Diagnostic Accuracy of Two rK39 Antigen-Based Dipsticks and the Formol Gel Test for Rapid Diagnosis of Visceral Leishmaniasis in Northeastern Uganda

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Visceral leishmaniasis (VL) or kala-azar affects an estimated 500,000 persons yearly, predominantly in the poor rural areas of India, Bangladesh, Nepal, Sudan, and Brazil (10). Most patients present with prolonged fever, weight loss, and splenomegaly. Other tropical diseases such as malaria, disseminated tuberculosis, or enteric fever can share the same clinical presentation. Therefore, laboratory testing is necessary to confirm the diagnosis of VL. Diagnostic tests for VL need to be highly sensitive and specific because of the fatal evolution of the disease without adequate treatment and the serious toxicity of antimonials (18), the most commonly used first-line therapy. The Direct Agglutination Test (DAT), developed in the 1980s (11, 12), has been validated in several areas of endemicity (1–3, 5). Various brands of rK39 dipsticks have been evaluated into a dipstick format. Validation studies have shown variable results depending on the location of the study site, the variable results depending on the location of the study site, the

The development of an accurate, practical, and affordable diagnostic test is essential to improve the management of visceral leishmaniasis (VL) in remote health centers. We evaluated the Formol Gel test (FGT) and two rK39 antigen-based dipsticks, the DUAL-IT L/M, and the Kalazar Detect for VL diagnosis in Amudat Hospital in Uganda. The DUAL-IT L/M was also evaluated for the diagnosis of malaria. All patients clinically suspect of VL were prospectively included in the study between October 2003 and March 2004. The gold standard used to define a VL case was a positive spleen aspirate or a direct agglutination test titer of >1:12,800 with an appropriate clinical response to antileishmanial therapy. A total of 131 VL and 112 non-VL patients were included in the analysis. The DUAL IT L/M was found to be more sensitive than the Kalazar Detect: 97% (95% confidence interval [95%CI] = 92 to 99%) versus 82% (95%CI = 74 to 87%). The Kalazar Detect and the DUAL IT L/M were highly specific (99% [95%CI = 95 to 100%] and 97% [95%CI = 92 to 99%], respectively). The FGT lacked both sensitivity (66% [95%CI = 57 to 73%]) and specificity (90% [95%CI = 83 to 94%]). The sensitivity of the DUAL IT L/M for malaria was only 57% (95%CI = 37 to 76%). The two rK39 dipsticks can be used for diagnostic confirmation of VL in this region. The DUAL-IT L/M without its malaria diagnostic component (DiaMed-IT LEISH) will be adopted as first-line test for VL in Uganda.

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endemic focus continues eastward in the West Pokot District of Kenya. Médecins Sans Frontières (MSF) has supported the clinical management of suspect VL patients in Amudat Hospital (AH) since 1997. The DAT and spleen aspiration were introduced in AH in 2000, and the DAT diagnostic thresholds (negative, borderline, positive) were validated in 2001 (unpublished data). However, cheaper and more practical diagnostic tests are needed to ensure a sustainable access to VL diagnosis in AH after the withdrawal of MSF support. Therefore, we conducted a diagnostic accuracy study in AH, comparing the performance of the FGT and two marketed rK39 dipsticks.

MATERIALS AND METHODS

Study area and population. The study was conducted in AH, a 120-bed hospital located in Pokot County, Nakapiripirit District, in northeastern Uganda. The aim was to enroll at least 100 true cases of VL and 100 true non-VL cases in the study to achieve adequate precision for the estimation of both the sensitivity and the specificity of the tests. The total sample size was fixed at 300 clinical suspects, taking into account an expected proportion of 40% true VL cases among clinical suspects.

