TOXOPLASMA SEROLOGY CONFIRMATION

LDBIO TOXO II IGG & LDBIO TOXO II IGM: two new confirmation tests help discriminate between specific and non specific antibodies in pregnant women

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INTRODUCTION

When *Toxoplasma gondii* infection is acquired during pregnancy, it may cause severe complications, such as miscarriage, stillbirths, congenital infections and late neonatal sequelae. The early and definitive detection of a recently acquired infection is critical for the clinical management of the mother and her fetus. IgG antibodies are often absent in early phases of infections, and IgM antibodies may be non specific and disappear at the end of pregnancy. Furthermore, therapy that is given as early as possible could affect antibody production, possibly as a consequence of a decrease in the parasitic load. Clearly, serological diagnosis needs to be improved if we have to distinguish early infections from non-specific antibody response. To this aim, we have evaluated LDBIO TOXO II IgG and LDBIO TOXO II IgM, two new confirmation immunoblots, in 24 pregnant women with suspected seroconversion.

PATIENTS and METHODS

Twenty four pregnant women with suspected seroconversion were referred to the clinic of the Infectious Diseases Department for further diagnostic workup. All women were negative for anti-*Toxoplasma* IgG (Etitoxok IgG DIASORIN Saluggia Italy,VIDAS toxo IgG II BIOMERIEUX Marcy l'Etoile, France) and positive for IgM with at least one of the two tests routinely used in the laboratory (Etitoxok IgM DIASORIN Saluggia Italy, Toxo IgM ISAGA BIOMERIEUX Marcy l'Etoile, France). They were followed weekly to detect the production of anti toxoplasma IgG. Spiramicyn was given from the first positive IgM test. All samples were also tested with LDBIO TOXO II IgG and LDBIO TOXO II IgM – the latter being not commercially available at this date (LDBIO DIAGNOSTICS, Lyon France). In 9 out of 24 women, lymphocyte stimulation was performed too, and CD25 and Stimulation Index were evaluated.

RESULTS

LDBIO TOXO II IgM was positive in twelve women. For 10 the seroconversion was proved with the appearance of specific anti-*Toxoplasma* IgG during the follow-up. In 4 they were already present in the first sample by using LDBIO TOXO II IgG. For the other 6, IgG antibodies were detected on subsequent samples earlier (at least two weeks) with LDBIO TOXO II IgG and later with traditional IgG serological tests. 6 of 10 were also positive in lymphocyte stimulation. Twelve women were LDBIO TOXO II IgM negative and no IgG was detected either with western blot nor with traditional tests on later samples, even after the treatment was discontinued based on this finding. In three cases, the lymphocyte stimulation confirmed these results. Two women resulted positive at LDBIO TOXO II IgM but constantly negative for IgG. One of them was also positive in lymphocyte stimulation.

CONCLUSIONS

In 22 of 24 patients, an early and correct diagnosis was reached with LDBIO TOXO II IgM. In all infected women LDBIO TOXO II IgG was positive earlier and confirmed the seroconversion several weeks before the other IgG serology tests. In all negative cases it was possible to stop safely the therapy and to reassure the women. On the other hand, no infected woman was missed, all positive women were given the appropriate therapy and prenatal diagnosis was offered. We had two false positive results at IgM (one also with lymphocyte stimulation), but the reliable evaluation of the specificity of LDBIO TOXO II IgM was not possible because of the small sample size.

CONGENITAL TOXOPLASMOSIS

IgG IgM profile comparison by Western-Blot in early diagnosis of congenital toxoplasmosis

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Congenital toxoplasmosis does not usually produce recognizable signs of infection at birth, and passively transmitted maternal antibodies could interfere in serological diagnosis. Most infected newborns are undetected by routine clinical and serological examinations at birth and remain untreated for many months and can develop later serious clinical sequelae such as chorioretinitis. So the infected children must be identified and treated as early as possible. The aim of this study was to evaluate diagnostic accuracy of IgG IgM Western-blot profile comparison (TOXOPLASMA WB IgG-IgM - LDBIO DIAGNOSTICS, Lyon France) on 238 newborns at risk of congenital toxoplasmosis.

Two hundred thirty eight neonates born from mother with suspected or certain infection in pregnancy were evaluated retrospectively with TOXOPLASMA WB IgG-IgM (LDBIO Lyon France). Serum obtained from all the newborns at birth was compared with maternal sample and then with samples obtained monthly during their first three months of life.

Furthermore all the sample were analyzed with routine assays: ELISA IgG IgM, IgA (Diasorin Saluggia Italy), IgG ELFA, IgM ISAGA (Biomerieux Marcy L'Etoile France). The patients were tested with all these routine assays monthly until seronegative and then at one year of age.

