LATERAL FLOW TESTS - ICT:
Point-of-care testing for Parasites Serology

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TOXOPLASMA ICT IgG IgM

Bicentric evaluation of a new ICT Toxoplasma IgG-IgM® (LDBIO Diagnostics) for the combined detection of toxoplasmic IgG and IgM : comparison with routine technique, the Architect. Caroline Mahinc, Coralie L'Ollivier, Pierre Flori, Cécile Guillerme, Hélène Raberin, Jamal Hafid, Stéphane Ranque. *J Clin Microbiol* **2017**. Accepted manuscript posted online 27 September 2017, doi: 10.1128/JCM.01106-17

A comparative study of the ICT Toxoplasma® IgG and IgM rapid diagnostic test (LDBIO Diagnostics, France) with the fully Architect automated test was performed on 767 non-selected samples, and on 235 selected sera, from Marseille and Saint-Etienne, collected between February and May 2015.
The ICT test is easily and quickly read and does not require the eye of an expert. The different groups were designed for the ICT expertise on each IgG and IgM parameter. The reference technique used as a confirmatory test for IgG is Toxo IgGII Western Blot (LDBIO Diagnostics). For the detection of IgM, the commercial kits Platelia Toxo IgM (Biorad) and Toxo-ISAGA (bioMérieux) used respectively in Saint-Etienne and Marseille, can be considered as reference techniques since there is not an accepted gold standard for the detection of IgM Toxoplasma antibodies. In addition, serological follow-up remains essential to monitoring the secondary occurrence of IgG.
From non-selected panel, the sensitivity and specificity of ICT were compared with those of the Architect IgG assay. The sensitivity is 100% for the ICT and 92.1% for the Architect (cut-off at 1.6 IU/ml). The Toxoplasma ICT is particularly interesting for equivocal results on the Architect. Indeed, when the results are in the Architect gray-zone (between 1.6 and 2.9 IU/ml), the test shows clear bands.
The selected panel evaluated the test performance for 92 sera with low IgG titres and 83 sera with specific or non-specific IgM. The 92 sera confirm a higher sensitivity of the ICT compared to the Architect. For IgM, the ICT is of great interest in the presence of IgM without IgG. It can distinguish non-specific IgM from specific Toxoplasma IgM. It is very sensitive and specific at the same time.
To conclude, this new rapid point of care test is easily carried out and interpreted; its reliability criteria make it an excellent second-line technique. It can overcome the crucial points of the Architect, as well as a large number of automated techniques, which are insufficient sensitivity for IgG and a specificity problem for IgM. It can be implemented in specialized and non-specialized laboratories.

Point-of-care testing for Toxoplasma gondii IgG/IgM using Toxoplasma ICT IgG-IgM test with sera from the United States and implications for developing countries. Begeman IJ, Lykins J, Zhou Y, Lai BS, Levigne P, El Bissati K, Boyer K, Withers S, Clouser F, Noble AG, Rabiah P, Swisher CN, Heydemann PT, Contopoulos-Ioannidis DG, Montoya JG, Maldonado Y, Ramirez R, Press C, Stillwaggon E, Peyron F, & McLeod R, *PLoS Negl Trop Dis* **2017** Jun 26;11(6):e0005670. doi: 10.1371/journal.pntd.0005670.

BACKGROUND: Congenital toxoplasmosis is a serious but preventable and treatable disease. Gestational screening facilitates early detection and treatment of primary acquisition. Thus, fetal infection can be promptly diagnosed and treated and outcomes can be improved. METHODS: We tested 180 sera with the Toxoplasma ICT IgG-IgM point-of-care (POC) test. Sera were from 116 chronically infected persons (48 serotype II; 14 serotype I-III; 25 serotype I-IIIa; 28 serotype Atypical, haplogroup 12; 1 not typed). These represent strains of parasites infecting mothers of congenitally infected children in the U.S. 51 seronegative samples and 13 samples from recently infected persons known to be IgG/IgM positive within the prior 2.7 months also were tested. Interpretation was confirmed by two blinded observers. A comparison of costs for POC vs. commercial laboratory testing methods was performed. RESULTS: We found that this new Toxoplasma ICT IgG-IgM POC test was highly sensitive (100%) and specific (100%) for distinguishing IgG/IgM-positive from negative sera. Use of such reliable POC tests can be cost-saving and benefit patients.
CONCLUSIONS: Our work demonstrates that the Toxoplasma ICT IgG-IgM test can function reliably as a point-of-care test to diagnose Toxoplasma gondii infection in the U.S. This provides an opportunity to improve maternal-fetal care by using approaches, diagnostic tools, and medicines already available. This infection has serious, lifelong consequences for infected persons and their families. From the present study, it appears a simple, low-cost POC test is now available to help prevent morbidity/disability, decrease cost, and make gestational screening feasible. It also offers new options for improved prenatal care in low- and middle-income countries.

Evaluation of the LDBIO point of care test for the combined detection of toxoplasmic IgG and IgM. Chapey E, Wallon M, & Peyron F, *Clin Chim Acta* **2017** Jan;464:200-201. doi: 10.1016/j.cca.2016.10.023.

The toxoplasma ICT IgG-IgM rapid diagnostic test for the simultaneous detection of specific toxoplasmic immunoglobulin (Ig) G and IgM was compared with the Architect fully automated chemiluminescence test. Four hundred sera were included, among which 248 scored negative in Architect. The cassettes were easily read with the naked eye. Diagnostic sensitivity and specificity were 97% and 96%, respectively. The test scored 8 false-positive IgG and yielded negative results in 3 sera displaying unspecific IgM in Architect. The LDBIO appears to be a reliable first line test, although the false-positive results for IgG deserve further investigation. Such an easily performed test could be used advantageously for screening for toxoplasmosis in pregnant women.

SCHISTOSOMA ICT IgG IgM

Accuracy of parasitological and immunological tests for the screening of human schistosomiasis in immigrants and refugees from African countries: An approach with Latent Class Analysis. Beltrame A, Guerriero M, Angheben A, Gobbi F, Requena-

Mendez A, Zammarchi L, Formenti F, Perandin F, Buonfrate D, & Bisoffi Z, *PLoS Negl Trop Dis* **2017** Jun 5;11(6):e0005593. doi: 10.1371/journal.pntd.0005593.

