EDITORIAL

VIII Congreso Colombiano de Enfermedades Infecciosas

Centro de Convenciones Gonzalo Jiménez de Quesada, Bogotá, D.C., 30 de mayo a 2 de junio de 2007

Con la octava edición del Congreso llegan también grandes compromisos, retos y expectativas. La puesta en escena de los congresos de la Asociación Colombiana de Infectología, ACIN, que lo preceden –tanto el nacional en Medellín, 2005, como el Encuentro de Investigadores en Armenia, 2006, impecable y ejemplarizante en sus resultados académicos y logísticos– dejan una obligada "costumbre" a la calidad y a la excelencia en las actividades de la sociedad.

Mas no es sólo el reto de intentar, al menos, emular el éxito pasado sino la responsabilidad de cumplir con el verdadero objetivo del Congreso: ser el medio por antonomasia para ofrecer la actualización de la más alta calidad y excelencia del vasto conocimiento de las enfermedades infecciosas. Este objetivo es el que nos desvela y nos compromete al saber que son muchos los sectores y actores de la salud que tienen puestos los ojos en el evento.

Por ello, hemos escogido como lema y orientador ideológico del Congreso la sentencia *Las infecciones nos tocan a todos.* Con ella pretendemos transmitir un mensaje directo a todos, bajo diferentes ángulos de interpretación:

- Al cuerpo médico en general, porque las infecciones no discriminan edad, sexo ni especialidad. Es imperativo nivelar por lo alto el conocimiento del enfoque, el manejo y la prevención de las diferentes patologías infecciosas en todas las áreas y especialidades.
- Al público en general, como un mensaje que nos recuerda la vulnerabilidad como individuos y como poblaciones a las diferentes amenazas de las enfermedades infecciosas, sean brotes, pandemias o casos aislados.
- A las entidades, instituciones hospitalarias y, en especial, al Gobierno, como emisor de las políticas de salud, a manera de invitación a reflexionar sobre las responsabilidades y acciones que deben asumirse en torno a las políticas de atención de la salud.
- A las universidades y asociaciones científicas para que su participación sea activa y directa en las actividades académicas y en la definición de pautas y protocolos de atención, y en la definición de necesidades y medios de investigación.

El Congreso también convoca a la industria farmacéutica que, a través de su desarrollo de investigación, introducción de nuevos antibióticos y soporte a la educación médica continua, no sólo han sido y serán importantes patrocinadores y contribuyentes en la financiación del congreso sino verdaderos partícipes del mismo.

Por todo lo anterior, el Comité Organizador ha hecho todos los esfuerzos posibles para lograr cumplir con estas expectativas y, en soporte de ello, se culminarán en el Congreso los siguientes objetivos:

- Lograr la participación de 40 invitados extranjeros de Europa y las Américas que, en conjunto, representan excelencia, experiencia y conoci-miento en una vasta área de las enfermedades infecciosas, en unión a otros cerca de 100 expertos nacionales.
- Brindar espacios amplios de revisión y discusión de los diferentes aspectos de las enfermedades infecciosas: desde lo clínico hasta lo molecular, o ambos en combinación como serán efectivamente tratados los temas de terapia, control y prevención de las enfermedades infecciosas hospitalarias y provenientes de la comunidad, como la tuberculosis y el sida, entre muchos otros.

- Dar oportunidad para la expresión y actualización en la mayoría de los temas de las enfermedades infecciosas.
- Crear foros de discusión intersectorial en temas cruciales de interés de salud pública, como la tuberculosis, el paludismo y la infección hospitalaria, de los cuales se espera encontrar puntos de interés común a problemas comunes entre los diferentes actores, como la Academia, el Ministerio de la Protección Social, las Secretarías de Salud, la Organización Panamericana de la Salud, las Empresas Prestadoras de Servicios, etc.
- Acoger propuestas de la Junta Directiva y del Comité Organizador de conceder generosos descuentos en la inscripción de profesionales de la salud vinculados a comités de infecciones hospitalarias, laboratorios de microbiología, estudiantes de posgrado de las área biomédicas, grupos de investigación y otros. De esta manera, queremos brindar facilidades para que el mayor grupo posible de personas asista a un evento que, desde el punto de vista de programación académica, ya consideramos un absoluto éxito.

Es así que consideramos que el ya inminente VIII Congreso Colombiano de Enfermedades Infecciosas en Bogotá logrará, sin duda, su objetivo de hacer que las infecciones nos toquen a todos, contribuyendo a su mejor conocimiento, prevención, control y manejo para el beneficio del paciente y de la comunidad.

Invitamos a todos a que consulten el programa completo, la lista de conferencistas nacionales e internacionales y toda la información detallada en la página web de la ACIN (www.acin.org) y a que, cuanto antes, aseguren su asistencia.

Bienvenidos al Congreso, nos vemos en Bogotá el 30 de mayo.

Ernesto Martínez Buitrago
Presidente, VIII Congreso Colombiano de Enfermedades Infecciosas

Toxoplasmosis research: time to take decisions

Jorge E. Gómez-Marín
Guest editor

Significant achievements in the field of toxoplasmosis research have increased immensely in the last few years. Now we know that Toxoplasma originated as a parasite in South America, and that we still have virulent strains predominating in this part of the world. At the same time, the most important drug that we use to treat pregnant women –spyramycin– has shown to have important shortcomings. It seems to have a positive effect only if given during the first weeks after seroconversion. This situation poses an enormous challenge to prenatal control programs since continuous follow-up and seroconversion detection in countries like Colombia with higher rates of infection but limited covertures of prenatal control are elusive goals.

Beyond any dispute whether prenatal programs are indicated or not, it is of the outmost importance to identify more effective drugs. Nonetheless, prenatal treatment has two important limitations: drug arsenals proved to be innocuous to the foetus are scarce (ethical restraints to test new drugs have to be taken into account), and even potent anti-*Toxoplasma* therapy is administered too late to prevent any probable damage.

Consequently, it is time to give priority to research on vaccine candidates. Natural immunity allows us to conclude that protective long lasting immunity is possible, and in toxoplasmosis we do not have the imperfect immunity problem as we do in malaria. We have gained a better understanding of how cellular immunity could be induced and monitored to identify which antigens are most important to trigger it. There are more than 14 antigen candidates including surface, ropthries and dense granule proteins, many antigen preparation methods (recombinant, DNA and peptides) and different immunizations strategies (nasal, intramuscular, oral) tested in animal models proving that they are efficient to induce immunity.

Intramuscular DNA immunization seems to be very promising. It should be noted that in the last two years many Chinese teams have focused and worked very intensely in this field. A future vaccine for toxoplasmosis will not be the definitive solution since previous experiences with other diseases have shown that vaccination strategies have their own limitations and difficulties, as we have seen with congenital rubeola, but it will certainly have an important impact reducing the burden of congenital disease.

The III International Congress on Congenital Toxoplasmosis is a great opportunity to summarize the collective experience on this field and to define the steps to be taken to undertake collaborative projects; this is the best tool to find significant answers which will ultimately control this terrible and invalidating disease.

Ackowledgements

The local organizing committee thanks the support of many enterprises that have supported this meeting: Asociación Colombiana de Infectología, Vélez lab, Third World Academy of Sciences, Colciencias, LDBio, Diasorin, Universidad del Quindío, Café Quindío and Biomerieux. Special thanks to Mrs. Amparo Arbeláez Escalante, gobernadora del Departamento del Quindío, and all her staff (Vicky Pardo, María Nelly Aponte, Luis Fernando Ramírez and the administrative staff).

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN PREGNANT PATIENTS FROM A LARGE PUBLIC HOSPITAL IN BOGOTÁ, COLOMBIA

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To determine the prevalence of IgG and IgM antibodies against *Toxoplasma gondii* in pregnant women in district 18 of Bogotá, Colombia.

Methods: Patient data was collected from Hospital Rafael Uribe Uribe, a large public hospital in the south of Bogotá, Colombia, from January 2005 to December 2006. IgG and IgM values were determined at the hospital using commercially available enzyme-linked immunosorbent kits (Diagnostic Systems Laboratories, Inc.).

Results and conclusions: Six thousand one hundred and three patients were surveyed during the period of study; 2,531 (41%) patients were IgG seropositive. In the case of 950 patients (16% of the total number of patients), IgM measurements were requested by the attending physician, and of this selected group of patients, 44 (4.6% of IgM tested) were IgM seropositive. We isolated DNA from 24 of the IgM seropositive blood samples. In one of these samples we were able to detect parasite DNA by amplification of the *T. gondii* B1 gene (Grigg and Boothroyd, 2001). Five of the IgM positive samples were submitted to IgG avidity measurements and exhibited high avidity.

We gratefully acknowledge the generous cooperation of Hospital Rafael Uribe Uribe which made possible this study.

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TOXOPLASMA GONDII: PRESENT KNOWLEDGE AND RISK ASSESSMENT OF FOODBORNE TOXOPLASMOSIS - CONCLUSIONS OF THE WORKING GROUP OF THE FRENCH FOOD SAFETY AGENCY

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Toxoplasmosis caused by *Toxoplasma gondii* is a common infection in France: approximately 50% of the adult population is infected and between 200,000 and 300,000 new estimated infections occur every year, including 2,700 cases in pregnant women. A number of 600 cases of congenital toxoplasmosis are estimated to occur annually, 175 with sequelae. The severity of toxoplasmosis is also related to the delayed risk of reactivation of a previously acquired infection, in situations of immunodepression.