RESULTS

At the end of the study 42 newborns were found infected. Thirty one were diagnosed at birth by the presence of IgM and/or IgA, in the other 11 diagnosis was made by antibody rebound or by IgG positivity at one year of age. The results of the test in comparison with traditional ISAGA IgM, plus ELISA IgA are shown in Tab 1.

CONCLUSIONS

IgG-IgM Western blot profile comparison showed a specificity (96,9) almost superimposable to the traditional tests (98,9). Sensitivity (95,2) was higher than the traditional tests (76,1) and the difference was statistically significative (P=0,02 Yates Corrected χ squared). WB profile comparison let us find out 9 infected newborns not detectable with traditional tests that could undergone an early treatment, while 190 not infected newborns avoided unnecessary therapy.

Tab.1

	IgM ISAGA+ IgA ELISA			IgG IGM Western blot		
	Pos	Neg	Tot	Pos	Neg	Tot
Infected	31	11	42	40	2	42
Not Infected	2	194	196	6	190	196
Tot	33	205	238	46	192	238
	Sens.76,1 Spec.98,9			Sens.95,2 Spec.96,9		

Predictive values of Western blot IgG and IgM profile comparison in early postnatal diagnosis of congenital toxoplasmosis in Poland and its relation to the population tested

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The prevalence of anti-*Toxoplasma gondii* IgG is 41% among Polish pregnant woman. The incidence of congenital toxoplasmosis (CT) is 1.5 /1000. Due to the lack of routine testing in pregnancy and so-called "wild screening" performed by various laboratories with different methods, there is an urgent need for an additional confirmation tool.

Considering that the predictive values of a test depend critically on the incidence of disease in the patients being tested, the goal was to evaluate TOXOPLASMA WB IgG-IgM test (LDBIO DIAGNOSTICS, Lyon, France) in two groups.

GROUP 1. covered woman with IgG and IgM detected during pregnancy (independently on the time of infection) or tested only after delivery. In the GROUP 2. woman with recent infection in pregnancy based on evolution of IgG and IgM curves (Bessieres, 1999) were included. There were 136 couples mother – cord blood (M-CB) of sera in the group 1 with 24 CT cases and 91 couples M-CB of sera with 12 children born with CT in the group 2. All samples were tested for specific IgG and IgM with VIDAS Toxo-IgG and Toxo-IgM (bioMerieux) and for IgA with Platelia Toxo-IgA (Biorad). Serological follow-up during the first year of life was a "gold standard" method for CT diagnosis.

RESULTS:

GROUP 1: The sensitivity (Se) of WB IgG was 75.3%, specificity (Sp) 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 97.2%. Se of WB IgM was 83.9%, Sp 98.5%, PPV 86.5% and NPV 98.1%. Se of WB IgG + WB IgM was 96.2%, Sp 98.2%, PPV 86.6% and NPV was 99.5%. Se of VIDAS IgM + Platelia IgA + WB IgG + WB IgM was 99.8%, Sp 97.0%, PPV 79.5% and NPV was 99.9%.

GROUP 2: Se of WB IgG was 54.6%, Sp 100.0%, PPV 100.0%, NPV, 91.7%. Se of WB IgM was 66.4%, Sp 98.3%, PPV 88.4%, NPV 93.6%.

CONCLUSION:

The predictive values of WB M-CB vary between the two groups of women. The use of WB M-CB improves the final diagnosis especially when used as a confirmation test in unscreened population.

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

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REVIEW Clin Microbiol Infect 2006; 12: 504–512
Because of its life cycle, the recovery of Toxoplasma gondii from biological samples is often impracticable. Consequently, a serological diagnosis represents the first and the most widely used approach to defining the stage of infection. The detection of IgG, IgM, IgA, IgE and IgG avidity by different methods offers this opportunity. However, the results may be affected by difficulties in interpretation, as the same antibody pattern may have a different valency, contingent upon subjects and clinical settings, e.g., pregnant women vs. neonates, and treated vs. untreated patients. This review describes the various factors that should be taken into account when performing serological tests for T. gondii, as well as the pitfalls that may be encountered during the interpretative process.