BACKGROUND: Schistosomiasis is a neglected infection affecting millions of people, mostly living in sub-Saharan Africa. Morbidity and mortality due to chronic infection are relevant, although schistosomiasis is often clinically silent. Different diagnostic tests have been implemented in order to improve screening and diagnosis, that traditionally rely on parasitological tests with low sensitivity. Aim of this study was to evaluate the accuracy of different tests for the screening of schistosomiasis in African migrants, in a non endemic setting.
METHODOLOGY/PRINCIPAL FINDINGS: A retrospective study was conducted on 373 patients screened at the Centre for Tropical Diseases (CTD) in Negrar, Verona, Italy. Biological samples were tested with: stool/urine microscopy, Circulating Cathodic Antigen (CCA) dipstick test, ELISA, Western blot, immune-chromatographic test (ICT). Test accuracy and predictive values of the immunological tests were assessed primarily on the basis of the results of microscopy (primary reference standard): ICT and WB resulted the test with highest sensitivity (94% and 92%, respectively), with a high NPV (98%). CCA showed the highest specificity (93%), but low sensitivity (48%). The analysis was conducted also using a composite reference standard, CRS (patients classified as infected in case of positive microscopy and/or at least 2 concordant positive immunological tests) and Latent Class Analysis (LCA). The latter two models demonstrated excellent agreement (Cohen's kappa: 0.92) for the classification of the results. In fact, they both confirmed ICT as the test with the highest sensitivity (96%) and NPV (97%), moreover PPV was reasonably good (78% and 72% according to CRS and LCA, respectively). ELISA resulted the most specific immunological test (over 99%). The ICT appears to be a suitable screening test, even when used alone.
CONCLUSIONS: The rapid test ICT was the most sensitive test, with the potential of being used as a single screening test for African migrants.

ASPERGILLUS WB IgG

Evaluation of the Aspergillus Western blot IgG kit for diagnosis of chronic aspergillosis.

Oliva A, Flori P, Hennequin C, Dubus JC, Reynaud-Gaubert M, Charpin D, Vergnon JM, Gay P, Colly A, Piarroux R, Pelloux H, & Ranque S, *J Clin Microbiol* **2015** 53(1):248-54. doi: 10.1128/JCM.02690-14.

Immunoprecipitin detection (IPD) is the current reference confirmatory technique for anti-Aspergillus antibody detection; however, the lack of standardization is a critical drawback of this assay. In this study, we evaluated the performance of the Aspergillus Western blot (Asp-WB) IgG kit (LDBio Diagnostics, Lyon, France), a recently commercialized immunoblot assay for the diagnosis of various clinical presentations of chronic aspergillosis. Three hundred eight serum samples from 158 patients with aspergillosis sensu lato (s.l.) were analyzed. More specifically, 267 serum samples were derived from patients with Aspergillus disease, including 89 cases of chronic

pulmonary aspergillosis, 10 of aspergilloma, and 32 of allergic bronchopulmonary aspergillosis, while 41 samples were from patients with Aspergillus colonization, including 15 cystic fibrosis (CF) and 12 non-CF patients. For blood donor controls, the Asp-WB specificity was 94%, while the kit displayed a sensitivity for the aspergillosis s.l. diagnosis of 88.6%, with a diagnostic odds ratio (DOR) of 119 (95% confidence interval [CI], 57 to 251). The DOR values were 185.22 (95% CI,78.79 to 435.45) and 43.74 (95% CI, 15.65 to 122.20) for the diagnosis of Aspergillus disease and Aspergillus colonization, respectively. Among the patients, the sensitivities of the Asp-WB in the diagnosis of Aspergillus colonization were 100% and 41.7% in CF and non-CF patients, respectively. The Asp-WB yielded fewer false-negative results than did IPD. In conclusion, the Asp-WB kit performed well for the diagnosis of various clinical presentations of aspergillosis in nonimmunocompromised patients, with an enhanced standardization and a higher sensitivity than with IPD, which is the current reference method.

Diagnosis of pulmonary aspergilloma by serological Aspergillus antibody detection: comparison of a new commercial immunoblot-based test with detection by immunoprecipitation, G. Paugam, D. Toubas, E. Dannaoui, P. Le Pape, M. Cornet, & F. Persat, **2013** *TIMM, Copenhaguen.*

Diagnosis of pulmonary aspergilloma is based on chest radiographic images (fungus ball) and specific antibody detection by serologic procedures. Concerning Aspergillus serology the reference method remains detection by immunoprecipitation although this method lacks standardization.
Objective. To compare the commercially available IMB kit " Aspergillus WB IgG" (LBDBio Diagnostics, Lyon, France) with the immunoprecipitation method used in our laboratory. A panel of sera from patients with proven aspergilloma (lung biopsy showing mycelium and culture of Aspergillus fumigatus) was tested using the two methods in parallel.
Methods. We used a total of patient 32 sera. Nine were collected at our hospital and the 23 sera were provided by 4 other hospitals. IMB was used in accordance with manufacturer's instruction. A serum was considered as positive if a minimum of 2 bands were detected among the 4 specific Aspergillus antigen bands (16, 18-20, 22, 30 kD). IPD used commercial somatic and metabolic antigens (BioRad, Marne-La-Coquette, France) and a commercial Aspergillus positive control serum (bioRad,Marne-La-Coquette,France). A serum was considered as positive if one somatic or metabolic antigen was precipitated.
Results. Of these 32 sera 19 were positive with IPD (detection from one to nine antigens). The sensitivity was 59%. Thirty-one sera were positive with IMB (detection from 2 to 4 bands). The two bands the most often observed were P16 and P18-20. The sensitivity was 97%. IMB showed significantly higher sensitivity than IPD. Specificity of IMB previously performed on blood donors (n = 213) was evaluated to 96%. IMB offers important advantages over IPD: small amounts of serum are required (15 µl versus 60µl) and in contrast to IPD the criteria for interpreting positivity is simpler. The antigen-positive bands are much easier to detect than precipitated antigens.
Conclusion. IMB appears a better method to diagnose pulmonary aspergilloma.