A working group was set up within the *Agence Française de Sécurité Sanitaire des Aliments* (AFSSA), with the main objectives to gather together and analyse the scientific data to enable a risk assessment of foodborne toxoplasmosis and to provide the health authorities with the decision-making elements required for the promotion of an appropriate prevention.

The experts conducted a review of the literature on all the elements which might be involved in the contamination of water or food with *T. gondii* and then analysed the available data on the effectiveness of water treatment procedures, and of packaging and cooking in controlling the parasite.

The data gathered was then used to begin a process of quantitative risk assessment, to enable an estimation of the implication or impact of the consumption of potentially contaminated foods on the incidence of toxoplasmosis in pregnant women and on congenital toxoplasmosis. The accumulated data was used to review the pertinence and the conditions of application of the preventive measures currently recommended.

This led the group to suggest several priority areas for investigation or action for a better risk-assessment of foodborne toxoplasmosis and improvement of primary prevention, such as:

- Improving the assessment of levels of *T. gondii* contamination in foodstuffs and water, notably to estimate the respective parts played by different types of food in human infection.
- Putting in place a quantitative risk assessment process focusing on evaluating the impact of the consumption of potentially contaminated foods on the incidence of toxoplasmosis in pregnant women and on congenital toxoplasmosis.
- Improving information on toxoplasmosis and its prevention.

Such priorities justify setting up a national initiative, agreed between the different health professionals and public health services involved in the prevention of congenital infections.

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Seroprevalence of *Toxoplasma gondii* antibodies in sheep of Puebla, Mexico

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An extremely variable prevalence of *Toxoplasma gondii* infection has been found in sheep from different parts of the world. The most important source for sheep and goats kept mostly on pasture, are oocysts shed by cats with their feces. In México, only two studies have been carried out in sheep raised in small flocks during the 1990´s; they revealed prevalences from 20% to 55% in three central states of México, all of them with a dry warm climate.

It is known that *T. gondii* prevalence is higher in moist-warm places than in other environments. For this reason, we sampled sheep from an experimental farm located in the Puebla State, which has propitious climate for this protozoan to disperse.

One hundred and four serum samples of sheep were analyzed by ELISA and Western blot in order to determine anti-*T. gondii* antibodies. The seroprevalence was 71.1% (CI: 62.4 - 79.8%) by ELISA and the results were confirmed by a commercial kit (agreement, 87.5%). At least eight bands ranging from 20 to 180 kd were detected in the positive sera by immunoblotting. Fifty six of these animals were sampled ten months later and were tested again. The prevalence did not change significantly, and an 80% correlation was observed in absorbance values. One sheep became positive in the second sampling indicating an incidence rate of around 2.1% per year.

Higher prevalence rates of toxoplasmosis in moist warm compared to cold atmosphere is attributed to the longer viability of *T. gondii* oocysts. This may explain the high *T. gondii* prevalence in Puebla State, which apparently has favorable climatic conditions for the transmission of this protozoan.

In conclusion, our results confirm the presence of anti-*T. gondii* antibodies in sheep and suggest that this species may constitute one of the main reservoirs of *T. gondii* among domestic animals. Current studies are in progress to isolate and genotype the parasites of positive animals.

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Antibody response during reactivation of latent toxoplasmosis: results of follow up of HIV-infected persons

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Reactivation of latent toxoplasmosis is frequently considered in diagnostic practice, especially if an increase of hitherto low antibody levels is detected. In pregnant women a risk of foetal infection is assumed. However, cases where the reactivation can be clinically confirmed are extremely rare. During a Czech cohort study, our team had the opportunity to follow up immunological parameters of 9 both *Toxoplasma* and HIV-infected, and until then asymptomatic persons, in whom toxoplasmic encephalitis broke out. Levels of anti-Toxoplasma antibodies using complement-fixation test (CFT), IgG and IgM ELISA as well as CD4 T lymphocyte counts were followed up for 0-85 months prior to the onset of symptoms of clinical toxoplasmosis, and 0-80 months afterwards.

The reactivation increased as the CD4 T lymphocyte counts dropped to 20-140 cells/µl. CFT titres were 1:4 to 1:64 at the time, IgG levels were low or intermediate and anti-toxoplasmic IgM was not detected in any of these cases. An increase of antibody levels was observed neither before nor after onset of clinical signs of reactivation; in 2 patients, a slight IgG or CFT increase occurred, but not earlier than a year after the reactivation. Even in patients treated subsequently with highly active antiretroviral therapy (HAART), whose CD4 counts returned to 400-500 cells/µl, the anti-*Toxoplasma* antibody levels remained low.

Our data suggests that latent toxoplasmosis can be reactivated in seriously immunocompromised per-

sons exclusively. Due to weak immune response, enhanced antibody levels resembling acute infection cannot be expected in cases of reactivated toxoplasmosis.

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PREVALENCE OF *TOXOPLASMA GONDII* IN CATS FROM COLOMBIA, SOUTH AMERICA, AND GENETIC CHARACTERIZATION OF *T. GONDII* ISOLATES

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Cats are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete the environmentally-resistant oocysts. In the present study, prevalence of *T. gondii* was determined in serum, feces, and tissues of 170 unwanted cats from Colombia, South America.

Antibodies to *T. gondii* were assayed by the modified agglutination test and found in 77 of 170 (45.2%) cats with titers of <1:5 in 93, 1:5 in eight, 1:10 in 17, 1:20 in 10, 1:40 in 7, 1:80 in 4, 1:160 in 8, 1:320 in 6, and 1:640 or higher in 17 cats. *T. gondii* oocysts were not found in feces of any cat as ascertained by bioassay in mice.

Tissues (brain, heart, tongue) of 116 cats were bioassayed in mice or cats. *T. gondii* was isolated from tissues of 15 of the 42 cats with titers of 1:40 or higher and not from any of the 90 cats titers of 1:20 or lower. Of the 29 cats whose tissues were bioassayed individually, *T. gondii* was isolated from the tongues of 9, hearts of 8, and brains of 5. Mice inoculated with tissues of 12 of 15 infected cats died of toxoplasmosis; with nine *T. gondii* isolates all infected mice died. Overall, 65 of 92 (70%) of *T. gondii*-infected mice died of toxoplasmosis.

Genotyping of these 15 isolates using polymorphisms at the SAG1, SAG2, SAG3, BTUB, and GRA6 loci revealed that three isolates (TgCtCo1, 2, and 7) had type I alleles and one isolate (TgCtCo8) had type II allele at all five loci. Eleven isolates contained the combination of type I and III alleles and were divided into three genotypes, with TgCtCo3,5,6,9,12,13 and 15 had alleles I, I, III, I and III, TgCtCo4,10,11 had alleles I, III, III, I and I, and TgCtCo14 had alleles I, III, III, III, and III, at loci SAG1, SAG2, SAG3, BTUB and GRA6, respectively.

All infected mice from each group had identical genotype except one mouse infected with TgCtCo5 that had a type III allele at locus BTUB and a unique allele (u-1) at locus SAG1 indicating mixed infection for TgCtCo5, whereas the rest seven mice had a type I alleles at both loci.

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PREVALENCE OF *TOXOPLASMA GONDII* IN DOGS FROM COLOMBIA, SOUTH AMERICA, AND GENETIC CHARACTERIZATION OF *T. GONDII* ISOLATES

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The prevalence of *Toxoplasma gondii* in 309 un wanted dogs from Bogotá, Colombia, South America, was determined.

Antibodies to *T. gondii* were assayed by the modified agglutination test and found in 52 (16.8%) of 309 dogs with titres of 1:20 in 20, 1:40 in 6, 1:80 in 17, 1:160 in 3, 1:320 in 3, 1:1280 or higher in 3.

Some organs obtained after necropsy of dogs (hearts, tongues and brains, either separately or pooled) were used in bioassays carried out in mice (37 samples, of which 20 were assayed with separate organs and 17 were assayed with pooled organs), cats (pooled organs from six) and pooled organs of two dogs both in mice and cat.

Mice receiving dog tissues were examined for *T. gondii* infection. Feces of cats that received dog tissues were examined for oocyst shedding. In total, T. gondii was isolated from tissues of 20 dogs (16 by bioassays in mice, 3 by bioassay in cats and 1 by bioas-

say in mice and cat). All infected mice from 7 of 17 isolates bioassayed in this host died of toxoplasmosis during primary infection.

Only 10 of the 20 dogs whose tissues were bioassayed separately induced infections in mice. Interestingly, dog organs varied in their capacity to induce T. gondii infection in mice, hearts and tongues producing more positive results than the brain.

The 20 *T. gondii* isolates obtained from seropositive dogs were PCR-RFLP genotyped using polymorphisms at 10 nuclear markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and an apicoplast marker Apico. Ten genotypes were revealed. These genotypes are different from the three predominant types I, II and III lineages that are widely spread in North America and Europe.

A new allele denoted u-3 at PK1 locus was identified in three isolates. This result supports previous findings that *T. gondii* population is highly diverse in Colombia.

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Toxoplasma gondii infection of meat for human consumption detected by PCR assay in three cities from the coffee region of Colombia

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In Colombia the consumption of undercooked meat is a habit that can be found in 5% of the population, and it has been shown that it is an important risk factor for infection, accounting for up to 25% of *Toxoplasma* infections during pregnancy, as determined by previous studies. DNA detection of *Toxoplasma gondii* in meat for human consumption enables to measure the exposition level of this food to this protozoon.