Recent Developments for Diagnosis of Toxoplasmosis

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MINIREVIEW Journal of Clinical Microbiology, March 2004, p. 941-945, Vol. 42, No. 3

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

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Eur J Clin Microbiol Infect Dis 2003 Mar;22(3):174-80
The aim of this study was to evaluate a commercial Western blot IgG/IgM assay for use in the early serological diagnosis of congenital toxoplasmosis. This assay compares the immunological profile of mother and infant and allows differentiation between passive transmitted maternal antibodies and newly synthesized antibodies of the infant within the first 3 months

of life. Over a 6-year period (1995-2001), the sera from 169 mothers and their 175 off-spring (6 had twins) were examined for specific anti- Toxoplasma gondii IgG, IgM and IgA antibodies with an enzyme-linked immunosorbent assay or an immunosorbent agglutination assay. All mothers had primary Toxoplasma infection during pregnancy. Serological and clinical follow-up of the infants during the first year of life confirmed 36 cases of congenital toxoplasmosis. In 139 cases, infection could be ruled out. Three hundred fifty-one paired samples from 175 mother-child pairs were tested retrospectively for IgG and IgM patterns by Toxoplasma Western blot IgG/IgM (LDBIO Diagnostics, France). The results of conventional serological analysis (immunosorbent agglutination assay or enzyme-linked immunosorbent assay) to detect IgM or IgA were compared with the results of the Toxoplasma Western blot IgG/IgM on samples obtained within the first 3 months of life. The performance of the combination of the two methods was also assessed. At birth, the sensitivity values of conventional serological analysis and the Toxoplasma Western blot were 52% and 67%, with specificity values being 99% and 96%, respectively. Combination of the Western blot and conventional serological analysis increased the sensitivity at birth to 78% and within the first 3 months of life to 85%. Overall, the combination of both methods detected 94% of congenital infections. Therefore, this commercial Western blot represents a useful tool for early postnatal diagnosis of congenital toxoplasmosis.

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

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Eur J Clin Microbiol Infect Dis 2003 Feb;22(2):122-5
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.

Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies.

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J Clin Microbiol 2001 Jun;39(6):2267-71

In a study involving 14 laboratories supported by the European Community Biomed 2 program, we evaluated immunologic methods for the postnatal diagnosis of congenital toxoplasmosis (CT). Among babies born to mothers who seroconverted to positivity for toxoplasmosis during pregnancy, we analyzed 55 babies with CT on the basis of persistent anti-Toxoplasma immunoglobulin G (IgG) at 1 year of life and 50 control babies without anti-Toxoplasma IgG at 1 year of life in the absence of curative treatment with pyrimethamine-sulfonamides. We tested in-house methods such as the enzyme-linked immunofiltration assay (ELIFA) or Immunoblotting (IB) for the detection of IgG or IgM; these methods allowed comparison of the immunologic profiles of the mothers and the infants. We compared ELIFA and IB with a commercial enzyme immunoassay (EIA) or in-house immunosorbent agglutination assay (ISAGA) for the detection of IgM or IgA. The performances of combinations of methods were also assessed. A cumulative sensitivity of 98% during a 1-year follow-up was obtained with the ELIFA plus ISAGA combination. Only one case of CT was missed by the ELIFA plus ISAGA combination, whereas three cases were missed by the IB plus ISAGA combination, even though 48% of patients with CT were treated with pyrimethamine-sulfonamides, which are known to inhibit antibody neosynthesis. A similar performance was obtained with either ELIFA or IB in combination with EIA. The difference in performance between ELIFA plus ISAGA and IB plus ISAGA was not statistically significant ($P = 0.31$), and we conclude that both combinations of tests can be used for the diagnosis of CT in newborns.

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

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VIII European Multicolloquium of Parasitology, Poznan, 10-14 Sept. 2000
Objectives: Congenital toxoplasmosis is often subclinical and mainly in countries using screening programs. Without treatment, ocular sequels are frequent at short or long term and an earliest diagnosis is very important. In this context, we evaluated the performance of the kit *Toxoplasma* WB IgG-IgM (LDBIO DIAGNOSTICS 19A rue Louis Loucheur, 69009 Lyon). Methods: 106 mother-child couples were included in the study. All mothers had acquired toxoplasmosis during pregnancy. Children were tested at birth, D20, D60, and D90. Specific IgM and IgA were detected by ISAGA and IgG by EIA. 30/106 new-borns were congenitaly infected. The noninfected children had a follow up until the complete disappearence of maternal IgG. Results: At birth, the Positive Predictive Value (PPV) for WB G+M is 100%, the Negative Predictive Value (NPV) for WB G+M is 89.3%. PPV for Isaga is 95.2% and NPV 88.2% At D90, PPV for WB G+M is 100% and NPV 98.7%. For Isaga, PPV is 100% and NPV is 89.3%. False negative results in western blot assay at birth are mostly observed in new-borns treated by pyrimethaminesulfonamides in utero. Conclusions: The kit *Toxoplasma* WB Western Blot IgG-IgM greatly improves the performances of congenital toxoplasmosis serodiagnosis.