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Aspergillus serology: Have we arrived yet? Richardson MD, *Med Mycol* **2017** Jan 1;55(1):48-55. Review. PMID: 27816904

Aspergillus serology, from yesterday to today for tomorrow. Persat F, *Journal de Mycologie Médicale,* **2012**; 22 (1): 72‑82. doi:10.1016/j.mycmed.2012.01.004.

Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. De Pauw, Ben, Thomas J. Walsh, J. Peter Donnelly, David A. Stevens, John E. Edwards, Thierry Calandra, Peter G. Pappas, et al., *Clinical Infectious Diseases* **2008** 46 (12): 1813‑21. doi:10.1086/588660.

Pulmonary Aspergillosis: A Clinical Update. Zmeili, O. S., & A. O. Soubani, *QJM: Monthly Journal of the Association of Physicians* **2007**;100 (6): 317‑34. doi:10.1093/qjmed/hcm035.

Chronic Cavitary and Fibrosing Pulmonary and Pleural Aspergillosis: Case Series, Proposed Nomenclature Change, and Review. Denning, David W., Kostantinos Riniotis, Richard Dobrashian, & Helen Sambatakou, *Clinical Infectious Diseases* **2003** 37 Suppl 3: S265‑80. doi:10.1086/376526.

LDBIO TOXO II IgG – confirmation

Help in the Choice of Automated or Semi-automated Immunoassays for Serological Diagnosis of Toxoplasmosis: Evaluation of Nine Immunoassays by the French National Reference Center for Toxoplasmosis. Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, Paris L, Pelloux H, Villena I, & Candolfi E, *J Clin Microbiol* **2016** Dec 54(12):3034-3042.

Toxoplasmosis, a benign infection, is asymptomatic or paucisymptomatic in over 80% of cases, except in immunocompetent patients suffering from ocular toxoplasmosis or in immunocompromised patients with opportunistic or congenital toxoplasmosis. Diagnosis is based mainly on serology testing. Thus, we compared the performance of the nine most commonly used

commercial automated or semiautomated immunoassays for IgG and IgM Toxoplasma gondii antibody detection, that is, the Advia Centaur, Architect, AxSYM, Elecsys, Enzygnost, Liaison, Platelia, VIDAS, and VIDIA assays. The assays were conducted on four panels of serum samples derived during routine testing from patients with an interfering disease and who exhibited a low IgG antibody level in one of two clinical settings, namely, acute or chronic toxoplasmosis. As a result, IgG sensitivities ranged from 97.1% to 100%, and IgG specificities ranged from 99.5% to 100%. For IgG quantification, strong differences in IgG titers (expressed in IU/ml) were noted depending on the assay used. IgM sensitivities ranged from 65% to 97.9%, and IgM specificities ranged from 92.6% to 100%. For defining the best serological strategies to be implemented, it appears crucial to compare the diagnostic performance of the different tests with respect to their specificity and sensitivity in detecting the presence of IgG and IgM antibodies.

Serological diagnosis of Toxoplasma gondii infection: Recommendations from the French National Reference Center for Toxoplasmosis. Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, Paris L, Pelloux H, Villena I, & Candolfi E, *Diagn Microbiol Infect Dis* **2016** 84(1):22-33. doi: 10.1016/j.diagmicrobio.2015.09.009.

Toxoplasmosis manifests no clinical signs in 80% of cases in immunocompetent patient, causing immunization characterized by the persistence of cysts, particularly in brain, muscles, and retina. Assessing the serological status, based on testing for serum toxoplasma IgG and IgM antibodies, is essential in cases that are increasingly at risk for the more severe disease forms, such as congenital or ocular toxoplasmosis. This disease also exposes immunosuppressed patients to reactivation, which can lead to more widespread forms and increased mortality. By interpreting the serological results, we can estimate the risk of contamination or reactivation and define appropriate prophylactic and preventive measures, such as hygienic and dietetic, therapeutic, biological, and clinical follow-up, according to the clinical context. We hereby propose practical approaches based on serological data, resulting from a consensus of a group of experts from the French National Reference Center Network for Toxoplasmosis, according to both routine and specific clinical situations.

Utility of immunoblotting for early diagnosis of toxoplasmosis seroconversion in pregnant women. Jost C, Touafek F, Fekkar A, et al., *Clinical and vaccine immunology* **2011** 18(11):1908–1912. doi: 10.1128/CVI.05303-11.

Congenital transmission of Toxoplasma gondii occurs mainly when a mother acquires the infection for the first time during pregnancy. It was recently shown that although early treatment of the primary infection during pregnancy has little or no impact on the fetomaternal transmission rate, it does reduce the incidence of sequelae in infected infants. Seroconversion is defined by the appearance of IgG. Commercial reagents continue to vary considerably in detecting low concentrations of antibodies, as during early seroconversion. We compared two routinely used immunoassays (IA) (Platelia and Elecsys Toxo IgG) and an indirect immunofluorescence assay (IIF) with a qualitative test based on immunoblot analysis (Toxo II IgG) (IB) to assess their abilities to diagnose seroconversion at its earliest stages. This prospective study was carried out between January and November 2010. It included 39 pregnant women with monthly follow-up who seroconverted during pregnancy. On first sera that were IgM positive but IgG negative (or

equivocal) as detected by IA, IB diagnosed seroconversion twice as often as IIF (26/39 [66.7%] versus 13/39 [33.3%]; $P < 0.001$; $\chi(2)$ test). Serum samples were retaken 2 to 5 weeks later for the other 13 cases (IgG negative by IB on first serum). Seroconversion was demonstrated as follows: IB for 5 cases where IA remained negative or equivocal, IB and IIF for 5 cases where IA remained negative or equivocal, IA for 2 cases, and no method for 1 case (a third sample was necessary). In summary, IB permitted toxoplasmosis seroconversion diagnosis before other means in 92.3% of cases (36/39) and thus earlier therapeutic intervention.