We obtained a total of 180 meat samples from commercial stores at the capital cities of Caldas, Risaralda and Quindío departments of Colombia. We purchased 20 samples from each type of meat (pork, beef and chicken) in such cities. All samples were analyzed for the detection of the B1 gene of *T. gondii* by PCR assay.

Fifty three per cent of the samples were positive for specific DNA amplification of *Toxoplasma*; pork meat was proportionally the most infected (70%). There were important differences in the prevalence of infection in each the city: beef meat was more contaminated in Armenia (80%), chicken meat was more contaminated in Pereira (70%), and pork meat was more contaminated in Manizales (80%). No positive samples from chickens were found in Manizales. The overall prevalence of infection by city was: Pereira, 65%; Armenia, 60%, and Mani-zales, 33%.

Our results showed that there are high exposure levels to *Toxoplasma* in commercially available meats for human consumption in the coffee region of Colombia. These results make desirable public health measures to monitor infection in these products.

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Primary infection during pregnancy by Toxoplasma in Cuba

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Congenital toxoplasmosis is a consequence of a first infection during pregnancy. A descriptive study was carried out in a sample of 181 pregnant women. This sample was representative of a universe integrated by pregnant women from three policlinics of La Lisa municipality, Havana City, booked from March to September 2004, with the aim of identifying those with acute or recent toxoplasmosis infection. Follow-up and control of seronegative pregnant women were also conducted.

The sample size was determined through a simple, randomized sampling. Indirect immunofluorescence (IFI) was used to detect IgG and IgM anti-Toxoplasma antibodies, and the polymerase chain reaction (PCR) to confirm infection in blood or amniotic fluid.

One of the patients of this study, who was at her 18th week of gestation with acute infection and probable congenital transmission, showed serocon-version with IgG antibody titres of 1/256 and 1/2,048 in her first and second sera, respectively. The result of the IgM detection was of 1/32. These findings demonstrate a 44.2% of seroprevalence of T. gondii infection in a pregnant women population.

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NEURON SPECIFIC ENOLASE AND S-100B PROTEIN IN THE SERUM OF CONGENITALLY INFECTED CHILDREN

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Most newborns with *Toxoplasma gondii* congenital infection are subclinical at birth, but may develop sequels later in life, in some cases in spite of drug therapy. Destruction of cells and tissues before they cause clinical problems could partially explain this phenomenon. Neuron specific enolase (NSE) and S-100b protein (specific of astrocytes) have been used to predict clinical outcomes in stroke, cranial trauma and open heart operated persons after resuscitation.

By means of monoclonal-based antigen capture ELISA, we quantified NSE and S-100b titres in serum samples of newborns and infants with symptomatic and subclinical congenital toxoplasmosis, as well as in non-infected control babies born from women with and without chronic infection. We analyzed separately those samples positive for IgM and/or IgA anti-*T. gondii* antibodies from those presenting IgG only (i.e., "chronic").

Titres for both proteins were significantly higher in newborns with subclinical infection and IgM/IGA antibodies, as compared to the rest of the groups. Although these results are preliminary, they suggest that similar to other non-infectious disorders, NSE and S-100b are elevated in subclinical congenitally infected children, and thus could have a potential importance in prognosis.

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IGG AND 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. Thus, infected children must be identified and treated as early as possible. The aim of this study was to evaluate diagnostic accuracy of IgG and IgM Western blot profile comparison (TOXOPLASMA WB IgG-IgM - LDBIO DIAGNOSTICS, Lyon France) on 238 newborns at risk of congenital toxoplasmosis.

Patients and methods: Two hundred and thirty eight neonates born from mothers with suspected or certain infection in pregnancy were evaluated retrospectively with TOXOPLASMA WB IgG-IgM. Serum obtained from all the newborns at birth was compared with maternal samples and then with samples obtained monthly during their first three months of life. Furthermore, all the samples 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 were 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 the following table.

	IgM ISAGA+ IgA ELISA			IgG IgM Western blot		
	Positive	Negative	Total	Positive	Negative	Total
Infected	31	11	42	40	2	42
Not infected	2	194	196	6	190	196
Total	33	205	238	46	192	238

Sensitivity: 76.1; sensitivity: 95.2
Specificity: 98.9: specificity: 96.9

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 in the traditional tests (76.1)

and the difference was statistically significative (p = 0,02, Yates corrected x^2).

Western blot profile comparison allowed us to find out 9 infected newborns not detectable with traditional tests that could undergo an early treatment, while 190 not infected newborns avoided unnecessary therapy.

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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 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 (LDBIO DIAGNOSTICS, Lyon, France) test 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; group 2, woman with recent infection in pregnancy based on evolution of IgG and IgM curves (Bessieres, MH, 1999) were included.

There were 136 couples mother-cord blood of sera in group 1 with 24 congenital toxoplasmosis cases and 91 couples mother-cord blood of sera with 12 children born with congenital toxoplasmosis in 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 congenital toxoplasmosis diagnosis.

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 Western blot IgM was 83.9%; Sp, 98.5%; PPV, 86.5%, and NPV, 98.1%. Se of Western blot IgG + Western blot IgM was 96.2%; Sp, 98.2%; PPV, 86.6%, and NPV was 99.5%. Se of VIDAS IgM + Platelia IgA + Western blot IgG + Western blot IgM was 99.8%; Sp, 97.0%; PPV, 79.5%, and NPV, 99.9%. Group 2: Se of Western blot 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%, and NPV, 93.6%.

The predictive values of Western blot mother-cord blood vary between the two groups of women. The use of Western blot mother-cord blood improves the final diagnosis especially when used as a confirmation test in unscreened population.

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National control program for maternal toxoplasmosis in Montevideo, Uruguay: results on 11,543 mothers

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One of the most important threats for foetus is the acquisition of a first Toxoplasma infection during pregnancy. We developed during the period from October 2005 to January 2007, a national control program based on maternal screening for recent toxoplasmosis.

We used the ELFA technique to determine the prevalence of specific anti-*Toxoplasma* IgG and IgM antibodies on 11,543 mothers from the capital city of Uruguay, Montevideo. Confirmatory tests were performed by immunofluorescence and aviditiy assays.

A total of 5,887 mothers (51%) were negative by both IgG and IgM assays. We found 277 patients (2.4%) that were positive by IgM and IgG, at high levels, and we confirmed 23 cases (0.2%) as recent toxoplasmosis and 20 were treated. From these mothers, one child developed chorioretinitis, one died and 18 children were asymptomatic.

In conclusion, our program could detect recent infection during pregnancy and could detect congenitally infected children for the first time in a control program in Uruguay, showing that this program is feasible and can detect asymptomatic treatable children.

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Evaluation of the new VIDIA® toxoplasmosis IgG and IgM assays in women of childbearing age

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The aim of the present study was to evaluate the performance of the new VIDIA® toxoplasmosis IgG and IgM assays (Biomérieux, France), using the VIDIA system easy to use with a high level of traceability, with clinical specimens prospectively collected in women of childbearing age.

Methods: A total of 1,000 fresh serum samples consecutively obtained from 1,000 women aged 18 to 44 years (median = 30) during a period of 4 weeks were tested with ADVIA® Centaur™ toxoplasma IgG and IgM assays according to routine conditions. Serum aliquots stored at +4 °C were blindly rechecked within 24 hours with the VIDIA system for the same parameters. Discrepancies of results between both methods were resolved considering avidity test, direct agglutination (Toxo-Screen DA, Biomérieux), ISAGA (Biomérieux) and when available, the analysis of previous drawn serum samples.

Results: Among the 1,000 women screened with VIDIA, 89.4% had non detectable IgG and IgM (*T. gondii* seronegative) and 8.2% had a pattern of past acquired infection (positive IgG and no detectable IgM). Positive IgM were detected in 1.1% of them with VIDIA system versus 2% with ADVIA Centaur. Equivocal rate was 0.9% for VIDIA TOXO IgG and 0.5% for VIDIA TOXO IgM (versus 0.9% and 1.1%, respectively for the compared method).

For VIDIA TOXO IgG, the relative sensitivity and specificity were 96.7% and 99.7%, respectively. After the resolution of discrepancies, the sensitivity as well as the specificity was 100%.

For VIDIA TOXO IgM, the initial relative sensitivity and specificity were 64.7% and 100%, respectively. In fact, 4 of the 6 negative samples with VIDIA and positive with the compared method were found with high avidity index and the remaining two samples were negative with the reference test (ISAGA-IgM). Taking this into account, the absolute sensitivity was found 100%.

Conclusion: The two evaluated assays VIDIA TOXO IgG and VIDIA TOXO IgM have shown an excellent sensitivity and specificity and are well adapted to the routine screening of toxoplasmosis in pregnant women.

High levels of lactoferrin and secretory IgA in tear samples in patients with active toxoplasmic retinochoroiditis confirmed by intraocular synthesis of specific antibodies

Paul M.[1], Stefaniak J.[1],
Twardosz-Pawlik H.[2]

The aim of the study was to describe *Toxoplasma*-specific immunodiagnostic findings and clinical signs of infection in relation to the analysis of free tear lactoferrin and secretory IgA in patients with recent retinochoroiditis, presumably caused by toxoplasmosis.