OCULAR TOXOPLASMOSIS

Determinants of immunodiagnostic success in human ocular toxoplasmosis

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Parasite Immunology 2005,27, 61–68 Review Article
Ocular toxoplasmosis is a local manifestation of systemic infection in which Toxoplasma spreads into the eye, affecting mainlythe posterior segment of the eye. Reactivation of the initial retinal condition presumably results from the rupture of quiescent parasitic cysts lying adjacent to pre-existing scars and may secondarily involve the choroid (leading to retinochoroiditis). Although the molecular mechanisms underlying host–parasite interaction are largely unknown, toxoplasmic retinochoroiditis usually remains a local event, and does not necessarily evoke a detectable systemic immune response. Local immunotolerance mechanisms may likewise confound attempts to confirm the clinical diagnosis by serology.

Aqueous humour may be analysed for the presence of parasite DNA or of specific antibodies, but the DNA burden therein is low, and a more definite confirmation would require risky puncturing of the vitreous. Laboratory confirmation of the diagnosis is also frustrated by marked individual differences in the time elapsing between the onset of clinical symptoms and the activation of specific antibody production, resulting in a high proportion of false negative results. Whether a delay in the onset of local specific antibody production reflects immunotolerance in cases of congenital – but not obviously in those of acquired – infection remains an open question, but it could account for a relatively low confirmation rate in laboratory tests for local antibody production. Against this background, current diagnostic strategies need to be re-evaluated with a view to future improvements.

Aqueous Humor and Serum Immunoblotting for Immunoglobulin Types G, A, M, and E in Cases of Human Ocular Toxoplasmosis

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The purpose of this study was to compare the local and systemic *Toxoplasma*-specific humoral immune responses in individuals with ocular toxoplasmosis (OT). To this end, paired aqueous humor and serum samples from 46 individuals with active OT and from 30 individuals without inflammatory eye disease (controls) were analyzed by immunoblotting for anti-*Toxoplasma* immunoglobulin G (IgG), IgA, IgM, and IgE directed against 20- to 120-kDa antigens. The presence in the aqueous humor of a unique band, or of at least three bands that were at least three times more intense in aqueous humor than in serum, was taken as evidence of local antibody production. IgG bands were detected in 98% of the aqueous humor samples, while IgA bands were detected in 76%, IgM bands were detected in 8%, and IgE bands were not detected in any. Evidence of local production of specific antibodies was found in 32 cases (70%) (IgG in 23 [50%]; IgA in 16 [35%]). In 10 instances (22%), routine laboratory tests were not indicative of OT. In 14 cases (30%), no local antibody production was detected by immunoblotting; 3 of these cases yielded evidence of local antibody production according to the Goldmann-Witmer coefficient. Local antibody production was revealed for 7 of the 30 controls (23%). Hence, the sensitivity of immunoblotting for IgG and IgA is 70%, and the specificity is 77%. We conclude that immunoblotting for local specific IgG and IgA supports the clinical diagnosis of OT in 70% of cases. In 22% of these, the diagnosis is not confirmed by other laboratory tests. Hence, immunoblotting increases the sensitivity of routine laboratory tests and should be considered for samples that register negative by such tests.

Usefulness of immunoblotting and Goldmann-Witmer coefficient for biological diagnosis of toxoplasmic retinochoroiditis.

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Eur J Clin Microbiol Infect Dis 2004 Jan;23(1):34-8
Toxoplasmosis is a frequent cause of retinochoroiditis. Although the diagnosis relies mainly on ophthalmological examination, biological approaches are particularly useful in patients with atypical lesions. In a prospective study to determine the value of immunoblotting and immune load calculation in the diagnosis of active toxoplasmic retinochoroiditis, aqueous humor samples from 21 patients with retinochoroiditis and 5 control patients with cataracts were tested. Immune load was calculated on the basis of intraocular antibody production. The immune load ratio between aqueous humor and serum (Goldmann-Witmer coefficient) was significant (i.e. >2) in 9 of the 17 (53%) patients with retrospectively documented toxoplasmic retinochoroiditis. Immunoblotting suggested local antibody production in 10 of 17 (59%) patients. The combination of the two techniques gave a sensitivity of 71% (12/17). Both techniques were negative in the four patients in whom the final diagnosis of toxoplasmic retinochoroiditis was negative and in the five patients with cataracts. These results confirm the value of combining these two techniques. Moreover, immunoblotting has the advantages of being easy to perform and of requiring a very small sample.

Retinochoroiditis associated with congenital toxoplasmosis in children: IgG antibody profiles demonstrating the synthesis of local antibodies.