Discrepancies between a new highly sensitive Toxoplasma gondii ELISA assay and other reagents: interest of Toxo IgG Western blot. Leslé F, Touafek F, Fekkar A, Mazier D, & Paris L, *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology* **2011** 30(10):1207–1212. doi: 10.1007/s10096-011-1214-1.

Immunodiagnostic assays are commonly used to screen for maternal toxoplasmic seroconversion during pregnancy. The introduction to the market of a new highly sensitive IgG assay, the Elecsys Toxo IgG test, has resulted in discrepancy issues with other immunoassays because of a lack of standardisation. Western blot appears to be a good alternative gold standard to the dye test, as the latter is not routinely available. For the present prospective study, we compared the analytical performances of two immunoassays, Elecsys Toxo IgG (Roche Diagnostics) and Platelia Toxo IgG (Bio-Rad, Marnes la Coquette, France), to Toxo II IgG Western blot (LDBio, Lyon, France) using 231 consecutive sera with low or equivocal IgG titres. Of these 231 sera, 213 presented discrepancies, which showed the importance of a confirmation test. Of the Elecsys Toxo IgG-positive results, 100% were confirmed by the Western blot with a positive threshold of 30 IU/ml for Elecsys; in the equivocal area (1-30 IU/ml), Western blot is negative in 54% of cases. Our results suggest that the lower diagnostic cut-off of Platelia Toxo IgG should be further reduced. Our study indirectly confirms that monitoring, especially for pregnant women, must be done in the same laboratory using the same technique. The ability to diagnose very early seroconversion using Western blot merits further study.

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CONGENITAL TOXOPLASMA WB IgG-IgM

Comparison of mother and child antibodies that target high-molecular-mass Toxoplasma gondii antigens by immunoblotting improves neonatal diagnosis of congenital toxoplasmosis. L'Ollivier C, Wallon M, Faucher B, Piarroux R, Peyron F, & Franck J, *Clin Vaccine Immunol* **2012** 19(8):1326-8. doi: 10.1128/CVI.00060-12.

This retrospective study proposes a new reading of immunoblotting (IB) in the diagnosis of congenital toxoplasmosis. Our findings demonstrate that a three-IgM-band association at 75, 90, and 100 kDa called the IgM triplet increases the sensitivity to 95.8% when combined with prenatal and serological neonatal tests.

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Serological diagnosis of Toxoplasma gondii infection: Recommendations from the French National Reference Center for Toxoplasmosis. Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, Paris L, Pelloux H, Villena I, & Candolfi E, *Diagn Microbiol Infect Dis* **2016** 84(1):22-33. doi: 10.1016/j.diagmicrobio.2015.09.009.

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Evaluation of a commercial IgG/IgM Western blot assay for early postnatal diagnosis of congenital toxoplasmosis. Rilling V, Dietz K, Krczal D, Knotek F, & Enders G, *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology* **2003** 22(3):174–180.

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Congenital toxoplasmosis diagnosis by immunoblot; a prospective study using a new commercial immunobloting kit. J. Franck, F. Lanza-Silhol, L. Vuillemot, V. Pelissier, & H. Dumon, *VIII European Multicolloquium of Parasitology, Poznan,* **2000**.

OCULAR TOXOPLASMA WB IgG-IgA

Diagnostic approach to ocular toxoplasmosis.

Garweg JG[1], de Groot-Mijnes JD, & Montoya JG, *Ocul Immunol Inflamm* **2011** 19(4):255-61. doi: 10.3109/09273948.2011.595872.

Toxoplasmic retinochoroiditis is deemed a local event, which may fail to evoke a detectable systemic immune response. A correct diagnosis of the disease is a necessary basis for estimating its clinical burden. This is not so difficult in a typical clinical picture. In atypical cases, further diagnostic efforts are to be installed. Although the aqueous humor may be analyzed for specific antibodies or the presence of parasitic DNA, the DNA burden therein is low, and in rare instances a confirmation would necessitate vitreous sampling. A laboratory confirmation of the diagnosis is frustrated by individual differences in the time elapsing between clinical symptoms and activation of specific antibody production, which may result in false negatives. In congenital ocular toxoplasmosis, a delay in the onset of specific local antibody production could reflect immune tolerance. Herein, the authors attempt to provide a simple and practicable algorithm for a clinically tailored diagnostic approach in atypical instances.

Contributions of immunoblotting, real-time PCR, and the Goldmann-Witmer coefficient to diagnosis of atypical toxoplasmic retinochoroiditis. Talabani H, Asseraf M, Yera H, Delair E, Ancelle T, Thulliez P, Brézin AP, & Dupouy-Camet J, *J Clin Microbiol* **2009** 47(7):2131-5. doi: 10.1128/JCM.00128-09.

Ocular toxoplasmosis is a major cause of posterior uveitis worldwide. The diagnosis is based mainly on ophthalmological examination. Biological diagnosis is necessary in atypical cases, and this requires aqueous humor sampling by anterior chamber paracentesis. We evaluated real-time PCR targeting the Toxoplasma gondii 529-bp repeat element, the Goldmann-Witmer coefficient (GWC), and immunoblotting for the diagnosis of toxoplasmic retinochoroiditis in 54 patients with atypical uveitis. The results of these biological tests, applied to paired aqueous humor-serum samples, were compared to the clinical findings. Combining either PCR or the GWC with immunoblotting increased the sensitivity to 73% or 70%, respectively. Together, PCR and the GWC had 80% sensitivity. If feasible, sensitivity can be increased by combining the three methods (85% sensitivity). The interval between symptom onset and anterior chamber paracentesis strongly influenced the detection of specific intraocular antibody synthesis. The sensitivity of the GWC increased from 45% to 56% when sampling was performed 10 days after symptom onset, and that of immunoblotting increased from 53% to 72% when puncture was performed 30 days after symptom onset. PCR analysis of aqueous humor samples detected toxoplasmic DNA in 55% of patients. In contrast to the results of immunoblotting and the GWC, the results of PCR were not influenced by the interval between symptom onset and paracentesis. PCR was more informative than the GWC and immunoblotting for immunocompromised patients. Acute necrotizing retinal lesions were significantly larger in PCR-positive patients, with a mean of 3.5 optic disc diameters, than in PCR-negative patients, with a mean of 1.5 optic disc diameters.

