania* in visceral leishmaniosis and co-infected patients

Patient group	DAT titer				
	1:≤800	1:1600	1:3200–1:6400	1:12800–1:51600	1:≥102400
VL (n=93)	2 (2.1%)	5 (5.4%)	5 (5.4%)	6 (6.5%)	75 (80.6%)
VL /HIV (n=1)	–	1 (100%)	–	–	–
VL/HBV (n=6)	–	–	–	1 (16.7%)	5 (83.3%)

DAT, direct agglutination test; HIV, human immunodeficiency virus; HBV, hepatitis B virus. The sera were categorized according to the level of antibodies measured by DAT as follows: 1:≤800, negative; 1:1600, marginal; 1:3200–6400, weak; 1:12800–51600, moderate; 1:≥102400 strong.

HCV, and HIV co-infections among the two cohorts is shown in Tables 1 and 2. Eleven (11%) sera of VL patients were reactive for HBV when screened using ICT; this value was lower (8%) in the sera of the control group. HBV infection was confirmed in 6 (6%) VL patients and in 5 (5%) patients in the control group. Screening for HCV by the rapid test showed positive reactivity in 32 (32%) and 17 (17%) sera of VL and control groups, respectively. However, all reactive sera tested negative in ELISA. The reactive sera from the initial screening were retested by another rapid test (InTec Products, INC, Xiamen, China). The results were similar to those obtained by ELISA. Positive reactivity for HIV infection by ICT was demonstrated in 7 (7%) and 6 (6%) sera of VL and controls, respectively. Of these, only one patient from each group showed a positive result by ELISA.

Table 3 shows the levels of *Leishmania* antibodies, as measured by DAT in VL or co-infected sera. Out of the 93 VL sera, 75 (80.6%) had strong DAT titers (1:≥102400), 2 (2.1%) demonstrated the lowest DAT titers (1:≤800), and 5 (5.4%) had marginal DAT titers (1:1600). Of note, the VL/HIV co-infected serum had a negative antibody titer (1:1600). Out of the 6 VL/HBV co-infected sera, 1 (16.7%) and 5 (83.3%) demonstrated moderate (1:12800-1:51600) and strong (1:≥102400) DAT titers, respectively. Strong DAT titers, which were observed in the VL/HBV co-infected sera, were comparable to the DAT titers of the VL sera.

Discussion

VL co-infections with HIV and hepatitis B/C have been reported in several countries including resource-limited regions. This imposes an additional burden on the people in endemic areas where various infectious diseases are encountered due to many predisposition factors. These factors increase morbidity and mortality of infectious diseases [12]. The endemic areas of VL and other infections overlap [13–15], and the cases of VL co-infected with HIV and viral hepatitis have been extensively reported in several countries.

In this study, the prevalence of HBV among VL and controls was relatively low. This agrees with previously published data for the same region; for Gadaref, the value is 1.9% [16]. The same author has also reported the low prevalence of VL/HCV (1.3%) and VL/HIV (3.8%) co-infections. However, for other countries with a high endemicity of HBV, the higher prevalence of co-infections has been reported [17]. The low prevalence of co-infections reported in this study may be due to the difference in the accuracy of detection systems. It is known that the accuracy of different HBV assays considerably varies [18]. Indeed, the difference in HBV prevalence in various regions can be related to the variation in the degree of endemicity and the prevalence of diseases. Because in Sudan the prevalence of HBV is higher than that of HCV [15], it is not surprising that we did not detect HCV co-infection among the VL and control groups. In a previous study performed in Elobeid in Western Sudan, all screened specimens were non-reactive to HCV and considered negative [19]. However, in other studies, HCV infection was determined to be at a low level, i.e., 2.2–4.8% in the Gezira State in Sudan [20]. It has been shown that the seroprevalence of the HCV infection varied from region to region depending on the local medical practices and socioeconomic status of the countries. In India, the high prevalence of the HCV infection has been reported [21].