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Eur J Ophthalmol 2003 Jan-Feb;13(1):74-9
PURPOSE: Retinochoroiditis is generally diagnosed after the first year of life and the association with congenital toxoplasmosis presents a diagnostic dilemma. The detection of local intraocular specific antibodies could be useful for diagnosis. METHODS: We studied six patients (mean age 7 +/- 5 years) with retinochoroiditis which appeared after the first year of life. Aqueous and serum were analysed by immunoblotting for anti T. gondii IgG to diagnose toxoplasmosis. RESULTS: All serum samples were positive only for anti T. gondii IgG. The retinochoroiditis was active in three patients and inactive in the others. Immunoblot analysis of serum and aqueous from the patients with active lesions showed IgG versus the specific antigen of T. gondii. In the patients with inactive lesions the pattern was the same in the two compartments. In active forms, aqueous and serum Western blot patterns differed in proteins lower than 16kDa and higher than 116kDa: in aqueous the findings were typically positive for 30kDa. CONCLUSIONS: Aqueous humour analysis by the Western blot technique may be useful in the diagnosis of congenital toxoplasmosis. In the present small series, we nevertheless detected different patterns for inactive and active retinochor-

oiditis, confirming the diagnosis in the latter. Aqueous humour paracentesis may be indicated in a child with active retinochoroiditis with unusual clinical features, appearing after the first year of life, and with no clinical or serological evidence of congenital infection.

ECHINOCOCCUS

Human alveolar echinococcosis in Slovenia

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Clin Microbiol Infect 2007; 13: 544–546
Between January 2001 and December 2005, 1263 patients suspected of having echinococcosis were screened serologically by indirect haemagglutination assay (IHA). IHA-positive patient sera were then retested by western blot for confirmation and differentiation between Echinococcus granulosus and Echinococcus multilocularis infection. Of 43 sera confirmed as Echinococcus-positive, nine appeared to be specific for alveolar echinococcosis (AE) caused by E. multilocularis. AE-positive serological results corresponded to the clinical and/or imaging findings concerning the patients' liver cysts. The detected incidence of AE was 0.45/105 inhabitants, which suggests that clinicians and health authorities in Slovenia should give greater attention to AE in the future.

The immunodiagnosis of Echinococcus multilocularis infection

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REVIEW: Clinical Microbiology and Infection (OnlineEarly Articles).
doi:10.1111/j.1469-0691.2007.01665.x
Alveolar echinococcosis (AE) is a severe zoonotic disease caused by the metacestode stage of Echinococcus multilocularis. The infection can have fatal consequences in humans if treatment is not provided, so early diagnosis is fundamental for initiating treatment and reducing morbidity and mortality. In addition, detection of the parasite in the definitive host plays a central role in epidemiological studies and surveillance programmes for control of AE. This review presents an overview of the present situation regarding the immunodiagnosis of E. multilocularis infection. Special attention is given to the description of the native, partially

purified and recombinant antigens available currently for immunodiagnostic purposes. Recent advances in the primary serodiagnosis and follow-up of AE patients are highlighted, including the detection of specific cytokine profiles. Progress in the immunodiagnosis of intestinal E. multilocularis infection in definitive hosts, particularly the detection of excretory–secretory and integument products of the worm in faeces (copro-antigens) by ELISA, is also discussed.

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

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Jpn J Infect Dis. 2005 Apr;58(2):122-4.

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

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Kansenshogaku Zasshi 2004 Apr;78(4):320-6

Using serum specimens from patients with alveolar hydatidosis (AH) in Hokkaido, we assessed the usefulness of "Echinococcus Western Blot IgG" (the French immunoblot assay, FIA), which has recently been launched from Ldbio Diagnostics (Lyon, France) as new commercial immunoblot assay kit of immunodiagnosis of Echinococcus infections. Eighty serum specimens were used for the present study: 64 preoperative sera and nine postoperative sera, which were taken from AH patients in Hokkaido, and seven sera from persons who were ELISA (enzyme-linked immunosorbent assay)--positive in mass screening which was conducted for checking on Echinococcus infections in Hokkaido since 1982. When the 64 preoperative sera were examined by the Western blotting method (the Hokkaido method of Western blotting, HWB) which had been carried out at Hokkaido Institute of Public Health between 1987 and 1993, it was found that 53 cases were positive and six cases were quasi-positive, i.e. the rate of the positive cases including quasi-positive cases was 92.2%. From immunostaining patterns, HWB-positive sera could be grouped in two types: the complete type, which showed a pattern of multiple bands containing the 55 and 66 kDa bands, and the incomplete type, which showed pat-