Comparison of immunoblotting, calculation of the Goldmann-Witmer coefficient, and real-time PCR using aqueous humor samples for diagnosis of ocular toxoplasmosis. Fekkar A, Bodaghi B, Touafek F, et al., *Journal of clinical microbiology* **2008** 46(6):1965–1967.

We compared three biological methods for the diagnosis of ocular toxoplasmosis (OT). Paired aqueous humor and serum samples from 34 patients with OT and from 76 patients with other ocular disorders were analyzed by three methods: immunoblotting or Western blotting (WB), the calculation of the Goldmann-Witmer coefficient (GWC), and PCR. WB and GWC each revealed the intraocular production of specific anti-*Toxoplasma* immunoglobulin G in 81% of samples (30 of 37). PCR detected toxoplasmic DNA in 38% of samples (13 of 34). Nine of the 13 PCR-positive patients were immunocompetent. Combining the techniques significantly improved the diagnostic sensitivity, to 92% for the GWC-WB combination, 90% for the WB-PCR combination, and 93% for the GWC-PCR combination. The combination of all three techniques improved the sensitivity to 97%.

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Intraocular anti-Toxoplasma gondii IgA antibody production in patients with ocular toxoplasmosis. Ronday MJ., Ongkosuwito JV, Rothova A, & Kijlstra A, *Am. J. Ophthalmol* **1999** 127, 294–300.

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TOXOCARA WB IgG

Seroprevalence of Toxocara spp. in a rural population in Central African Gabon.

Lötsch F, Obermüller M, Mischlinger J, Mombo-Ngoma G, Groger M, Adegnika AA, Agnandji ST, Schneider R, Auer H, & Ramharter M, *Parasitol Int* **2016** Dec;65(6 Pt A):632-634. doi: 10.1016/j.parint.2016.09.001.

Toxocara spp. are zoonotic parasites with global distribution infecting humans by incidental ingestions of eggs shed in feces of dogs or cats. High seroprevalences have been reported from

several regions of Africa, however data from the Central African region remain limited. Although several clinical entities caused by larvae of Toxocara spp. have been described, the public heath impact of this infection has so far often been neglected. This study was conducted to estimate the prevalence in a rural central African population. The population based study was performed in volunteers in a rural region of Gabon. A two-step testing approach was applied using an ELISA as screening test and a Western Blot (immunoblot) as confirmatory assay. Basic demographic data and risk factors were collected and compared between seropositive and negative participants. In total, 199 out of 332 serum samples were tested positive for Toxocara spp. antibodies (59.9%). After standardization for age to the overall Gabonese population seroprevalence was 53.6% (95% CI 48.2-59.0%). There was a trend towards higher seroprevalence in participants with agricultural activity. Seroprevalence of antibodies against Toxocara spp. is high in this rural population in Gabon. These results are comparable with previous reports from other sub-regions of Africa and add to our understanding of the epidemiology of toxocariasis in Africa. Given the high prevalence of toxocariasis in tropical regions, it may be speculated that clinically relevant presentations (e.g. visceral or ocular larva migrans syndrome) may occur in considerable numbers. A formal assessment of the burden of disease and the public health impact of human toxocariasis is therefore warranted.

Ocular Toxocariasis: Clinical Features and Long-term Visual Outcomes in Adult Patients. Despreaux R, Fardeau C, Touhami S, Brasnu E, Champion E, Paris L, Touitou V, Bodaghi B, & Lehoang P, *Am J Ophthalmol* **2016** Jun;166:162-8. doi: 10.1016/j.ajo.2016.03.050.
PURPOSE: To investigate clinical characteristics and treatment outcomes of proven ocular toxocariasis (OT) in adult patients. DESIGN: Retrospective, consecutive, interventional case series. METHODS: setting: Institutional. STUDY POPULATION:Consecutive OT patients with positive serum serology and positive western blot (WB) on ocular sample. OBSERVATION PROCEDURES: Clinical features, optical coherence tomography (OCT), and treatment outcomes. MAIN OUTCOME MEASURES: Best-corrected visual acuity (BCVA) and OCT central foveal thickness (CFT).
RESULTS: Fourteen patients were included between 2011 and 2013. Mean age at diagnosis was 45.6 years. Mean duration between the first symptoms and diagnosis was 15.1 months. Uveitis was unilateral in all cases and all patients displayed vitreous inflammation. The main baseline findings were presence of ≥1 peripheral granulomas (57.1%), vasculitis (57.1%), vitreoretinal traction (57.1%), and chronic macular edema (ME) (71.4%). Delayed diagnosis (>8 months) seemed to be associated with higher rate of ME. All patients received albendazole. Systemic (n = 5) and/or local corticosteroids (CS) (n = 7) were administered in case of ME and/or posterior segment inflammation. Vitrectomy was performed when vitreous inflammation was severe and persistent despite CS or in case of threatening traction or visually significant epimacular membrane (28.6%). Overall, this regimen allowed significant decrease of CFT (P = .01). In the vitrectomy subgroup, mean BCVA increased (P = .01) and CFT decreased (P = .017).
CONCLUSION: While some features such as granuloma are typical signs of OT, atypical features can delay the diagnosis. In doubtful situations, WB on ocular samples seems to be more specific than serum antibodies alone. ME seems to be a common complication of longstanding OT in the adult.

Research on Toxocara canis antibodies obtained from patients with eosinophilia. Artinyan E, Uysal HK, Akgul O, Altiparmak S, & Oner YA, *Indian J Med Microbiol* **2014** 32(4):383-6. doi: 10.4103/0255-0857.142239.

