Immunoblots for Parasites Serology

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Utility of immunoblotting for early diagnosis of toxoplasmosis seroconversion in pregnant women.

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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; χ(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 Toxoplasma gondii highly-sensitive Elisa assay and other reagents: Interest of Toxo IgG Western Blot

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Immunodiagnostic assays are commonly used to screen for maternal toxoplasmic seroconversion during pregnancy. The introduction on the market of a new highly-sensitive
IgG assay, the Elecsys to xo IgG test, has resulted in discrepancy issues with other immu-
noassays. 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 Toxoplasma IgG (Roche diagno-
sics) and Platelia Toxo IgG (BioRad), to Toxo II IgG Western blot (LDBio) using 231
consecutive sera with low or equivocal IgG titres. 213/231 sera presented discrepancies
from which showed the importance of a confirmation test. 100% of the two immunoas-
says IgG positive results 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 di-
agnose very early seroconversion using Western blot merits further study.

Bicentric Evaluation of Six Anti-Toxoplasma Immunoglobulin G automated
immunoassays and comparison with the LDBio-Toxo II IgG Western Blot.
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Toxoplasma gondii serology in pregnant woman: characteristics and pitfalls
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LDBio-Toxo II immunoglobulin G Western blot confirmatory test for anti-
toxoplasma antibody detection.
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CONGENITAL TOXOPLASMOsis

Comparison of mother and child antibodies that target high-molecular-mass Toxoplasma gondii antigens by immunoblotting improves neonatal diagnosis of congenital toxoplasmosis.

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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.

Western blotting for the diagnosis of congenital toxoplasmosis.

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Toxoplasmosis is a common congenital infection. It does not usually produce recognizable signs of infection at birth so most infected newborns are not detected by routine clinical examination and remain untreated. Infected children without clinical symptoms should nonetheless be identified and treated as early as possible. Serological diagnosis of congenital toxoplasmosis is quite difficult. The aim of this study was to evaluate the utility of Western blot for the diagnosis of congenital toxoplasmosis. We compared the immunological profiles of mothers and children to differentiate between passively transmitted maternal antibodies and antibodies synthesized by the infants in the first three months of life. The method enabled us to diagnose congenital toxoplasmosis in cases in which the infection had not been detected by classical serology techniques.
Congenital toxoplasmosis: the importance of the western blot method to avoid unnecessary therapy in potentially infected newborns.

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Toxoplasma gondii infection in pregnancy: opportunities and pitfalls of serological diagnosis

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Recent Developments for Diagnosis of Toxoplasmosis

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Evaluation of a Commercial IgG/IgM Western Blot Assay for Early Postnatal Diagnosis of Congenital Toxoplasmosis.

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Usefulness of Western blot in serological follow-up of newborns suspected of congenital toxoplasmosis.


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The goal of the study reported here was to compare the results of Western blot with other serological methods for testing newborns suspected of having congenital toxoplasmosis. Western blot, enzyme-linked immunosorbent assay, immunoglobulin (Ig)M immunosorbent agglutination assay, and indirect immunofluorescence assay were performed on the
sera of 126 neonates collected at birth and at 1 and 3 months of life. Western blot was more sensitive than IgM detection with the immunosorbent agglutination assay (82.6% vs. 69.6%), and the specificity of the two methods was 96.1% and 92.2%, respectively. Among the serological techniques tested, the combination of Western blot (IgG and IgM) with IgM immunosorbent agglutination assay achieved the greatest improvement in the sensitivity of early (postpartum) diagnosis of congenital toxoplasmosis.


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Congenital toxoplasmosis diagnosis by immunoblot; a prospective study using a new commercial immunoblotting kit

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VIII European Multicolloquium of Parasitology, Poznan, 10-14 Sept. 2000
OCULAR TOXOPLASMOsis

Comparison of immunoblotting, calculation of the Goldmann-Witmer coefficient, and real-time PCR using aqueous humor samples for diagnosis of ocular toxoplasmosis.

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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%.

Determinants of immunodiagnostic success in human ocular toxoplasmosis
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Parasite Immunology 2005;27:61–68

Aqueous Humor and Serum Immunoblotting for Immunoglobulin Types G, A, M, and E in Cases of Human Ocular Toxoplasmosis
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Usefulness of immunoblotting and Goldmann-Witmer coefficient for biological diagnosis of toxoplasmic retinochoroiditis.
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Retinochoroiditis associated with congenital toxoplasmosis in children: IgG antibody profiles demonstrating the synthesis of local antibodies.