Thirty-one immunocompetent patients aged between 5 and 62 years (mean, 25.4 years) were strongly suspected of ocular toxoplasmosis, based on ophthalmic assessment. Clinical signs observed on admission were unilateral white focal chorioretinal lesions associated with old pigmented retinal scars, mostly located in the same eye (n = 29) or rarely in a previously healthy eye (n = 2). Fresh macular lesions were observed in 13 patients. Bilateral inactive scars were found in 10 cases.

In all patients, a puncture of ocular anterior chamber of affected eyes was performed for a comparative immunological profile analysis to confirm a local synthesis of specific anti-*T. gondii* antibodies in aqueous humour shown by the IgM-IgG Western blot assay. In 30 of the 31 examined patients, a routine serology showed an immunological profile for chronic *Toxoplasma* infection. In 22 of the 31 patients (71%), the local production of *T. gondi*-specific IgG bands of different antigenic specificity when compared with serum samples was discovered in their intraocular fluids.

Levels of lactoferrin ranged between 95.5 and 6,010 ng/ml (mean [SD], 3,889 [3,367] ng/ml) in tears, and 80-4,730 ng/ml (mean [SD], 2,244 [1,546] ng/ml) in serum samples. The mean ratio of lactoferrin levels in tears to levels in serum samples from patients with confirmed intraocular synthesis of *Toxoplasma*-specific IgG was 6.5 times higher than in cases without the immunological evidence of the parasitic infection in the affected eye. Secretory IgA concentrations in tear fluids were significantly elevated, attaining levels of 434-1,145 mg/ml (mean [SD], 781 [307] mg/ml).

Active chorioretinal lesions with coexisting positive serology are not enough evidence to confirm ocular *T. gondii* infection. Elevated concentrations of lactoferrin and sIgA in tears seem to be valuable

complementary markers of active ocular toxoplasmosis. Evidence of the local synthesis of specific antibodies in the aqueous humour, demonstrated by the Western blot assay, is required for the final confirmation of retinal lesions of toxoplasmic origin.

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P30 PEPTIDE RECOGNITION PATTERN IN PATIENTS WITH OCULAR TOXOPLASMOSIS

de la Torre A.[1], Cardona N.[1], Siachoque H.[2], Gómez-Marín J.E.[1]

Aim: P30 is the major antigenic protein of *Toxoplasma gondii*. Immune response in animal models has shown that recognition by antibodies preferentially occurs at the carboxy end peptides of the protein. Recognition pattern of peptides by human serum antibodies is unknown. We aimed to determine what peptides from the P30 protein were recognized by human infected with *Toxoplasma*.

Methods: Immunoenzymatic assays with serum from patients with different clinical forms were carried out. We used 9 peptides from the amino and the carboxy ends derived from the P30 protein of *T. gondii.*

Results: Initial assays with serum from congenital, ocular and chronic asymptomatic toxoplasmosis indicated that they only recognized peptides from the carboxy end of the protein, and that sera from patients with ocular toxoplasmosis has a higher absorbance against the peptide Pep17 as compared to other sera with congenital or chronic asymptomatic infection.

We also tested the peptide Pep17 in 32 patients with toxoplasmosis, 13 without ocular infection, 13 with inactive chorioretinal scars, and 6 with active ocular infection. All sera recognized this peptide and there were no differences in the absorbance levels between groups.

Conclusions: As it occurs in mice, human serum antibodies are directed against the carboxy terminal peptides of the P30 major protein. We are currently working on the cellular immune response against the same peptides in order to determine if there is dichotomy between humoral and cellular immune responses during ocular infection in human toxoplasmosis.

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DIAGNOSTIC VALUE OF NESTED-POLYMERASE CHAIN REACTION IN PERIPHERAL BLOOD IN ACTIVE OCULAR TOXOPLASMOSIS

López-Castillo C.A., de la Torre A., Lora F., Gómez-Marín J.E.

Aim: To determine the diagnostic value of nested-polymerase chain reaction (n-PCR) through the amplified B1 gene of *Toxoplasma gondii* in peripheral blood of patients with retinochoroiditis by toxoplasmosis.

Design: Test of a test; gold standard: clinical diagnosis based on fundus examination performed by an ophthalmologist and positive specific anti-*Toxoplasma* IgG in sera.

Methods: Patients with uveitis were distributed in three groups: group I: uveitis patients with an active ocular toxoplasmosis episode (10 patients); group II: patients with inactive ocular toxoplasmosis (9 patients), and group III: uveitis patients without toxoplasmosis (5 patients). Nested PCR was carried out using two pairs of primers targeting the *T. gondii* B1 gene of, was done in two cycles.

Results: We included 24 blood samples from the same number of patients, seen at a referral center for uveitis in Armenia. B1 gene amplification was obtained in 4 out of 10 (40%) isolates from group I, 1 out of 9 (11%) isolates from group II, and in 2 out of 5 isolates (40%) from group III with positive IgG anti-*Toxoplasma* results.

Conclusions: Nested PCR in peripheral blood does not differentiate between active and inactive cases with ocular toxoplasmosis. It is possible to amplify B1 gene in blood from patients with IgG anti-*Toxoplasma* titres, independent of whether they present or not ocular symptoms.

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TREATMENT OF ACTIVE RETINAL TOXOPLASMOSIS WITH INTRAVITREAL CLINDAMYCIN AND TRIAMCINOLONE

Castro A.[1]

Retinal toxoplasmosis can produce severe an extensive damage and it is not infrequent to have significant visual loss as a sequelae. Recovery with sys-

temic treatment can take several weeks and additional damage to important areas of the retina can occur as a consequence of this prolonged response. Because of this potential for severe damage, management of active severe retinochoroiditis-vitreitis as an endogenous endophthalmitis is postulated.

Nine of 35 cases treated with intravitreal clindamycin (1-5 mg) followed by intravitreal triamcinolone (4 mg) one week later, are presented. In most cases the intravitreal treatment is used as adjunct to systemic treatment of clindamycin, 1,200 mg per day plus trimetoprim 160 mg-sulfametoxasole, 800 per day. In some cases of systemic intolerance, intravitreal drug was the only treatment. These cases demonstrate how intravitreal therapy improves response to treatment and accelerate visual recovery.

It is suggested that severe retinal toxoplasmosis should be treated more aggressively by combining intravitreal antibiotic and anti-inflammatory therapy. The ideal would be to have a slow and prolonged release of the drug in the vitreous cavity; thus, effective local treatment might be an alternative to the standard systemic treatment, avoiding secondary effects.

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High frequency of active retinochoroiditis lesions in newborns with congenital toxoplasmosis identified by the neonatal screening program in Minas Gerais, Brazil

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A ratio of one infected baby per 1,590 live births was observed In neonatal screening for toxoplasmosis in Belo Horizonte (2003-04) and 68.4% (13/19) of these children have presented ocular involvement. Only one newborn from the total had shown active retinochoroiditis. In other regions of the world, applying the same strategy, prevalence of ocular involvement at diagnosis is about 20%, and active retinochoroidal lesions are rarely reported.

Objective: To evaluate ophthalmological involvement in newborns with congenital toxoplasmosis identified by newborn screening in the state of Minas Gerais.

Materials and methods: Prospective study of children with congenital toxoplasmosis identified by newborn screening (IgM anti-*Toxoplasma gondii*, Q-PrevenÒ) in Minas Gerais from November 2006 to January 2007. Confirmatory serology (mother/baby) was carried out and newborns presenting IgM and/or IgA and IgG, or IgG positive associated to ocular lesions and positive IgM and IgG in the mother were considered positive.

Indirect binocular ophthalmoscopy was performed by an experienced professional. The audiometric examination, the determination of mother/baby immunological profile and the isolation of toxoplasma strains are in progress.

Results: A total of 59,113 children were tested and 47 were positive, leading to a ratio of one infected baby per 1,258 live births in Minas Gerais. One case of vertical transmission was identified during the prenatal examination, and the mother received spiramycin. Ophthalmological examination had already been performed in thirty-eight children at an average age of 47.6±12.8 days. Retinochoroiditis was detected in 63.2% (24/38); active lesions in at least one eye were shown in 75% (18/24). Bilateral lesions were detected in 58% (14/24). Involvement of macula occurred in 16 children (66%), and bilaterally in 12 (75%). Specific treatment was instituted in all children, and systemic corticosteroid was associated in those children with macular active lesions.

Conclusion: High prevalence of active retinochoroiditis lesions in newborns with congenital toxoplasmosis submitted to the neonatal screening in Minas Gerais was observed. Early diagnosis of infection has allowed treatment of active lesions and an improvement in vision prognostic is expected in the long term. These results suggest that a strategy emphasizing early diagnosis and treatment of congenital toxoplasmosis should be adopted.

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Sponsored by the Secretaria de Estado de Saúde de Minas Gerais.

Recurrences in ocular toxoplasmosis: frequency and clinical related factors

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Aim: To determine the frequency and clinical related factors for recurrences in toxoplasmic retinochoroiditis.

Setting: Referral clinical consultation for uveitis at the Universidad del Quindío medical center (Colombia) between September 2005 and November 2006.

Study design and inclusion criteria: Case series analysis was based on clinical charts. Patients with retinochoroidal lesions by fundoscopy, positive IgG anti-*Toxoplasma* serological assay, and at least two years of follow-up were included in the study. Data were analyzed for demographic and clinical characteristics: age, sex, socioeconomical level, number of scars, sinechiae, papilar exacavation size, specific IgG and IgM anti-*Toxoplasma* results, PCR assay results, drug therapy, congenital or acquired origin and retinal localization area.