BACKGROUND: Eosinophils may suggest the presence of a great variety of anomalies whereupon allergies, malignancies, certain tissue disorders, idiopathic hypereosynophilic syndrome and parasitic infections (with the exception of protozoons) can be cited as a few. Although the clinical manifestations may differ, the eosinophils level is quite an important data in cases related to the helminth infections. Similarly, in parasitic infections related to larva migrans (visceral, cutaneous, ocular), the eosinophils level is again a primary indicator and its evident cause is the roundworm Toxocara spp.
AIM: The aim of this study was to evaluate the significance characteristics of Toxocara spp. in patients with eosonophilia.
MATERIALS AND METHODS: In our study, serums were collected from 93 patients of various age groups with eosonophilia (10% and above) while visiting Istanbul University Medical Faculty due to various complaints.
RESULTS: Serum samples were treated with Toxocara IgG ready ELISA kit and Toxocara western blot IgG ready kit. Based on the ELISA method; out of 93 patients, 30 patients (32.3%) were positive. Western blot technique; 45 (48.4%) were positive with anti-toxocara IgG antibodies.
CONCLUSION: Results point out to western blot technique being more sensitive and superior on a scale of ($P < 0.0001$) when compared with the ELISA method.

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Investigation of anti-Toxocara antibodies in epileptic patients and comparison of two methods: ELISA and Western blotting. Zibaei M, Firoozeh F, Bahrami P, & Sadjjadi SM, *Epilepsy research and treatment* **2013** 2013:156815. doi: 10.1155/2013/156815.

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Association between epilepsy and cysticercosis and toxocariasis: a population-based case-control study in a slum in India. Singh G, Bawa J, Chinna D, et al., *Epilepsia* **2012** 53(12):2203–2208.

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Evaluation of immunodiagnostics for toxocarosis in experimental porcine cysticercosis. García HH, Cancrini G, Bartalesi F, et al., *Tropical medicine & international health* **2007** 12(1):107–110.

Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature. Eberhardt O, Bialek R, Nägele T, & Dichgans J, *Clinical neurology and neurosurgery* **2005** 107(5):432–438.

Seroprevalence of Toxocara antibodies among patients suspected of ocular toxocariasis in Slovenia. Logar J, Soba B, Kraut A, & Stirn-Kranjc B, *The Korean journal of parasitology* **2004** 42(3):137–140.

Highlights of human toxocariasis. Magnaval JF, Glickman LT, Dorchies P, & Morassin B, *The Korean journal of parasitology* **2001** 39(1):1–11.

LEISHMANIA WB IgG

Western blot analysis as an aid for the diagnosis of cutaneous leishmaniasis due to Leishmania major. Pomares C, Despierres L, del Giudice P, Delaunay P, Michel G, Ferrua B, & Marty P, *Trans R Soc Trop Med Hyg* **2012** 106(7):452-4. doi: 10.1016/j.trstmh.2012.03.001.

Cutaneous leishmaniasis (CL) due to Leishmania major is endemic in the Old World. To evaluate the diagnostic value of Western blot (WB) compared with IFAT, we tested serum samples from 45 patients with proven CL. Twenty-one (47%) patients were positive by IFAT and all patients were positive by WB with specific bands against 14kDa and/or 18kDa Leishmania antigens. Our results suggest that WB could be a useful non-invasive tool for the diagnosis of CL caused by L. major.

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Surveillance of leishmaniases in France, 1999 to 2012. Lachaud L, Dedet JP, Marty P, Faraut F, Buffet P, Gangneux JP, Ravel C, Bastien P, Working Group for the Notification of Human Leishmanioses in France, *Euro Surveill* **2013** 18(29):20534.

Mucosal Leishmania infantum leishmaniasis: specific pattern in a multicentre survey and historical cases. Faucher B, Pomares C, Fourcade S, Benyamine A, Marty P, Pratlong L, Faraut F, Mary C, Piarroux R, Dedet JP, & Pratlong F, *J Infect* **2011** 63(1):76-82. doi: 10.1016/j.jinf.2011.03.012.

Asymptomatic carriage of Leishmania in family members of patients with visceral leishmaniasis in Central Tunisia. Saghrouni F, Khammari I, Kaabia N, et al., *Pathologie-biologie* **2012** Oct;60(5):e55-8. doi: 10.1016/j.patbio.2011.11.001.

Asymptomatic bearing of Leishmania infantum among Tunisian HIV infected patients. Kallel K, Ammari L, Kaouech E, et al., *Pathologie-biologie* **2007** 55(10):521–524.

ECHINOCOCCUS WB IgG

Serological Evidence of Exposure to Globally Relevant Zoonotic Parasites in the Estonian Population. Lassen B, Janson M, Viltrop A, Neare K, Hütt P, Golovljova I, Tummeleht L, & Jokelainen P, *PLoS One* **2016** Oct 10;11(10):e0164142. doi: 10.1371/journal.pone.0164142. eCollection 2016.

We investigated Estonian population and its selected subgroups for serological evidence of exposure to Ascaris lumbricoides, Echinococcus spp., Taenia solium, Toxocara canis, Toxoplasma gondii, and Trichinella spiralis. Serum samples from 999 adults representing general population, 248 children aged 14-18, 158 veterinarians, 375 animal caretakers, and 144 hunters were tested for specific immunoglobulin G antibodies against the selected parasites using commercial enzyme immunoassays (ELISA). Sera yielding positive or twice grey zone Echinococcus spp, T. solium, T. canis, and T. spiralis results were subjected to western blot (WB) analysis. In the general population, based on the ELISA results, the A. lumbricoides seroprevalence was 12.7%, Echinococcus spp. seroprevalence was 3.3%, T. solium seroprevalence was 0.7%, T. canis seroprevalence was 12.1%, T. gondii seroprevalence was 55.8%, and T. spiralis seroprevalence was 3.1%. Ascaris lumbricoides seroprevalences were higher in children and in animal caretakers than in the general population, and T. canis seroprevalence was higher in animal caretakers than in the general population. Compared with the general population, Echinococcus spp. seroprevalence was higher in children. By contrast, T. gondii seroprevalence was higher in animal caretakers, and lower in children, than in the general population. In the general population, the WB-confirmed Echinococcus spp. seroprevalence was 0.5%, T. solium cysticercosis seroprevalence was 0.0%, Toxocara spp. seroprevalence was 14.5%, and Trichinella spp. seroprevalence was 2.7%. WB-confirmed Toxocara spp. seroprevalence was higher in animal caretakers than in the general population. We found serological evidence of exposure to zoonotic parasites in all tested groups. This calls for higher awareness of zoonotic parasitic infections in Estonia.