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**ECHINOCOCCUS**

Serological confirmatory testing of alveolar and cystic echinococcosis in clinical practice: results of a comparative study with commercialized and in-house assays.

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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.

Evaluation of a commercial Echinococcus Western Blot assay for serological follow-up of patients with alveolar echinococcosis.

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A total of 20 patients with alveolar echinococcosis in different clinical stages according to the WHO-PNM staging system (P, parasitic mass in the liver; N, involvement of neighboring organs; M, metastasis) were followed up serologically with the commercial Echinococcus Western Blot IgG assay and a crude antigen extract enzyme-linked immunosorbent assay (ELISA). The cohort included patients after curative resection and patients who had unresectable lesions with stable disease or progressive infection. There were visible correlations of the crude antigen ELISA index and the presence and intensity of diagnostic bands in the Western blot. In most patients after curative resection, bands at 7, 16, and 18 kDa markedly decreased or vanished after 1 to 4 years. In a patient with a nonviable lesion (it died out), bands at 16 and 18 kDa vanished after 4 years. Among individuals with unresectable lesions but stable disease under antiparasitic chemotherapy, a decrease of all diagnostic bands was visible after 2 to 3 years in half of the patients, whereas the other half had unchanged blot results after 4 to 6 years. Patients with progressive disease showed increasing intensities of bands at 16, 18, and 7 kDa. The change of banding patterns was not influenced by the PNM stage in patients after curative surgery or with unresectable lesions. Our data indicate a correlation of the 7-, 16-, and 18-kDa-Western blot bands with disease activity independent of the PNM stage. This study demonstrated the usefulness of the Echinococcus Western Blot IgG assay as an additional serological test for the follow-up of patients with alveolar echinococcosis.

Serological evidence for human cystic echinococcosis in Slovenia.
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BMC Infect. Dis. 2008 May;9(8):63.

Comparison of several commercial serologic kits and Em18 serology for detection of human alveolar echinococcosis.
Bart JM, Piarroux M, Sako Y, Grenouillet F, Bresson-Hadni S, Piarroux R, Ito A.
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Contribution of Western blotting to the diagnosis of hydatidosis
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Muscular cystic hydatidosis: case report.
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Human alveolar echinococcosis in Slovenia

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Active alveolar hydatidosis with sero-negativity for antibody to the 18 kDa antigen.

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Laboratory evaluation of commercial immunoblot assay kit for serodiagnosis of Echinococcus infections using sera from patients with alveolar hydatidosis in Hokkaido

Furuya K, Kawanaka M, Yamano K, Sato N, Honma H
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Kansenshogaku Zasshi 2004 Apr;78(4):320-6

Immunodiagnosis of Echinococcus infections: confirmatory testing and species differentiation by a new commercial Western Blot.

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The Echinococcus Western Blot IgG (LDBIO Diagnostics, Lyon, France), using a whole larval antigen from Echinococcus multilocularis, was evaluated for serodiagnosis and differentiation between two human parasitic infections of worldwide importance: cystic echinococcosis, due to Echinococcus granulosus, and alveolar echinococcosis, due to E. multilocularis. Fifty and 61 serum samples from patients with cystic and alveolar echinococcosis, respectively, were used for assessing diagnostic sensitivity. The sensitivity of the
assay was compared with those of screening tests used for these applications. Sera used for assessing cross-reactivities were from 154 patients with other diseases, either parasitic or not. The assay allowed the detection of serum immunoglobulin G antibodies in 97% of Echinococcus-infected patients. It had a higher sensitivity than screening assays for the detection for each echinococcosis. The assay allowed us to correctly distinguish between E. granulosus- and E. multilocularis-infected patients in 76% of cases. It did not allow us to distinguish active from inactive forms of both echinococcoses. The occurrence of cross-reactivities with neurocysticercosis indicates the necessity for retesting sera with species-specific antigens, for rare patients with neurologic disorders. This study shows the usefulness of the commercially available Echinococcus Western Blot IgG for the serological confirmation of human echinococcosis.