terns of only few bands containing the AH-specific polysaccharide antigen named C antigen. Forty-three of the 53 HWB-positive sera were of the complete type and the residue was of the incomplete type. On the other hand, when the 64 preoperative sera were examined by FIA, 60 sera (93.8%) were judged to be positive and the others as negative sera. On the basis of the interpretation of immunostaining patterns described in the instruction manual, 47 (78.3%) of the 60 positive sera were regarded as pattern P3, five (8.3%) as pattern P4, and eight (13.3%) as pattern P5. All of the complete-type sera were regarded as P3, indicating high antibody titers. Contrarily, most of the incomplete-type or quasi-positive sera resulted in other patterns such as P4 and P5, indicating low antibody titers. Of 5 HWB-negative sera, two were FIA-positive (which showed P3 and P5 patterns respectively), however their immunoreactions were significantly low. Therefore, apart from interpretation of pathological conditions of cases with exceedingly low antibody titers, FIA may be able to give a serologically clear interpretation to HWB-quasi-positive cases, indicating that it is a highly sensitive and useful method for immunodiagnosis of Echinococcus infections.

Molecular confirmation of human alveolar echinococcosis in Poland.

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Clin Infect Dis 2003 Oct 15;37(8):121-5

Infections of humans with Echinococcus multilocularis, the causative agent of alveolar echinococcosis (AE), a zoonosis, have been described with increasing frequency in Poland since 1994. In the attempt to verify these reports, we analyzed specimens obtained from a representative group of Polish patients. Liver lesions in patients with AE that was diagnosed on the basis of results of histological and serological tests contained E. multilocularis DNA, as shown by the presence of specific microsatellite sequences and mitochondrial 12S rDNA. The same tests clearly distinguished between AE and cystic echinococcosis, which is caused by Echinococcus granulosus. These data are unequivocal proof that human infections with E. multilocularis occur in Poland.

Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for differential diagnosis of alveolar echinococcosis: results of a blind test.

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J Clin Microbiol 2002 Nov;40(11):4161-5
Alveolar echinococcosis (AE) is the most potentially lethal parasitic zoonosis of the non-tropical areas in the northern hemisphere, where cystic echinococcosis (CE) is also endemic. Both AE and CE are highly endemic in China, and both serologic detection of echinococcosis, either AE or CE, and differentiation of AE from CE are crucial problems. Evaluation of Western blot analysis (WB) and enzyme-linked immunosorbent assay (ELISA) for the Em18 antigen, using affinity-purified and recombinant Em18, was carried out "blindly" using 60 human sera from patients diagnosed in France. The results were compared with those obtained using a commercially available Echinococcus WB immunoglobulin G (IgG) kit developed in France. The Em18 WB and Echinococcus WB IgG showed very similar results for detection of AE. Both affinity-purified Em18 or a recombinant Em18 WB and Echinococcus WB IgG seem useful for identification of AE, and the latter seems appropriate for both AE and CE, whereas affinity-purified Em18 ELISA and the newly developed recombinant Em18 ELISA appear to be suitable for detection of AE, especially for epidemiological surveys.

Laboratory diagnosis of cystic hydatic disease.

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World J Surg 2001 Jan;25(1):10-4
The main purpose of this article is to answer the questions about which test to perform for hydatic diagnosis and when. Several techniques for biologic diagnosis and follow-up of human cystic hydatidosis are reviewed. The specificity and sensitivity of immunologic reactions are reported. The differential diagnosis between Echinococcus granulosus and E. multilocularis is examined. The characteristics of the immunologic diagnosis according to the stage and the treatment of hydatidosis disease is discussed. Laboratory diagnosis of cystic hydatic disease is complementary to the clinical data. A judicious association of the usual techniques (indirect immunofluorescence assay, indirect hemagglutination assay, immunoelectrophoresis, co-electrophoresis with antigen 5 identification) confirms the diagnosis in 80% to 94% of hepatic hydatidosis cases and in 65% of pulmonary hydatidosis cases. Special techniques (enzyme-linked immunosorbent assay, Western blot, polymerase chain reaction) must be used for other localizations or when cysts are calcified. A serologic survey is necessary for the follow-up of operated medically treated patients. Despite poor standardiza-

tion, purified antigens can distinguish between E. granulosus and E. multilocularis infections, although false-positive results are observed during other helminthiases, such as cysticerocosis.

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

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J Clin Microbiol 2000 Oct;38(10):3718-21
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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Clinical and Diagnostic Laboratory Immunology, Apr. 2005, p. 548–551 Vol. 12, No. 4
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.

TRICHINELLA

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

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Clin Diagn Lab Immunol 2003 Sep;10(5):793-6
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.