Serological confirmatory testing of alveolar and cystic echinococcosis in clinical practice: results of a comparative study with commercialized and in-house assays. Reiter-Owona I, Grüner B, Frosch M, et al., *Clinical laboratory* **2009** 55(1-2):41–48.

Sera of 50 patients with either cystic (CE) or alveolar echinococcosis (AE) in different clinical stages were examined for the presence of anti-Echinococcus-antibodies. Antibody-screening was performed with ELISA, IHA and IFAT, and confirmatory testing was done by the commercialized E. multilocularis-specific Em2plus-ELISA versus an in-house E. multilocularis-specific Em10-ELISA. Sera with discrepant confirmatory results were subjected to a commercial Echinococcus IgG Western blot (WB). In sera from patients with CE, the Em2plus-ELISA showed cross-reactions in 23.5%, whereas the Em10-ELISA did not exhibit any cross-reactivity. Cross-reactivity paralleled active infection with high antibody titers in the screening assays. In sera from patients with AE, confirmation by both ELISAs was achieved in 57.6%, mostly in patients with an advanced stage of the disease and high antibody titers in the screening assays. False-negative reactions of both ELISAs occurred in 30.3%, mostly in patients who had low antibody levels in the screening tests. The Em2plus-ELISA exhibited fewer false-negative reactions than the Em10-ELISA. The WB confirmed the positive results of either assay and was the assay with the highest reliability at different stages of CE and AE, followed by the Em2plus-ELISA for AE. High antibody titers in the screening assays will favour the detection of species-specific antibodies in either form.

Contribution of Western blotting to the diagnosis of hydatidosis. Makni F, Hachicha L, Mseddi F, et al., *Bulletin de la Société de pathologie exotique* **2007** 100(3):171–173.

The aim of this study is to evaluate the contribution of the immunoWesternblot for the diagnosis and the post surgical follow-up of the hydatidosis. 71 sera from patients with hydatidosis confirmed by surgery were studied. All had a negative hydatic serology by screening tests (enzyme-linked immunosorbent assay, hemagglutination, electrosyneresis). 12 patients with sera in pre and post operative were monitored for 2 years. The Echinococcus Western blot IgG permitted to rectify the diagnosis of hydatidosis in 67.6 %. The rate of positivity was 100 % for the multivesicular liver cysts, 60 % for the young cysts and 50 % for the calcified cysts. Western blot permitted to rectify the diagnosis of lung cysts in 62.5 % of cases and in 50 % of cranial-spinal localizations. Analysis of Western Blot evolution in the 12 patients followed in pre and post-surgical revealed the disappearance of the bands 16, 18 and 26-28kDa in 8 month in the 8 patients with complete exeresis. This study proved the value added of Westernblot compared to the other traditional techniques for the immunodiagnostic and the post-surgical monitoring of hydatidosis.

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Human echinococcosis in Poland in 2003-2010 according to the serological tests results of NIPH-NIH. Wnukowska N, Salamatin R, & Gołab E, *Przegląd epidemiologiczny* **2011** 65(3):455–458. PMID:22184948

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Immunodiagnosis of Echinococcus infections: confirmatory testing and species differentiation by a new commercial Western Blot. Liance M, Janin V, Bresson-Hadni S, et al., *Journal of clinical microbiology* **2000** 38(10):3718–3721.

CYSTICERCOSIS WB IgG

Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis. Gekeler F, Eichenlaub S, Mendoza EG, et al., *European journal of clinical microbiology & infectious diseases* **2002** 21(3):227–229.

In patients with neurocysticercosis (NCC), clinical manifestations and the results of neuroimaging procedures vary widely and often do not facilitate a definite diagnosis. In order to determine the value of immunodiagnosis for NCC, 222 serum and cerebrospinal fluid samples from patients with NCC and healthy subjects were examined. The samples represented patients from various endemic regions, those with other neurological disorders from an endemic area (Mexico), persons with various helminth infections other than NCC, and a group of healthy volunteers. All specimens were tested by enzyme-linked immunosorbent assay and immunoblot for the presence of Taenia solium-specific antibodies. The sensitivities of the enzyme-linked immunosorbent assay and the immunoblot test in NCC patients were almost identical (80% and 81.7%, respectively). For both tests, the sensitivity was higher when cerebrospinal fluid (86%) was tested compared with serum (75%). The overall specificity of enzyme-linked immunosorbent assay was only 75.3% because of frequent false-positive results in patients with other helminth infections, especially in those with echinococcosis. The specificity (99.4%) of the immunoblot test was clearly superior. It is concluded that enzyme-linked immunosorbent assay as a screening method and immunoblot as a confirmatory test contribute considerably to the diagnosis of NCC.

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The Serological Survey for Human Cysticercosis Prevalence in Mbulu District, Tanzania. J. Mwang'onde B, *Advances in Infectious Diseases* **2012** 02(03):62–66.

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TRICHINELLA E/S WB IgG

Outbreaks of human trichinellosis, still a challenge for the public health authorities in Bulgaria. Iskra Rainova, Iskren Kaftandjiev, Rumen Harizanov, Nina Tsvetkova, Diana Jordanova, Irina Marinova, Rossitza Kurdova, Todor Kantardjiev, & Nikolai Lalkovski, *Journal of Public Health* **2016** August 24(4):291–297

Aim: Human trichinellosis is an important food-borne zoonotic disease that causes financial losses and health problems for the population. Sporadic cases and outbreaks of different intensities are recorded each year in Bulgaria. With this work we attempt to clarify the main reasons leading to outbreaks of trichinellosis in the country and to compare the recorded incidence with that in other European countries.
Subjects and methods: In the present study, the epidemiological, clinical, and laboratory data of the trichinellosis outbreaks recorded in the country from 2008 to 2014 were analyzed. Epidemiological data based on a standard protocol with full case descriptions were collected for each region of the country and analyzed at the National Center of Infectious and Parasitic Diseases in Sofia.
Results: Between 2008 and 2014, 29 outbreaks were recorded in Bugaria. Of 1670 people who consumed meat or meat products contaminated with Trichinella larvae, 710 were infected. The annual incidence of human trichinellosis for the period varied from 0.22 to 5.82 per 100,000 population. Studies using the polymerase chain reaction technique identified Trichinella spiralis and Trichinella britovi as the causative agents of trichinellosis among humans in Bulgaria.
Conclusions: Of all food-borne parasitic diseases, trichinellosis has the most pronounced negative effect on human health in the Republic of Bulgaria, and the country is still one of the European Union member states with a high human morbidity rate from trichinellosis.