SCHISTOSOMA

Development and Evaluation of a Western Blot Kit for Diagnosis of Schistosomiasis

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We evaluated the performance of Western blot (WB) analysis using commercially available antigen strips and compared the results with those of indirect hemagglutination (IHA) and indirect immunofluorescence (IFAT) for the serodiagnosis of human schistosomiasis. The antigen preparation was a crude extract of Schistosoma mansoni. The WB profile characteristics of schistosomiasis were characterized by comparing the results for 58 serum samples from patients with parasitologically proven S. mansoni (n = 12) and S. haematobium (n = 46) infections and 37 individuals with probable cases of schistosomiasis but with only positive serology results. The specificity of WB analysis was assessed by testing 12 serum samples from healthy subjects, 67 serum samples from patients with other proven helminthic and protozoan infections, and 16 serum samples from patients with autoantibodies. Six immunodominant bands (65, 70, 80, 95, 110, and 120 kDa) were revealed with sera from patients with schistosomiasis. The presence of three or more bands in the range 65 to 120 kDa, with the exception of the 100-kDa band, was considered diagnostic for Schistosoma infection and had a specificity of 100% in our series. In patients with proven schistosomiasis, the sensitivity of WB analysis was 84.5%, whereas those of IFAT and IHA were 65.5 and 72.9%, respectively. For serologically proven cases, the sensitivity of WB analysis was 97.3%. The overall sensitivity and specificity for both groups of patients were 89.5 and 100%, respectively, with positive and negative predictive values of 100 and 91.3%, respectively. We conclude that WB analysis is a useful technique for the immunological diagnosis of schistosomiasis.
A major trichinellosis outbreak suggesting a high endemicity of Trichinella infection in northern Laos.

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Trichinellosis is an important and under-recognized food-borne zoonosis in Southeast Asia. After 30 years of no reports, a small outbreak was described in Central Lao PDR (Laos) in 2003. Here we report a large outbreak of at least 650 estimated patients in Udomxay (northern Laos) in June 2005. Trichinella ELISA assays on serum from 133 patients and Western blot assays on 16 patients were positive in 67.6% and 81.2%, respectively. No deaths were recorded. Consumption of uncooked or fermented pork at funeral and wedding ceremonies was the main source of infection. Larvae of Trichinella spiralis were found in 1 of 11 local pigs not involved in this outbreak. The results suggest that trichinellosis may be an under-recognized but important endemic disease in Laos and reinforces the need to urgently implement veterinary and educational programs.

Development and evaluation of a Western blot kit for diagnosis of human trichinellosis.

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We evaluated industrially prepared Western blot strips designed to avoid the cross-reactions observed with indirect immunofluorescence and enzyme-linked immunosorbent assays used for the serodiagnosis of trichinellosis. The antigen preparations were crude extracts of Trichinella spiralis. The Western blot profile characteristic of trichinellosis was characterized by comparing 60 sera from patients infected by Trichinella to 11 sera from healthy subjects, 51 sera from patients with other proven parasitic diseases (cysticercosis, schistosomiasis, strongyloidosis, fascioliasis, toxocariasis, liver amebiasis, anisakiasis, filariasis, toxoplasmosis, hydatidosis, or malaria), and 23 sera from patients with autoantibodies. Specific 43- to 44-kDa and 64-kDa bands were obtained with all of the sera from 51 patients with acute trichinellosis, in 4 out of 9 patients at the early stages of the disease, and in only 1 control patient, who had suspected anisakiasis and in whom trichinellosis could not be ruled out by muscle biopsy.
Cutaneous manifestations of human toxocariasis.

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Human toxocariasis is a parasitic disease characterized by the presence of larvae of the genus *Toxocara* in human tissues. *T canis* and *T cati*, the adult roundworms of which are found in dog and cat intestines, respectively, are the most common causative agents of the disease. Toxocarial larvae usually cause two severe syndromes: visceral larva migrans and ocular larva migrans, depending on the location of the larvae. Two other syndromes, covert toxocariasis and common toxocariasis, which are less typical and not as severe, have also been described. During the last two decades, cutaneous manifestations such as chronic urticaria, chronic pruritus, and miscellaneous eczema, in patients with *Toxocara* antibodies, have been studied by different authors. In some cases, these cutaneous manifestations are the only signs indicating the presence of the disease, and they are cured after antiworm treatment when there is good patient compliance. In this review, we focus on these particular skin manifestations regarding their clinical description, diagnosis, and treatment.