Results: Out of 60 analyzed cases, 27 fulfilled the inclusion criteria. A total of 44 recurrences were found during a total of 3,063 months of follow up, indicating that there is a 1.6 recurrent episode every 10 years. We standardized the number of recurrences by follow up months, and we found that subconjunctival injection of steroids, azythromycin therapy and use of systemic steroids were related with a greater number of recurrences.

Conclusions: The number of recurrences in our population is similar to those previously described in other studies. Subconjunctival or systemic steroids and azythromycin were related significantly with the nuember of recurrences.

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Dehydroepiandrostenione sulphate levels during ocular toxoplasmosis

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Reduction of the levels of dehydroepiandrostenione sulphated hormone (DHEAS) has been related to increased susceptibility to *Schistosoma* and *Plasmodium* infections, and there is not any previous study in ocular toxoplasmosis.

We analyzed the relationship between DHEAS levels in chronic asymptomatic and active retinochoroiditis by *Toxoplasma*. We used the chemoluminescence automated immulite assay to determine DHEAS levels.

Four groups of patients were studied: (i) chronic asymptomatic infection patients with a positive test for IgG anti-*Toxoplasma* and without ocular lesions (n=16); (ii) chronic asymptomatic patients with retinal scars of retinochoroiditis by *Toxoplasma* (n = 16); (iii) acute symptomatic patients with active retinochoroiditis by *Toxoplasma* (n = 26), and (iv) individuals with negative assays for specific IgG anti-*Toxoplasma* (n = 14).

We compared DHEAS levels between groups adjusted by age and sex; non-parametric Kruskall-Wallis statistical tests were applied to determine differences in the median of DHEAS levels.

We found significantly lower levels in patients with active ocular toxoplasmosis compared to patients without active lesions ($p = 0.022$). There were also significant differences between the infected group without ocular lesions and the non infected group of individuals ($p = 0.022$).

Our results suggest that DHEAS levels might be reduced during active ocular toxoplasmosis, further studies are needed to clarify its role in *Toxoplasma* infection.

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Recurrence characteristics in human ocular toxoplasmosis

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Ocular toxoplasmosis is associated with a substan tial number of patient visits each year, thus reinforcing an interest for a better understanding of the disease.

Patients and methods: In this descriptional case series, 139 patients with ocular toxoplasmosis were retrospectively contacted with the approval of the Local Institutional Ethical Committee, and 63 completed a questionnaire. The data was reconfirmed with that from clinical records. Parameters of interest included patient-reported age at first manifestation, and in our records we documented age at first presentation and of recurrences. Patients were then categorized according to median age at first ocular toxoplas-

mosis manifestation. For all comparisons, the level of significance was set at p = 0.05.

Results: The mean reported age at first ocular toxoplasmosis was 23.9 (median = 20.9, range: 0-70.5, ± 12.9) years. The clinical diagnosis was made 3.5 years later (p < 0.0008). The mean follow-up time had been 6.5 (median = 5.0; range: 0.6-49.9, ± 7.6) years. The two groups (group 1: < 20.9 years; n = 35; group 2: = 20.9 years; n=28) differed in their recurrence rates with 66% for the former and 39% for the latter (x^2 test, p < 0.05).

Patients reporting only one episode (n=16) had a mean age of 26.9 (median= 25.6; range: 10.6-70.5; ±14.3) years, those reporting two episodes (n=19) were younger at first presentation [mean age=17.9 (median=19.5; range: 5.9-33.9; ± 7.8) years; p < 0.05]. The portion of patients developing a recurrence after their last episode remained stable in 50%-70%, as did the time to recurrence with 1.0-1.7years.

Conclusion: Younger patients are at a higher risk for recurrences of their ocular toxoplasmosis. After each ocular toxoplasmosis episode two thirds of patients will develop a next episode after a surprisingly short time to recurrence.

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CORRELATION OF MORPHOLOGY AND VISUAL FUNCTION IN HUMAN OCULAR TOXOPLASMOSIS

GARWEG J.G., SCHERRER J.N., ILIEV M., HALBERSTADT M.[1]

Visual acuity and visual field testing have not been systematically assessed regarding their capability to detect a functional impact of ocular toxoplasmosis. Therefore, in a series of patients with ocular toxoplasmosis, we wished to compare these functional tests with clinical and photographic documentation of ocular findings.

Patients and methods: In this prospective, cross-sectional study, 139 consecutive patients with inactive ocular toxoplasmosis were invited to a voluntary assessment of their eyes, with approval of the Local Institutional Ethical Committee. Sixty one patients were finally included into this analysis. From all patients, a complete ophthalmological examination was taken including best corrected Snellen visual acuity, automated visual field testing (SAP; Octopus perimeter, program G2), slit lamp examination and dilated examination of the fundus of both eyes.

The inclusion criteria were: inactive stage of the disease, and reliability factor in the visual field =25%. We classified visual acuity as normal (=20/25), mildly (20/25 to 20/60), moderately (20/60 to 20/400, low vision), and severely impaired (<20/400, legal blindness). Visual field damage was correspondingly graded as mild (mean defect <4 dB), moderate (mean defect=4-12 dB), or severe (mean defect >12dB).

Results: Eight patients (13%) presented with bilateral ocular toxoplasmosis, thus a total of 69 eyes were included. Visual field damage was encountered in 65 eyes (94%), while only 28 eyes (41%) had a reduced visual acuity, revealing perimetric findings to be more sensitive (p<0.001). Correspondence with the morphology of chorioretinal scars was better for the visual field (in 70% of the instances) than for visual acuity (33%). Moderate to severe functional impairment was registered in 65.2% for the visual field, and 27.5% for visual acuity.

Conclusion: In its quiescent stage, ocular toxoplasmosis had a functional impact on the 30° in the visual field in more than 94% of studied eyes. Hence, it may be used for the detection of intercurrent, functionally represented, subclinical reactivation. Standard automated perimetry represents a sensitive means of surveilling ocular toxoplasmosis, and is superior to visual acuity as a functional test in the clinical assessment of prophylactic and therapeutic strategies.

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HIGH PARASITE BURDEN IS ASSOCIATED WITH SEVERITY OF CONGENITAL TOXOPLASMOSIS IN POLAND

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Recently estimated incidence of congenital toxoplasmosis in Poland (1.5-2.0/1,000) shows the magnitude of the problem. In France disseminated form of congenital toxoplasmosis with cerebral lesions, hepatosplenomegaly and ocular manifestation are very rare but higher parasite concentrations in amniotic fluid were associated with severe outcome of disease in a fetus and neonate (Romand S., 2004). Previously,

genotype II parasites strains were identified as the major source of *Toxoplasma gondii* infection in France and the only identified type in Poland.

We determined the number of parasites in amniotic fluid and cerebrospinal fluid samples from congenital toxoplasmosis cases diagnosed at the Research Institute Polish Mother's Memorial Hospital in Lodz (Poland). Real-time quantitative PCR assays were performed at the Laboratoire de Parasitologie-Mycologie in Marseille (France).

Ten confirmed cases of congenital toxoplasmosis were analyzed, including 9 severe cases and 1 asymptomatic. All severe cases were diagnosed in the third trimester of pregnancy. Symptoms included hydrocephaly (in 9 cases), intracerebral calcifications (8), chorioidoretinitis (6), microophtalmia (6), microcephaly (2), hepatosplenomegaly (4), pneumonitis (4), growth retardation (3), thrombocythopenia (2), ascites (2), petechiae (2), and death (4).

Parasite concentration was evaluated in 8 amniotic fluid samples obtained during pregnancy by amniopuncture and 2 cerebrospinal fluid samples from neonates. The higher parasite load was related to the severe outcome of congenital toxoplasmosis. The number of *T. gondii* 529 bp repeat region ranged from 5 to 8,400 in amniotic fluid and from 25 to 100 in cerebrospinal fluid copies/ml, with a mean of 2,142 copies/ml. In 40% of the samples the number of *T. gondii* DNA copies was higher than 1,000 copies/ml. In the asymptomatic case the 7.5 copies in 1ml of amniotic fluid was detected.

Number of symptoms ($p = 0.03$), death ($p = 0.03$), hepatosplenomegaly ($p = 0.042$), pneumonitis ($p = 0.042$), and preterm delivery ($p = 0.03$) and the parasite concentration were assocciated with the parasite load. There was no relation found between microcephaly ($p = 0.28$), ascites ($p = 0.28$), petechiae ($p = 0.077$) and thrombocythopenia ($p = 0.077$) and the *T. gondii* concentration.

The study showed a high prevalence of cases with severe symptoms in the group with congenital toxoplasmosis in Poland. The outcome of the infection was strongly related to the parasite load.

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Outcome of treatment and clinical follow up of children with congenital Toxoplasma gondii infection diagnosed by neonatal screening: a 10-year experience

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The study aimed to evaluate the efficiency of sero logical screening of Polish newborns, followed by intensive postnatal anti-parasitic treatment for preventing clinical relapses of congenital *Toxoplasma gondii* infection in childhood.

Thirty-five infected children with a follow up period of 6.9 to 10.5 years (mean, 8.4 years) were included into the clinical study. The patients were diagnosed during the first 3 days of life by the regional screening programmes detecting T. gondii-specific IgM (1996-1998) or both IgA and IgM antibodies (1998-2000) in filter-paper dried blood specimens. Twenty-five children identified independently of neonatal screening as having at least one of typical clinical signs at paediatric examination, and confirmed by serology constituted a control group. The final diagnosis of congenital toxoplasmosis was established by analysis of comparative immunological profiles of the mother/neonate serum pairs using the IgM/IgG Western blot assay.