HIV co-infection with VL represents a major challenge in endemic areas, which considerably increases the risk of conversion from asymptomatic VL to clinical disease; HIV considerably contributed to the spread of leishmaniosis in Europe

[22,23]. In India, which is another hyperendemic country, HIV co-infection with VL represents approximately 5.5% of cases, with a high percentage of cases reported among sexually active men [24]. In the hyperendemic areas of VL in Ethiopia, up to 40% of VL patients may be co-infected with HIV [22,25,26]. In the hypoendemic areas of VL, such as in South Europe, the prevalence of HIV among leishmaniosis patients is believed to be high, i.e., 37% [27].

Serological tests vary in their accuracy and are less sensitive in immunocompromised hosts. Few studies have focused on the performance of serology in immunocompromised patients co-infected with VL and HIV in East Africa. In general, the sensitivity of serology in HIV patients is lower than that in HIV-negative cases. However, the *Leishmania* antibody response does not seem to be affected by HBV co-infection. In a study from Ethiopia, both DAT and rK39 immunochromatographic tests showed lower sensitivity when the sera of VL and HIV co-infection were used [28]. In Sudan, similar findings have been reported, which demonstrated a considerable decrease in the *Leishmania* antibodies in VL/HIV co-infected subjects from France and thus a low sensitivity for VL [10]. Herein, the serological evidence of low antibodies to *Leishmania* in VL/HIV co-infection was reported. Indeed, the decreased detectable level of specific antibodies in VL may affect the performance of *Leishmania* serology. Considering the low sensitivity, serological tests should not be used alone to confirm the diagnosis of VL in known HIV cases. Such co-infected patients tend to produce low titers [29]. However, other studies have shown the potential of DAT for detecting VL in HIV co-infected subjects [30–32]. Nevertheless, there is considerable variation in the sensitivity of serological tests for VL in HIV co-infected patients; the tests are better in East Africa than in Europe [33].

VL co-infection with HIV and hepatitis B/C is low in endemic areas in Eastern Sudan but may create a diagnostic difficulty. VL/HIV co-infected patients may have low levels of *Leishmania* antibodies; thus, alternative methodologies (e.g., antigen tests) may help the diagnosis.

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References

[1] Marlet M.V.L., Wuillaume F., Jacquet D., Quispe W., Dujardin J.C., Boelaert M. 2003. A neglected disease of humans: a new focus of visceral leishmaniosis in Bakool, Somalia. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 97: 1-5.

[2] Alvar J., Canavate C., Gutierrez-Solar B., Jimenez M., Laguna F., Lopez-Velez R., Molina R., Moreno J.1997. *Leishmania* and human immunodeficiency virus coinfection: The first 10 years. *Clinical Microbiology Reviews* 10: 298-318.

[3] Diro E., Lynen L., Ritmeijer K., Boelaert M., Hailu A., van Griensven J. 2014. Visceral leishmaniosis and HIV coinfection in East Africa. *PLoS Neglected Tropical Diseases* 8: e2869. doi:10.1371/journal.pntd.0002869

[4] Meheus F., Abuzaid A.A., Baltussen R., Younis B.M., Balasegaram M., Khalil E.A., Musa A.M. 2013. The economic burden of visceral leishmaniosis in Sudan: an assessment of provider and household costs. *American Journal of Tropical Medicine and Hygiene* 89: 1146-1153.

[5] de Araújo V.E., Morais M.H., Reis I.A., Rabello A., Carneiro M. 2012. Early clinical manifestations associated with death from visceral leishmaniasis. *PLoS Neglected Tropical Diseases* 6: e1511. https://doi.org/10.1371/journal.pntd.0001511

[6] Caccamo G., Saffioti F., Raimondo G. 2014. Hepatitis B virus and hepatitis C virus dual infection. *World Journal of Gastroenterology* 20: 14559-14567.