Evaluation of immunodiagnostics for toxocarosis in experimental porcine cysticercosis.

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Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature.

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Seroprevalence of Toxocara antibodies among patients suspected of ocular toxocariasis in Slovenia

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*Korean J. Parasitol.* 2004 Sept;42(3):137-140
CYSTICERCOSIS

Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid.

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Neurocysticercosis (NC), caused by the larval stage of Taenia solium, is one of the most common parasitic diseases of the central nervous system. The diagnosis of NC is mostly based on costly brain neuroimaging (computed tomography and/or nuclear magnetic resonance), which is rarely accessible in most affected areas. The most sensitive and specific tools for NC diagnosis are imagery techniques. The identification of specific antibodies and antigens is currently used only to support NC diagnosis due to their limited specificity and sensitivity. This study was performed to compare immunodiagnostic assays (antibody detection by enzyme-linked immunosorbent assay [ELISA] and enzyme-linked immunoelectrotransfer blotting [EITB] and HP10 antigen detection by ELISA) with the detection of parasite DNA by PCR amplification of a repetitive element of the parasite genome in the cerebrospinal fluid (CSF) of 121 radiologically and clinically characterized NC patients. Patients were divided into six groups according to the stage of the parasites and their localization. The CSF cellularity of each patient was also recorded. When all patients were considered, PCR exhibited the highest sensitivity (95.9%) and variable specificity (80% or 100%) depending on the controls used. The sensitivities of antibody detection by ELISA and EITB were not significantly different, and ELISA identified HP10 antigen mostly when vesicular cysticerci were located in the subarachnoidal basal cisterns. These results can help in the selection of different individual assays or combinations of assays to be used in NC diagnosis according to different requirements.

Seroprevalence of human Taenia solium cysticercosis in Haiti.

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Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis.

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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.

**LEISHMANIA**

Mucosal Leishmania infantum leishmaniasis: specific pattern in a multicentre survey and historical cases

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**OBJECTIVE:**
Leishmania infantum mucosally restricted leishmaniasis was rarely reported, so that diagnostic and treatment strategies remain debated. A long-term multicentric survey appeared thereby necessary.

**METHODS:**
Cases were prospectively collected over 12 years in 3 academic hospitals of Southern France. Predisposing factors, clinical findings, diagnostic procedures, treatment and outco-
RESULTS:
Ten new cases and 40 historical reports were collected. Respectively 10/10 and 35/40 patients were adult males. Immunodeficiency was frequent (5/10 and 18/40). No previous cutaneous lesion was reported. Leishmaniasis affected mostly larynx (5/10 and 19/40), but also mouth (2/10 and 19/40) and nose (3/10 and 5/40). Lesions were highly polymorph. Mucosa histological examination provided respectively 1/10 and 2/40 false negative results, contrary to serum immunoblotting and PCR on mucosal biopsy. Although local response was always satisfactory even using topical treatment, subsequent visceral spreading was observed in 2/10 and 1/40 cases.

CONCLUSION:
L. infantum mucosally restricted leishmaniasis exhibits a specific pattern, marked by tropism for adult males, high clinical and histological polymorphism. Immunoblot screening and PCR confirmation of suspected lesions are necessary because of direct examination occasional false negative results. The risk of visceral spreading sustains systemic therapy.

SUMMARY:
Leishmania infantum mucosal leishmaniasis mostly affects adult males, half of them immunodeficient. Clinical and histological polymorphism makes the diagnosis difficult, stressing the need for immunoblot screening and mucosa PCR analysis of suspected cases. Possible visceralization sustains systemic therapy.

FASCIOLA

Cross-sectional serological survey of human fascioliasis in haiti.
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Fasciola hepatica, the aetiological agent of fascioliasis in the Caribbean region, occurs throughout the major islands of the Greater Antilles and in localised zones on two islands (Martinique and Saint Lucia) of the Lesser Antilles. However, apart from Puerto Rico, information regarding human fascioliasis in islands of the Caribbean is out of date or unavailable, or even nonexistent as in Haiti. 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.