Combined treatment with pyrimethamine plus sulfadiazine with or without alternation of spiramycin was prescribed for 12-24 months (mean, 13.7 months), according to the severity of infection and immunological status of the patients.

Twenty-four infants (68.6%) presented a subclinical form of congenital *T. gondii* infection or demonstrated some non specific symptoms or signs at birth, related to prematurity or adaptative respiratory disorders. The clinically overt form of toxoplasmosis, mild or moderate, occurred in 7 cases; in 2 other infants coexisting patent cytomegalovirus infection was diagnosed.

Only one of the 35 children developed severe sequelae of congenital infection during the prenatal and neonatal period with generalized parasitaemia and a central nervous system damage, leading to the death at 12 months of age. In another child, who presented some neurological signs at birth, non febrile seizures confirmed by a generalized paroxysmal activity in electroencefalography without specific signs in brain imaging examinations were demonstrated at the age of 6.2 years.

New pathognomonic signs of congenital infection were diagnosed in 2 cases (small intracranial calci-

fications or peripheral chorioretinal scar) at the age of 2.5 and 7.7 years, respectively.

Serological screening of neonates followed by the longterm postnatal anti-parasitic chemotherapy seems to be promising in the successful prevention of severe clinical sequelae of congenital *T. gondii* infection in school-age children.

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Anti-*Toxoplasma* IgG and IgM seroprevalence in mothers and newborn children from two geographic zones of Boliva: Yacuiba-Tarija and Alto La Paz from November 2006 to November 2007

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Since 1990, no studies have been performed in Bo livia about the seroprevalence of toxoplasmosis (either IgG or IgM) or the rate of congenital infection. We carried out a one year survey to evaluate prevalence of IgG in pregnant women and of IgM in newborn children using blood samples on paper filter. Prevalence of toxoplasmosis was determined in pregnant women and in newborn children, at the moment of childbirth, in two regions of Bolivia: El Chaco (Hospital de Yacuiba, departmento de Tarija) in the southern part of Bolivia and in Altiplano (Hospital de La Paz y Hospital de El Alto, departmento de La Paz) in the center of Bolivia at a higher altitude.

One blood sample was taken on filter paper from the infant's heel. We used the neonatal *Toxoplasma gondii* IgM fluorometric enzyme immunoassay (FEIA, Anilabsystems®) to measure IgM titers in newborn infants and the technical modified IgG (DSL®) to measure IgG titres in the mother.

In the case of a FEIA positive result, the titration measurement was again carried out and then confirmed with a specific test (ISAGA, BioMérieux, France, cut off point =6) and a Western blot.

In Chaco (Yacuiba), IgM prevalence was 0.5% (2/365) in infants, and IgG in pregnant women of 84% (303/365). In Altiplano (La Paz and El Alto), we observed a 0.3% (2/578) prevalence of anti-*Toxoplasma* IgM in infants, and 20% (112/591) for anti-*Toxoplasma* IgG in pregnant women. Although there were significant differences in IgG prevalence among pregnant women between the two regions, the frequency of congenital toxoplasmosis was near the same.

Prevalence of newborn congenital infection is high compared to reports from others countries. The survey will continue in these localities as well as in other parts of Bolivia.

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Pilot study of newborn screening for congenital toxoplasmosis in the Republic of Ireland

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Early treatment of congenital toxoplasmosis may improve neurological outcome and reduce chorioretinitis. Early clinical detection is problematic and treatment may be delayed.

Aim: To assess feasibility of newborn congenital toxoplasmosis screening and to determine congenital toxoplasmosis incidence.

Methods: A two year pilot screening programme began in July 2005. Testing with parental consent was added to the routine national newborn screening programme using dried blood spots obtained from the babies' heel 72-120 hours after birth.

A quantitative assay for toxoplasma IgM antibody was first performed and if greater than a predetermined threshold value, a confirmatory ISAGA IgM test was performed on the same blood spot. Positive cases were confirmed with paired mother/infant serology analysed at The Toxoplasma Reference Laboratory, Swansea, U.K., confirmed positive infants underwent detailed clinical evaluation and received 1 year antiprotozoal treatment.

Clinical and ophthalmology follow up of identified cases is ongoing.

The programme was implemented following six weeks of education for staff collecting blood spot samples. Screening failure rate will be determined by undetected cases subsequently referred to the paediatric infectious diseases service.

Results: From 07/05-12/06, of 93,510 births, 93,248 infants were screened, 0.03% opted out. Twenty five cases required confirmatory serology. Congenital toxoplasmosis was confirmed in 11/25 infants; 10 asymptomatic, 1 symptomatic with unilateral absence of central vision and fixation.

Three out of ten asymptomatic cases had congenital toxoplasmosis related abnormalities on further investigation: unilateral retinochoroidal lesion in 1 case, intracranial calcification in 1 case and bilateral retinochoroidal lesions with intracranial calcification in 1 case.

Ten out of eleven cases started treatment. One untreated infant with equivocal early serology and negative Western blot analysis at 3 months of age had congenital toxoplasmosis diagnosed at 1 year based on persisting IgA with a rising dye test.

Ten out of fourteen false positive screening tests were attributed to prior maternal infection and confounding maternal antibody. In the other 4 there was no serologic evidence of previous maternal infection.

Conclusion: Eleven infants were found to have congenital toxoplasmosis, an incidence of approximately 1 in 8,400. Only 1 case (9%) was symptomatic. Congenital toxoplasmosis screening can be successfully added to existing newborn screening programmes. To date no screen negative case has been diagnosed clinically.

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Compliance with the French screening programme for maternal Toxoplasma infections in pregnancy: data on 34,290 pregnancies

Wallon M.[1], Cornu C.[2], Bissery A.[3], Ecochard R.[3], Gaillard S.[2], Peyron F.[1]

To assess compliance with the French programme for preventing congenital toxoplasmosis that requires 1) to identify in the first trimester of pregnancy women who are not immune against *Toxoplasma* infection, and 2) to follow them through monthly serological tests until delivery. This programme, which aims at reducing the number of children with severe congenital toxoplasmosis, has never been evaluated in terms of compliance or efficacy.

Patients and methods: This descriptive transversal study was based on data collected for reimbursement purposes by the Rhone-Alpes health system on all pregnant women who delivered between July 1st 2002 and June 30th 2003 and had at least one serology for toxoplasmosis. Details on tests, prescribing physicians, laboratories performing the tests and pregnant women were available. Outcomes were i) first test performed in the first trimester, and ii) overall number of tests and mean between-test time intervals. Patients characteristics associated with a poor compliance were studied.

Results: Data from 34,290 pregnancies was analyzed. The first test was performed after the first trimester of pregnancy in 25% of cases (8,430). Women had on average 5.7 tests during pregnancy; 23% (7,883) achieved the minimum of 7 recommended tests. The median between-test interval was 32.7 days and 80% had at least one between-test interval >35 days. Independent predictors for a delayed first test were free medical coverage, a younger age and prescription by a gynaecologist rather than a general practitioner. Free medical coverage, delivery in a public hospital, tests prescribed by general practitioners only using repeated single prescriptions, a first test performed behind schedule after a long interval between prescription and testing, were independent predictors for a lower number of tests and long between-tests intervals.

Discussion: Efforts toward pregnant women, especially those who are young and in poor social condition, and toward their physicians are necessary to promote earlier and more frequent serological tests. Planning all tests in one prescription at the start of pregnancy might be a simple way to increase compliance. Adjunction of health education messages on the tests prescriptions could also emphasize the need for primary prevention.

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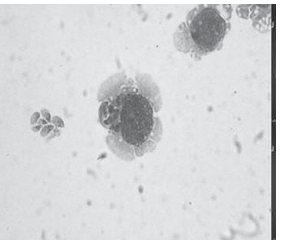

PERSISTENT LOW *TOXOPLASMA*-SPECIFIC IgG AVIDITY IN PREGNANT WOMEN

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An estimation of the time of seroconversion is of utmost importance for counselling pregnant women who have been diagnosed with toxoplasmosis. Among serological markers, low avidity of immunoglobulin G (IgG) has been reported to be a useful marker of recent infection with *Toxoplasma*. Nevertheless, discrepant results on the maturation of avidity over time have been reported. We investigated persistent low IgG avidity after *Toxoplasma* seroconversion and looked for factors that could influence the evolution of its maturation over time.

Methods: From our hospital database, we extracted 120 patients who presented with a low avidity index and who had had a positive serological test at least 3 months before sampling. IgG avidity was studied retrospectively in 309 sera from 117 pregnant women who seroconverted during pregnancy. Interpatient variations in the evolution of IgG avidity and factors that potentially influence maturation, such as gestational age at infection and treatment with spiramycin, were investigated.

Results: Persistent low avidity was found in some patients even after a median follow up period of 6 years. The avidity index of IgG was significantly heterogeneous and ranged from 7.8 to 35.3% for 95% of the women 12 weeks after infection ($p<0.05$). After logarithmic transformation, evolution of the avidity index plotted against time displayed heterogeneous patterns with slopes between -0.017 and 0.051 for 95% of the women ($p=0.011$). Maturation of avidity decreased when gestational age at infection increased ($p=0.03$) and increased when the delay between infection and onset of treatment increased ($p=0.0003$).