Clinical suspect patients were defined as patients with a history of fever of ≥14 days with either clinical splenomegaly or wasting syndrome. All clinical suspect patients, adult and pediatric, who presented at the Outpatient Department of AH were enrolled in the study between October 2003 and March 2004. Thirty-three patients were excluded from the analysis for the following reasons: failure to meet the definition of clinical suspect (21 patients), a difference of more than two dilution titers between AH and the Royal Institute of Tropical Medicine in Amsterdam, Holland, laboratories (10 patients), or death before completion of diagnostic investigations (one patient) or antileishmanial treatment (one patient). A total of 243 patients, 131 with VL (54%) and 112 with another diagnosis (46%), were included in the analysis. The demographic and clinical characteristics of the 131 VL and 112 non-VL patients are compared in Table 1. VL was

FIG. 1. Diagnostic algorithm of visceral leishmaniasis in Amudat Hospital, Pokot County.
confirmed by a positive spleen aspirate in 15 (11%) patients or by a DAT of \( \geq 1:25,600 \) with a good response to antileishmanial treatment in 116 patients (89%). The final diagnosis of the 112 non-VL patients was hyper-reactive malarial splenomegaly in 68 patients (61%), smear-proven malaria in 17 patients (15%), brucellosis in 8 patients (7%), liver disease in 3 patients (3%), and another or unknown illness in 16 patients (14%). None of the initially diagnosed non-VL patients were diagnosed with VL within a 6-month period after discharge from AH.

The DUAL IT L/M was significantly more sensitive than the Kalazar Detect: 97% (95%CI = 92 to 99%) versus 82% (95%CI = 74 to 87%) (Table 2). The Kalazar Detect and the DUAL IT L/M were both highly specific: 99% (95%CI = 95 to 100%) and 97% (95%CI = 92 to 99%), respectively. The FGT lacked both sensitivity (66% [95%CI = 57 to 73%]) and specificity (90% [95%CI = 83 to 94%]). The performance of the test was not significantly influenced by the mode of confirmation of VL diagnosis (data not shown).

### DISCUSSION

The DUAL IT L/M rK39 antigen-based dipstick from DiaMed AG, Switzerland, was found to be highly sensitive (97%) and specific (97%) for the diagnosis of VL in northeastern Uganda. The Kalazar Detect dipstick was also highly specific (99%) but substantially less sensitive (82%). The FGT was less sensitive (66%) and specific (90%) than the rK39 dipsticks.

Our study is the first to evaluate the performance of these tests in Uganda and is one of the few diagnostic accuracy studies in VL using a prospective design in a clinical setting.
The reference standard we used was a composite one: case ascertainment was based either on direct examination of Giemsa-stained splenic aspirates smears (close to 100% sensitivity and specificity) or on a combination of serology with observation of response to treatment. The diagnostic accuracy of DAT is known to be excellent and, since the therapeutic spectrum of antimonials is so narrow, a good response to it can safely be regarded as evidence of visceral leishmaniasis (25). All DAT results and spleen aspirates were reviewed in reference laboratories, and patients with discordant results were excluded from analysis. Therefore, we can safely claim that little or no misclassification occurred in the present study with regard to disease status.

The DiaMed-IT LEISH, the equivalent of the DUAL IT L/M but without the malaria diagnostic component, has been previously evaluated in India and Sudan. In India, the sensitivity of the DiaMed-IT LEISH was 99% and its overall specificity was 94% when tested on blood from patients with VL, patients with other diseases, and controls from areas of high and low endemicity (20). In Sudan, the DiaMed-IT LEISH was found to be 81% sensitive and 97% specific in a group of 341 clinical suspect individuals (16). Low sensitivity of rK39 antigen-based dipsticks was already reported in another study from Sudan, and the authors of that study suggested that Sudanese VL patients develop lower titers of antibodies against the K39 antigen (27). Other researchers suggest that the format of the immunochromatographic assay might be the cause (16).

Therefore, we believe that the results of diagnostic accuracy studies for VL should only be extrapolated with caution between different epidemiological and ecological regions.