TOXOCARA

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

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Clin Neurol Neurosurg. 2005 Aug;107(5):432-8. Epub 2004 Nov 18.
Toxocariasis is a worldwide-occurring parasitic infection leading to tissue damage in various organs due to wandering Toxocara larvae (visceral larva migrans). More than 40 cases of CNS involvement in children and immunocompetent adults have been documented in detail to date. Here, we present evidence of eosinophilic meningomyelitis in an adult without known risk factors and with positive Toxocara antibody response in CSF, but not in blood. Toxocariasis has to remain among the differential diagnosis in patients with eosinophilic CNS infection even if serological tests in blood are negative. Adult cases seem to be more frequent than previously thought (about 60%).

Evaluation of immunodiagnostics for toxocarosis in experimental porcine cysticercosis.

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Trop Med Int Health. 2007 Jan;12(1):107-10.
We assessed whether immunodiagnostic tests for cysticercosis can cross-react with the currently available immunodiagnostic tests for Toxocara canis in an established animal model for cysticercosis infection in pigs, known host for Toxocara. We examined by TES-enzyme-linked immunosorbent test and immunoblot assay for toxocarosis and cysticercosis the baseline and final follow-up sera of 10 pigs, before and after (3 months) infection with Taenia solium. After successful cysticercosis infection, the nine evaluable pigs became seropositive to T. solium (enzyme-linked immunoelectrotransfer blot assay), but did remain seronegative for Toxocara in both assays, documenting the lack of cross-reactivity with anti-T. solium antibodies in both T. canis assays. These findings should help clinicians better interpret serology for toxocariosis and cysticercosis in endemic areas for both helminth infections.

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

Ocular toxocariasis named also ocular larva migrans is caused by larvae of the roundworm Toxocara spp. The purpose of this study was to find out the seroprevalence of Toxocara antibodies in patients suspected of ocular toxocariasis. Between January 2001 and December 2003, sera from 239 ocular patients, aged 3 to 80 years, were examined by ELISA and confirmed by Western blot test. Out of the 239 patients, 172 (72%) were seronegative and 67 (28%) were Toxocara seropositive; 95% CI (22-34%). The median age of Toxocara seropositive patients was 37.6 years. There was no significant difference in the number of Toxocara positive sera between the younger age group (≤14 years) and the older age group (>14 years), $p>0.05$. A high rate of Toxocara seropositivity in ocular patients should alert the ophthalmologists in Slovenia to include toxocariasis in the differential diagnosis of eye diseases more frequently.

A study on some epidemiologic and paediatric aspects of toxocarosis in Hungary

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Journal of Thrombosis and Haemostasis 2003; 1 Supplement 1 July:

Objectives:Toxocarosis is the most frequent human helminthosis in Hungary. Most of the clinical symptoms are nonspecific, thus, its diagnosis depends considerably on laboratory methods, such as ELISA and Western blot (WB) techniques. The objectives of our study was to determine the frequency and distribution of Toxocara seroprevalence in the different regions and the different age groups of Hungary. Special reference will be given to children in whom anti-Toxocara antibodies were determined for differential diagnostic reasons, such as exanthemata and/or asthmatic symptoms.

Methods: Sera were obtained from 6985 asymptomatic individuals representing all age groups of the population of Hungary distributed to 20 different regions. Sera of 1427 symptomatic children aged <1–14 years were also obtained from different regions of Hungary. Seroprevalence of the symptomatic children were compared with 1605 asymptomatic children. Toxocara seroprevalence was measured with NOVATEC Toxocara ELISA IgG kit. In case of negative or low-positive values obtained by ELISA, the results were confirmed by WB method (LDBIO).

Results: The sera of 1977 persons of 6985 asymptomatic individuals were found to be positive for Toxocara IgG antibody (28.3%). The lowest prevalence (17.6%) was found in Buda-

pest, the highest ones were found in Szabolcs-Szatmár-Bereg (39.4%) and Hajdú-Bihar (38.2%) countries. Seropositivity rapidly increases by age reaching 37.0% at the age of 10–14 years. A significantly higher percentage (31%, P = 0.01) of Toxocara seropositivity was found in children suffering from bronchial asthma when compared with the 17% seropositivity measured in asymptomatic control groups of children. The borderline anti-Toxocara IgG ELISA results were found to be positive with WB method.
Conclusions: In Hungary, 28.3% of the population has anti-Toxocara IgG antibodies. The highest positivity (39.4%, 38.2%) were found in the most under-developed regions of Hungary, the lowest one (17.6%) was found in the capital. Probably, this reflects the differences in the hygienic conditions. The Toxocara seroprevalence rapidly increases by the age. This can be explained by the frequent contact of children with contaminated soil. A significantly higher percentage of Toxocara seropositivity was found in asthmatic children compared with the asymptomatic children. The Western blot technique is very useful in confirming the borderline and negative anti- Toxocara IgG values obtained by ELISA method.