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Trichinellosis. J. Dupouy-Camet, H. Talabani, & T. Ancelle, *Rev Prat* **2010** 60(2) :159-164.

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A major trichinellosis outbreak suggesting a high endemicity of Trichinella infection in northern Laos. Barennes H, Sayasone S, Odermatt P, et al., *The American journal of tropical medicine and hygiene* **2008** 78(1):40–44. World distribution of Trichinella spp. infections in animals and humans. E. Pozio, *Vet. Parasitol* **2007** 149(1-2):3-21.

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Comparative evaluation of a latex agglutination test, two Elisa tests and a Western blot test for the serodiagnosis of human trichinellosis. Andiva S, Yera H, Haeghebaert S, et al., *Annales de biologie clinique* **2002** 60(1):79–83.

SCHISTOSOMA WB IgG

Evidence for a permanent presence of schistosomiasis in Corsica, France, 2015.

Berry A, Fillaux J, Martin-Blondel G, Boissier J, Iriart X, Marchou B, Magnaval JF, & Delobel P, *Euro Surveill* **2016** 21(1). doi: 10.2807/1560-7917.ES.2016.21.1.30100.

We present a case of acute schistosomiasis acquired in Corsica after bathing in the Cavu River during the summer of 2015. The diagnosis was made following epidemiological, laboratory and serological assessments. After a previous outbreak of urogenital schistosomiasis during the summer of 2013, when more than 120 infections were diagnosed, this further case indicates transmission was still effective in 2015, thus suggesting a permanent presence of schistosomiasis in Corsica.

Introgressive hybridizations of Schistosoma haematobium by Schistosoma bovis at the origin of the first case report of schistosomiasis in Corsica (France, Europe).

Moné H, Holtfreter MC, Allienne JF, Mintsa-Nguéma R, Ibikounlé M, Boissier J, Berry A, Mitta G, Richter J, & Mouahid G, *Parasitol Res* **2015** 114(11):4127-33. doi: 10.1007/s00436-015-4643-4.

This study concerns the first urinary schistosomiasis case observed in Corsica (France, Europe) occurring in a 12-year-old German boy. The aim was to identify the relationship between this

Schistosoma haematobium infection and other schistosomes of the Schistosoma group with terminal-spined ova. Morphological and molecular analyses were conducted on the ova. The results showed that the schistosome responsible for the emergence of schistosomiasis in Corsica was due to S. haematobium introgressed by genes from S. bovis.

Accuracy of indirect haemagglutination and western blot assays for the detection of anti-Schistosoma antibodies in non-severe febrile patients in two Tanzanian hospitals.
Bevilacqua N, Pane S, Vairo F, Nicastri E, Paglia MG, Ame SM, Schepisi MS, Kitua A, Mangi S, Racalbuto V, Meschi S, & Ippolito G, *Scand J Infect Dis* **2012** 44(6):453-8. doi: 10.3109/00365548.2011.645505.

BACKGROUND: The diagnosis of schistosomiasis is usually based on clinical data associated with the detection of eggs in stool, urine, and/or rectal and bladder biopsy specimens. However antibody detection can be useful to indicate Schistosoma infection in those for whom eggs cannot be demonstrated. The aim of this study was to assess the seroprevalence of schistosomiasis and to evaluate the accuracy of indirect haemagglutination (IHA) and Western blot (WB) assays for the detection of anti-Schistosoma antibodies in 2 peripheral hospitals of the United Republic of Tanzania.
METHODS: Between February and March 2007 blood samples were collected from 297 non-severe febrile outpatients who attended Chake Chake Hospital, Pemba Island and Tosamaganga Hospital, Iringa region in Tanzania. The samples were processed for Schistosoma antibodies by IHA and WB assays in Italy.
RESULTS: Two hundred and sixty-two of 297 patients were schistosomiasis antibody-positive by IHA (88.2%). Of 142 patients positive by IHA, only 22 (12.4%) cases were confirmed by WB assay. The WB assay confirmed all 35 negative cases previously identified by IHA. The seroprevalence of Schistosoma at Chake Chake Hospital was lower than in Tosamaganga Hospital (9/97, 9.3% vs 13/80, 16.2%).
CONCLUSIONS: Schistosomiasis is endemic in Tanzania, being more prevalent on the mainland than on Pemba Island. The implications of this study are of public health relevance and suggest the need for increased efforts in large-scale chemotherapy-based morbidity control programmes, integrated with those for other soil-transmitted helminthiases, in these 2 peripheral areas of the United Republic of Tanzania.

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An Unusual Case of Hematuria in a French Family Returning from Corsica. Brunet Julie, Alexander W. Pfaff, Yves Hansmann, Guillaume Gregorowicz, Bernard Pesson, Ahmed Abou-Bacar, & Ermanno Candolfi, *International Journal of Infectious Diseases* **2015** 31: 59‑60. doi:10.1016/j.ijid.2014.10.024.

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The authors conducted a retrospective, cross-sectional serological survey in Port-au-Prince using a Western blotting test (LDBIO Diagnostics) on human fascioliasis in Haiti. A total of 216 serum samples obtained from apparently healthy adults were tested. The frequency of antibodies in serum samples of the study population was 6.5% (14/216). The immunodominant bands recognised in Western blots were 27-28 kDa (100%), 42 kDa (64%), 60 kDa, and 8-9 kDa (28%). This is the first survey to reveal a relatively low proportion of asymptomatic F. hepatica-infected humans in Haiti.

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