The Kalazar Detect dipstick from Inbios International, Inc., Seattle, Wash., has been previously evaluated (sometimes under another trade name) in the Indian subcontinent and Latin America, with reported sensivities and specificities of 87 to 100% and 71 to 100%, respectively (5–7, 9, 19). The present study is the first published evaluation of the validity of the Kalazar Detect among African patients clinically suspect of VL. We found a disappointingly low sensitivity (81%) but an excellent specificity (99%). An ongoing multicenter diagnostic study conducted by the World Health Organization in several East African countries will add data on the validity of the Kalazar Detect. According to the manufacturer, a more sensitive generation of the Kalazar Detect dipstick is under development.

The poor sensitivity of the FGT confirmed the findings of previous studies (5, 8). The specificity of the FGT was good (90%) but lower than recently described in Nepal (5), most likely because hyper-reactive malarial syndrome, a classical cause of polyclonal hypergammaglobulinemia, was a frequent diagnosis (61%) in our non-VL patients. We considered the cause of polyclonal hypergammaglobulinemia, was a frequent cause of antibody against the K39 antigen (27). Other researchers suggest that the format of the immunochromatographic assay might be the cause (16). In summary, the DUAL IT L/M was found to be a highly accurate and practical diagnostic test for VL in Uganda. The malaria diagnostic component was not found to be useful.

Therefore, we introduced the DiaMed IT LEISH as a first-line test for patients presenting with clinical suspicion of VL at AH in the first trimester 2005. If an ongoing 3- to 6-month post-study validation phase confirms the findings of the present study, the dipstick will replace the DAT for the diagnosis of VL in this setting in Uganda. Because of the long persistence of antibodies against rK39 antigen in the serum after treatment (27), spleen puncture will remain necessary to confirm the diagnosis of relapse in patients with a previous history of VL and a positive dipstick. The introduction of the dipstick in more peripheral health centers, leading to an earlier diagnosis and a positive dipstick. The introduction of the dipstick in more peripheral health centers, leading to an earlier diagnosis of VL patients and control of VL strategies depends mainly on the cost of hospitalization and treatment (4). The relatively high packaging volume of the DUAL IT L/M can indirectly increase transport cost if high numbers of dipsticks are needed.

We found no added value of the malaria antigen (pLDH) detection line present on the DUAL IT L/M in our population of VL suspect patients. Only few non-VL patients were diagnosed with smear-proven malaria (8.4%), and the sensitivity of the DiaMed DUAL IT L/M among these patients was only 57%. This poor sensitivity was most likely due to the high proportion (43%) of patients with low parasitemia (<200 parasites/mm3). A low sensitivity of the Optimal test (DiaMed AG, Switzerland), using the same technology, has been previously reported in malaria patients with low parasitemia (15, 21). It must be emphasized that malaria has a clear seasonal pattern in the study area and that our study took place during a low-transmission season. The prevalence of smear-proven malaria and the intensity of parasitemia is likely to be higher during the higher transmission rainy season, thus influencing the performance of the malaria component of the DUAL IT L/M.

In summary, the DUAL IT L/M was found to be a highly accurate and practical diagnostic test for VL in Uganda. The malaria diagnostic component was not found to be useful. Therefore, we introduced the DiaMed IT LEISH as a first-line test for patients presenting with clinical suspicion of VL at AH in the first trimester 2005. If an ongoing 3- to 6-month post-study validation phase confirms the findings of the present study, the dipstick will replace the DAT for the diagnosis of VL in this setting in Uganda. Because of the long persistence of antibodies against rK39 antigen in the serum after treatment (27), spleen puncture will remain necessary to confirm the diagnosis of relapse in patients with a previous history of VL and a positive dipstick. The introduction of the dipstick in more peripheral health centers, leading to an earlier diagnosis and a better treatment outcome, should be implemented. The major remaining challenge is for the Ugandan Ministry of Health to create and ensure a sustainable supply of this diagnostic tool to AH.

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REFERENCES