[7] Lindoso J.A., Cunha M.A., Queiroz I.T., Moreira C.H. 2016. Leishmaniosis-HIV coinfection: current challenges. *HIV and AIDS Review* 8:147-156. doi:10.2147/HIV.S93789

[8] Abubakr O.A. Adam, Mohamed M.M. Dafalla, Hatim A.A. Mohammed, Mohamed Y. Elamin, Brima M. Younis, Mona E.E. Elfaki, Ahmed M. Musa, Ahmed M. Elhassan, Eltahir A.G. Khalil. 2014. Visceral leishmaniosis-hepatitis B/C coinfections: a rising necessity to triage patients for treatment. *Annals of Saudi Medicine* 34: 143. doi:10.5144/0256-4947.2014.143

[9] Abass E., Bollig N., Reinhard K., Camara B., Mansour D., Visekruna A., Lohoff M., Steinhoff U. 2013. rKLO8, a novel *Leishmania donovani*-derived recombinant immunodominant protein for sensitive detection of visceral leishmaniosis in Sudan. *PLoS Neglected Tropical Diseases* 7: e2322. doi:10.1371/journal.pntd.0002322

[10] Abass E., Kang C., Martinkovic F., Semiăo-Santos S.J., Sundar S., Walden P., et al. 2015. Heterogeneity of *Leishmania donovani* parasites complicates

diagnosis of visceral leishmaniosis: comparison of different serological tests in three endemic regions. *PloS One* 10: e0116408. https://doi.org/10.1371/journal.pone.0116408

[11] Harith A.E., Chowdhury S., Al-Masum A., Semião-Santos S.J., Karim E., El-Safi S., Haque N. 1995. Evaluation of cleaving agents other than trypsin in direct agglutination test for further improving diagnosis of visceral leishmaniosis. *Journal of Clinical Microbiology* 33: 1984-1988. doi:10.1128/JCM.33.8.1984-1988.1995

[12] Tette E.M., Sifah E.K., Nartey E.T. 2015. Factors affecting malnutrition in children and the uptake of interventions to prevent the condition. *BMC Pediatrics* 15: 189.

[13] Lemoine M., Nayagam S., Thursz M. 2013. Viral hepatitis in resource-limited countries and access to antiviral therapies: current and future challenges. *Future Virology* 8: 371-380. https://doi.org/10.2217/fvl.13.11

[14] Sousa-Gomes M.L., Romero G.A., Werneck G.L. 2017. Visceral leishmaniosis and HIV/AIDS in Brazil: are we aware enough? *Plos Neglected Tropical Diseases* 11: e0005772. https://doi.org/10.1371/journal.pntd.0005772

[15] Mudawi H.M. 2008. Epidemiology of viral hepatitis in Sudan. *Clinical and Experimental Gastroenterology* 1: 9-13.

[16] Mohammed H.B., Ali A.A., Idriss M.I., Gasmelseid K.M., Yousif M.M. 2016. Prevalence of hepatitis B, hepatitis C, HIV and malaria co-infection among patients infected with visceral leishmaniosis in Gedarif, Eastern Sudan. *Global Journal of Infectious Diseases and Clinical Research* 2: 021-024. doi:10.17352/2455-5363.000010

[17] Hoffmann C.J., Thio C.L. 2007. Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. *Lancet. Infectious Diseases* 7: 402-409. doi:10.1016/s1473-3099(07)70135-4

[18] Krajden M., McNabb G., Petric M. 2005. The laboratory diagnosis ofhepatitis B virus. *Canadian Journal of Infectious Diseases and Medical Microbiology* 16: 65-72. https://doi.org/10.1155/2005/450574

[19] Elfaki A., Eldour A., Elsheikh N. 2008. Seroprevalence of immunodeficiency virus, hepatitis B and C and syphilis among blood donors at ElObeid Teaching Hospital, West Sudan. *Sudan Journal of Medical Sciences* 3: 333-338. https://doi.org/10.4314/sjms.v3i4.38555

[20] Mudawi H.M., Smith H.M., Rahoud S.A., et al. 2007. Epidemiology of HCV infection in Gezira state of central Sudan. *Journal of Medical Virology* 79: 383-385. doi:10.1002/jmv.20780