Conclusion: These results demonstrated that maturation of *Toxoplasma* IgG avidity in pregnant women is highly variable and that persistent low avidity can be observed. Evolution of avidity over time is influenced by gestational age at maternal infection and delay in the onset of treatment. In pregnant women who present with specific IgG and IgM, acute *Toxoplasma* infection cannot be diagnosed reliably solely on the basis of low IgG avidity. When the first sample is drawn late in pregnancy, estimation of the time of infection must be based on several tests using different techniques.

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TOXOPLASMA GONDII SPECIFC ANTIBODY CLASES AND SUBCLASES IN MOTHER/ NEWBORN PAIRS

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Most cases of congenital toxoplasmosis are asymptomatic at birth. Immune control is mainly mediated by the cellular arm, but antibodies of the IgG1 and IgG3 classes bind to macrophages and natural killer cells through Fcg receptors, which are effective in destroying the parasite. These antibodies are expected in mothers or newborns protected from vertical transmission or from parasite-induced damage. Conversely, IgG2 and IgG4 are induced or enhanced by Th2 cytokines, and their presence could be associated with poor clinical outcome.

Fifty six mother/newborn pairs of sera were selected from banks previously studied, which included cases from high risk gynecology hospitals or screening programs. All newborns were of minutes to 40 days of age. They were classified according to infection probability as "immune" (no congenital infection) or "infected", by a serology panel of one, three and two different techniques for IgA, IgM and IgG antibodies, respectively, and total antibodies, assayed by capture and indirect ELISA, IFAT, immunoblot and dye test. All IgG subclasses were tested by indirect ELISA against a crude antigen.

The results of 41 negative control pairs were used to determine the cut off points for each subclass. IgG1 was the most frequently recognized antibody in mothers and children; among "infected", its presence in the mothers was related to poor clinical outcome in the offspring. All mothers were negative for IgG3 antibodies, while there were three positive newborns, all "infected" and with clinical symptoms. IgG2 was a marker

of vertical transmission if present in the newborn sera, while the presence of IgG4 in the mothers or children was associated to newborn clinical problems. Passive transfer of IgG1 (mostly), IgG2 and IgG4 was supported by correlation of values between mothers and offspring in some pairs of the "immune" group.

The role of different subclasses in congenital infection by *Toxoplasma gondii* could be different to that expected from their cytokine control and effector mechanisms.

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Serological rebounds in children with congenital *Toxoplasma gondii* infection treated since the neonatal period

Paul M, Stefaniak J.[1]

The purpose of the study was to evaluate the role of immunological recurrences in assessing potential risk of clinical relapses in children with congenital toxoplasmosis who were not treated during the prenatal period.

Thirty-five infected patients identified during the first days of life by regional screening based on the detection of *Toxoplasma*-specific IgM and/or IgA antibodies eluted from neonatal Guthrie cards were included in the serological study. The final diagnosis was confirmed by a comparative analysis of serum samples from the mother and neonate using Western blotting. Serological testing for anti-*T. gondii* IgA, IgM and IgG serum antibodies was repeated every 2 months during the first year of life, then every 4 months after the cessation of treatment in early childhood, and twice a year during the school period.

Toxoplasma-specific IgG showed decreasing titres in all the infants during anti-parasitic treatment. In 4 prematurely born infants, a transient negativisation of specific antibodies was observed at 2-10 months of the age (mean, 7.3 months) during intensive therapy. In 22 infants, asymptomatic serological rebounds of IgG (n=4), IgG and IgA (n=17) or IgA alone (n=1) occurred firstly at 16-35 months of life (mean, 24.5 months) or between 2-48 months (mean, 8.7 months) after the end of initial pyrimethamine/sulphadiazine therapy.

During the follow up period of 6.9-10.5 years (mean, 8.4 years), 16 children had multiple serological recurrences (range, 2-5; mean, 2.6 rebounds). Patients with eye and/or brain lesions had no more serological reactivations (mean, 1.8) than other children with the asymptomatic infection (mean, 2.1).

After 6 years of life, serological rebounds were detected less frequently (26.1%) in comparison to the early infancy and pre-school period (91.3%). In one patient, a rising titre of specific IgG antibody was observed 8 weeks before a primary attack of non-febrile seizures confirmed by electroencephalography, but intracranial calcifications were not found in brain computerized tomography scans performed at the age of 10 and 76 months.

Serological reactivations are frequent in children with congenital toxoplasmosis who were diagnosed by neonatal screening and did not receive prenatal treatment but they are not associated with an increasing risk of clinical relapses.

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Newborn screening for congenital toxoplasmosis in Minas Gerais, Brazil: clinical and epidemiological results in 59,113 screened babies

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Brazilian researches have been showing ratios from one infected baby per 500 to 3,000 live births in neonatal screening for toxoplasmosis. In regions with high prevalence, prenatal screening has been suggested as an eligible strategy to the early treatment and diagnosis of infected newborns.

To evaluate the incidence of infection and the newborn screening as an strategy for early treatment and diagnosis of infected newborns in the State.

Material and method: Prospective study of newborns with congenital toxoplasmosis identified by neonatal screening (IgM anti-*Toxoplasma gondii*, Q-Preven® in dried blood spot) was carried out in Minas

Gerais from November 2006 to January 2007. Confirmatory serology (mother/baby) was performed, and newborns presenting IgM and/or IgA and IgG, or IgG positive associated to ocular lesions and positive IgM and IgG in the mother were considered positive. Indirect binocular ophthalmoscopy, audiometric examination, determination of mother/baby immunological profile and the isolation of toxoplasma strains were carried out at diagnosis.

Results: A total of 59,113 newborns (98% of live births in the period) were tested and 47 were positive, leading to a ratio of one infected baby per 1,258 live births in Minas Gerais. The incidence in the state varied between 1:526 and 1:3,284, predominating in the north and northeast, where the human development index is lower. Mothers of newborns carried out prenatal (97.5%) with an average 6.06 (±2.25) medical appointments, most were asymptomatic (75%) and had at least one serological exam for toxoplasmosis during pregnancy (62.5%).

All children were identified as infected by *Toxoplasma gondi*i exclusively after neonatal screening. Toxoplasmosis was identified in the prenatal period in only one pregnant woman, but the baby was not identified as infected during this period. This neonate has presented unilateral macular retinochoroiditis. Retinochoroiditis lesions were found in 63.2% (24/38) and from this total, 75% (18/24) showed active lesions. Specific treatment was instituted in all children.

Conclusion: The data shows that neonatal screening for congenital toxoplasmosis, when associated to newborn screening programs for other diseases, is feasible and can contribute to early diagnosis of the infection, even in regions with high prevalence of the disease without difficult access to public prenatal assistance services.

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

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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 subse-

quent samples earlier (at least two weeks) with LDBIO TOXO II IgG and later with traditional IgG serological tests. Six 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 or 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.

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Detection of *Toxoplasma gondii* DNA in peripheral blood of infants for diagnosis of congenital toxoplasmosis: a preliminary report

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Serological methods may be inaccurate for diagnosis of congenital toxoplasmosis. Polymerase chain reaction (PCR) assays have been successfully used for diagnosis of fetal toxoplasmosis. There are few studies regarding its use in peripheral blood samples of infants with suspected congenital toxoplasmosis.

Objective: To evaluate the usefulness of detection of *T. gondii* DNA in peripheral blood for diagnosis of congenital toxoplasmosis.

Methods: Forty infants with suspected congenital toxoplasmosis due to maternal/infant serology or clinical findings were enrolled. Diagnosis was confirmed by the persistence/rise of specific IgG antibodies >1 year of age. Peripheral blood samples were obtained at the first clinical evaluation, before initiation of treatment. A real time PCR fluorescence assay by the Sbyr green I method using a primer set which amplifies the B1 gene with a lower detection limit of 1.6 parasites/µl was performed in all samples.

Results: Twelve infants were diagnosed with congenital toxoplasmosis; 28 were uninfected. Six out of twelve (50%) mothers of infected infants received treatment during gestation. Seven out of twelve (58.3%) infected infants had overt clinical disease, and all had ophthalmologic and/or neurological involvement. PCR was performed within 2 weeks of birth (median, 1 day) in 7 infected and 20 uninfected infants. In the remaining 13 infants, it was done later (median, 87 days). *T. gondii* DNA was detected in a single infant tested in the postnatal period (1/7; 14.3%). None of the 5 infected infants tested >14 days nor any of the 28 uninfected infants had positive DNA results.

Conclusions: Although the DNA detection assay can be further improved, it is likely that this method is not useful for diagnosis when applied to peripheral blood samples of infants. Time of sampling and previous institution of treatment may influence the test performance.

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Synthesis and evaluation of some pyrimidine analogs against toxoplasmosis

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Toxoplasmosis is a world wide infection caused by obligate intracellular protozoan parasite which is *Toxoplasma gondii.* Some pyrimidine analogs were synthesized to be evaluated for their antitoxoplasma effects in the experimental animals.

Results were assessed through studying the parasite density, estimation of serum *Toxoplasma* antigen, studying the ultrastructural changes of the parasite and the histopathological changes of the affected organs.

The results showed that four out of twelve synthesized compounds have promising antitoxoplasma potentials. The animals that received these four compounds showed statistically significant decrease in the

mean number of the parasite count in the liver and the spleen when compared to the corresponding control group. Moreover, *Toxoplasma* antigen was very low or even absent in the serum of animals receiving these compounds.