Immunodiagnosis of ocular toxocariasis using Western-blot for the detection of specific anti-Toxocara IgG and CAP for the measurement of specific anti-Toxocara IgE.

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J Helminthol 2002 Dec;76(4):335-9
A prospective multicentric study was carried out to assess both the performance of Western-blot (WB) detecting specific anti-Toxocara IgG and that of CAP measuring specific IgE titre for the immunodiagnosis of ocular toxocariasis. For 14 outpatients presenting ophthalmic symptoms (choroiditis, chorioretinitis, papillar oedema, hyalitis, retinal detachment and/or uveitis), samples of serum and aqueous fluid (AF) were sent to the Department of Parasitology, University Hospitals, Toulouse, France. All patients but two tested positive with WB on the serum; 13 WB tests were performed on the AF, 12 of which were positive. The two patients who had a negative WB serum result tested positive for the AF. Specific IgE detection was considered as a complementary test of WB. Two patients showed a greater specific IgE titre in the AF than in the serum, and one had a positive result in the AF, but not in the serum. These six patients were considered as clear cases of ocular toxocariasis. Western-blot coupled with specific anti-Toxocara IgE detection appeared therefore to be an accurate procedure for the immunodiagnosis of ocular toxocariasis, provided the testing was simultaneously performed on the serum and AF.

Highlights of human toxocariasis.

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Service de Parasitologie, Centre Hospitalier Universitaire Rangueil 31403 Toulouse 4, France.

Korean J Parasitol (Korea 2001 Mar;39(1):1-11
Human toxocariasis is a helminthozoonosis due to the migration of Toxocara species larvae through human organism. Humans become infected by ingesting either embryonated eggs from soil (geophagia, pica), dirty hands or raw vegetables, or larvae from undercooked giblets. The diagnosis relies upon sensitive immunological methods (ELISA or western-blot) which use Toxocara excretory-secretory antigens. Seroprevalence is high in developed countries, especially in rural areas, and also in some tropical islands. The clinical spectrum of the disease comprises four syndromes, namely visceral larva migrans, ocular larva migrans, and the more recently recognized "common" (in adults) and "covert" (in children) pictures. Therapy of ocular toxocariasis is primarily based upon corticosteroids use, when visceral larva migrans and few cases of common or covert toxocariasis can be treated by anthelmintics whose the most efficient appeared to be diethylcarbamazine. When diagnosed, all of these syndromes require thorough prevention of recontamination (especially by deworming pets) and sanitary education.

Is toxocariasis an etiology of unexplained chronic prurigo, pruritus and urticaria?

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VIII European Multicolloquium of Parasitology, Poznan, 10-14 Sept. 2000
Objectives: Evaluation of the prevalence of toxocarosis infection in patients suffering from chronic prurigo, pruritus or urticaria and in a control population living in the same area (Franche-Comté, France).
Methods: 128 inpatients (Department of dermatology, Besançon) were included from March 1999 to December 1999 : 8 suffered from chronic prurigo, 30 had chronic pruritus, and 90 presented with chronic urticaria.
All patients had a clinical and biological screening to detect any disturbance known to be associatedwith these conditions, such as alimentary allergy, autoimmune disease, ectoparasitosis etc.. 60 sera, initially collected for a systematic screening for toxoplasmosis performed before marriage, during pregnancy or before corneal graft, were selected as a control group. This group had identical sex ratio and age distribution than the dermatological patients group. Toxocarosis serological diagnosis were performed using an immunoblot assay (LDBio Diagnostics, Lyon, France).
RESULTS: Clinical examination and biological tests led to an explanation of symptoms in 22 cases of urticaria, 13 cases of pruritis and 1 case of prurigo. Other patients are considered to have unexplained cause of their condition. Immunoblot test for toxocarosis was positive in

22% (13 / 60) of control group versus 41% (28 / 68) of unexplained urticaria cases (p = 0.02, Chi2 test), 76% (13 / 17) of unexplained pruritus cases (p = 0.00003, Chi2 test), and 57% (4 / 7) of unexplained prurigo cases (not significant).
CONCLUSIONS: Our results strongly support the involvement of toxocarosis as an etiology of unexplained chronic pruritus and chronic urticaria. A prospective case-control study is ongoing to confirm these findings.

CYSTICERCOSIS

Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis.

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Eur J Clin Microbiol Infect Dis 2002 Mar;21(3):227-9
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.