[21] Singh S., Dwivedi S.N., Sood R., Wali J.P. 2000. Hepatitis B, C and human immunodeficiency virus infections in multiply-injected kala-azar patients in Delhi. *Scandinavian Journal of Infectious Diseases* 32: 3-6. https://doi.org/10.1080/00365540050164137

[22] Alvar J., Aparicio P., Aseffa A., Den Boer M., Canavate C., Dedet J.P. et al. 2008. The relationship between leishmaniosis and AIDS: the second 10 years. *Clinical Microbiology Reviews* 21: 334-359. doi:10.1128/CMR.00061-07

[23] van Griensven J., Carrillo E., Lopez-Velez R., Lynen L., Moreno J. 2014. Leishmaniosis in immunosuppressed individuals. *Clinical Microbiology and Infection* 20: 286-299. doi:10.1111/1469-0691.12556

[24] Coutinho J.V.S.C., Santos F.S.D., Ribeiro R.D.S.P., Oliveira I.B.B., Dantas V.B., Santos A.B.F.S., Tauhata J.R. 2017. Visceral leishmaniosis and leishmaniosis-HIV coinfection: comparative study. *Revista da Sociedade Brasileira de Medicina Tropical* 50: 670-674. doi:10.1590/0037-8682-0193-2017

[25] Hurissa Z., Gebre-Silassie S., Hailu W., Tefera T., Lalloo D.G., Cuevas L.E., Hailu A. 2010. Clinical characteristics and treatment outcome of patients with visceral leishmaniosis and HIV co-infection in northwest Ethiopia. *Tropical Medicine and International Health* 15: 848-855.

[26] Mengistu G., Ayele B. 2007. Visceral leishmaniasis and HIV co-infection in patients admitted to Gondar University Hospital, Northwest Ethiopia. *The Ethiopian Journal of Health Development* 21: 53-60.

[27] Lopez-Velez R. 2003. The impact of highly active antiretroviral therapy (HAART) on visceral leishmaniosis in Spanish patients who are co-infected with HIV. *Annals of Tropical Medicine and Parasitology* 97 (Suppl. 1): 143-147. doi:10.1179/000349803225002615

[28] Bezuneh A., Mukhtar M., Abdoun A., Teferi T., Takele Y., Diro E. et al. 2014. Comparison of point-of-care tests for the rapid diagnosis of visceral leishmaniosis in East African patients. *American Journal of Tropical Medicine and Hygiene* 91:1109-1115. doi:10.4269/ajtmh.13-0759

[29] Cota G.F., de Sousa M.R., Demarqui F.N., Rabello A. 2012. The diagnostic accuracy of serologic and molecular methods for detecting visceral leishmaniosis in HIV infected patients: meta-analysis. *PLoS Neglected Tropical Diseases* 6: e1665. https://doi.org/10.1371/journal.pntd.0001665

[30] ter Horst R., Tefera T., Assefa G., Ebrahim A.Z., Davidson R.N., Ritmeijer K. 2009. Field evaluation of rK39 test and direct agglutination test for diagnosis of visceral leishmaniosis in a population with high prevalence of human immunodeficiency virus in Ethiopia. *American Journal of Tropical Medicine and Hygiene* 80: 929-934.

[31] Hailu A,, Berhe N. 2002. The performance of direct agglutination tests (DAT) in the diagnosis of visceral leishmaniosis among Ethiopian patients with HIV co-

infection. *Annals of Tropical Medicine and Parasitology* 96: 25-30. https://doi.org/10.1179/000349802125000475

[32] Ritmeijer K., Dejenie A., Assefa Y., Hundie T.B.., Mesure J. et al. 2006. A comparison of miltefosine and sodium stibogluconate for treatment of visceral leishmaniosis in an Ethiopian population with high prevalence of HIV infection. *Clinical Infectious Diseases* 43: 357-364. doi:10.1086/505217

[33] Deniau M., Canavate C., Faraut-Gambarelli F., Marty P. 2003. The biological diagnosis of leishmaniosis in HIV-infected patients. *Annals of Tropical Medicine and Parasitology* 97 (Suppl. 1): 115-133. doi:10.1179/000349803225002598

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