Light microscopic examination of the peritoneal exudates of animals receiving these compounds showed stoppage of movement and deformity in shape of the tachyzoites, whereas, by scanning electron microscope, the organisms lost their crescent shape, with dimples and deep ridges on their surfaces. Very mild histopathological changes were noticed in liver, spleen and lungs of the groups of animals receiving these compounds in comparison to the other groups.

Thus, these compounds proved their effectiveness in eradication of experimental *Toxoplasma* infection.

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CLONING AND PURIFICATION OF A NITRIC OXYDE SYNTHASE IN *TOXOPLASMA GONDII*

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Nitric oxide is a gas molecule involved in signal transduction process of eukaryotic cells. Its role as a second messenger molecule has been described in a diversity of multicelular and unicelular organisms.

We previously described a putative nitric oxide synthase genomic sequence on *Toxoplasma gondii;* this was the first genomic sequence described for a pathogen protozoan. Now, we report the cloning, expression and purification of this protein in a *Baculovirus* gen expression system based on the DNA sequence.

We obtained a protein of approximately 15 kd that was active in a colorimetric functional assay for nitric oxide by using L-arginine as a substrate. This protein produced 3.28 nmol/ml per second of nitrites per microgram of purified protein. Functional studies should be made in order to define the substrates and physiological role of this enzyme in this human pathogenic protozoan.

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A MODEL FOR THE DYNAMIC OF CONGENITAL TOXOPLASMOSIS TRANSMISSION

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This work describes the dynamics of the congenital toxoplasmosis transmission by means of a model of differential equations structured by age and linked to the space-temporal model obtained by Trejos and Duarte (2004) where toxoplasmosis dispersion in cats was described.

For this work a function is used which deals with the risk of infection transmission to the fetus depending on the week of pregnancy.

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USEFULNESS OF A RECOMBINANT DENSE GRANULE GRA2 PROTEIN FOR SERODIAGNOSIS OF ACUTE *TOXOPLASMA* INFECTION: COMPARISON OF SERA FROM FRENCH AND IRANIAN PREGNANT WOMEN

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Three fusion systems (pELB leader, glutathione-s-transferase and thioredoxin) were tested to express in bacteria, the (Hisx6)-tagged mature GRA2 protein, a highly immunogenic protein of *Toxoplasma gondii.* The recombinant proteins were purified in a single step on Ni2+-NTA resin. The most efficient expression of soluble GRA2 was obtained for GRA2 expressed in fusion with thioredoxin. On immunoblots, recombinant proteins were recognized by an anti-GRA2 monoclonal antibody as well as by sera from Iranian *T. gondii* infected pregnant women.

An enzyme-linked immunosorbent assay was developed to evaluate the reactivity of sera from both French and Iranian pregnant women to the TRX-His-GRA2 fusion protein. Specificity of the test was 96.4%. Sensitivity of the GRA2 ELISA ranged from 95.8% (sera collected in France) to 100% (sera collected in Iran) for sera of acute infection and from 65.7 (sera collected in France) to 71.4% (sera collected in Iran) for

sera of chronic infection, respectively. Moreover, the mean absorbency value of acute sera was markedly higher than the mean of chronic sera and the difference was extremely significant.

These results suggest that recombinant GRA2 could advantageously complement other previously described *T. gondii* antigens for the serodiagnosis of acute *Toxoplasma* infection.

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Identification of trypsin like proteases genomic sequences in Toxoplasma

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Proteolytic processing plays a significant role in the process of invasion by the obligate intracellular parasite *Toxoplasma gondii.*

We found three putative genes (55.m05020, 65.m01132, 80.m02288) encoding for trypsin-type serine proteases in *T. gondii* tachyzoites genome. These proteins are homologous to other Apicomplexan, plants and bacterial trypsin (DegP or HtrA).

The *T. gondii* sequences conserved the catalytic triad (histidine-acid aspartic-serine) as well as the motif QIDAAINPGNSGGPA owned of the family trypsin S1 and a PDZ domain.

We obtained the amplification of the genes 65.m01132 and 80.m02288 used in PCR. The phylogenetic analysis suggests that these genes are of eubacterial origin, obtained from mitochondria endosymbiosis in the beginning of eukaryotic cells.

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Oocyst detection of toxoplasma in water: an example of detection from water in Champagne-Ardenne, France

Villena I.[1]

Toxoplasma gondii is a protozoan parasite capable of infecting a variety of birds and mammals, including humans. Several recent outbreaks of toxoplasmosis were related to drinking water. We propose a strategy for *Toxoplasma* oocysts detection, based on a multirisk waterborne parasitic approach including *Giardia* and *Cryptosporidium* recovery by the *Agence Francaise de Normalisation* (AFNOR) method on the same sample.

Our strategy involves three basic steps: (i) concentration and filtration of the water sample to recover small numbers of *Toxoplasma* oocysts, (ii) elution and purification on a density gradient, and (iii) detection. Water samples are filtered to recover *Toxoplasma* oocysts, and purified on a sucrose density gradient. Detection is based on PCR and mouse inoculation (bioassay), to determine the presence and the infectivity of recovered oocysts.

This detection strategy was then applied to 241 environmental water samples collected over a 3 year period. A total of 241 water samples from 80 sites were tested by PCR and mouse inoculation. The turbidity of raw surface water samples ranged from 0.30 to 30.1 nephelometric turbidity units, while that of public drinking water samples ranged from 0.03 to 1.9 units. Among the 241 samples, 12 (5%) were positive for *Toxoplasma* DNA and 213 were negative; the other 16 samples contained PCR inhibitors. Inhibitors were more frequent in raw surface water (12 of 55; 22%) than in untreated water (4 of 88; 5%) or public drinking water (0 of 98). None of the samples were positive by bioassay.

Finally, among the 225 interpretable samples, we detected *Toxoplasma* DNA in 12 cases (5.3%). Three cases involved raw surface water, whose environmental matrices may be contaminated by soil washing after peaks in rainfall. This could also be the case for the untreated water samples (eight positive samples), which were chosen by local public health officials because of frequent pathogen recovery (including *Giardia* spp. and *Cryptosporidium* spp.). The detection of *Toxoplasma* DNA in one public drinking water was more surprising, since none of the samples were positive by bioassay. In a previous study, *T. gondii* identification after filtration was based on mouse inoculation. Mouse bioassay is still the reference method to detect viable

oocysts, but 7 days is required for sporulation, before mouse inoculation, and 4 additional weeks are required to obtain the immunological results.

We need to develop tools for rapid detection of *T. gondii* oocysts in water, like those proposed for detection of *Giardia* cysts and *Cryptosporidium* oocysts.

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GENOTYPES OF *TOXOPLASMA GONDII*

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Multiple genetic markers, from PCR-RLFP to sequencing or microsatellites, have been developed to differentiate *Toxoplasma* strains and analyse the parasite population structure. The consensus of the first studies was that the vast majority of isolates belong to three clonal lineages, designated as type I, II or III. However, these isolates were predominantly obtained from humans and domesticated species in Europe and North America. Extension of epidemiological screening across a wider geographical and host range, together with a multilocus genotyping approach, reveals a more complex pattern.

Toxoplasma strains observed in Europe and USA belong to three main lineages. Among these three lineages, the type II is largely predominant. In France, it is observed in more than 90% of human congenital toxoplasmosis, but also in all isolates originating from a large variety of animals.

Strains circulating in South America are different from the archetype strains and more diverse. They exhibit different mixtures of type I and III alleles, and unique polymorphisms. Type II alleles are less frequently detected. Unique polymorphisms are present especially in strains isolated in the wild part of the Amazonian forest. The few strains isolated from Africa or from Caribbean islands also possess a mixture of type I and III alleles. Occasionally, atypical genotypes with unique polymorphisms may also be found in the wild life of North America.

The population structure has consequences in terms of possible links between phenotypes and genotypes. In case of a clonal structure, biological information obtained from one isolate may successfully predict the others because of their identical genetic background. If strains have different genetic background, the association between genotype and phenotype can not be predicted and continued recombination may lead to strains that acquire new pathogenic mechanisms.

Relationships between human genotype and human disease certainly exist, but are still difficult to assess because of the host immune status and genetic background, and because of other factors of virulence such as the infecting dose or parasite stage. Type II strains are "virulent" for immature foetuses and for immunocompromised patients, but are also responsible for many asymptomatic or benign toxoplasmosis in more mature foetuses and probably for most of the asymptomatic infections in immunocompetent patients in Europe. This may also be the case for type III strains, although we have very few reports of human disease associated with this genotype. Type I strains have been associated with higher virulence in some patients (acquired ocular disease, cases of disseminated congenital toxoplasmosis), but their detection in cases of reactivation of chronic infection in immunocompromised patients or in placentas with no congenital infection suggests that it can also be responsible for asymptomatic infections in immunocompetent patients.

The situation for atypical or recombinant strains in humans is even more complex. However, among nearly 400 isolates collected in France from human cases

of toxoplasmosis, these recombinant or atypical isolates (acquired mainly outside Europe) were found essentially in cases of severe toxoplasmosis acquired by immunocompetent patients or of severe congenital toxoplasmosis after late maternal infection.

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Virulence shift in a sexual clade of wild Toxoplasma gondii infecting marine mammals

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Toxoplasma gondii-associated meningoencephalitis is a significant disease of California sea otters (*Enhydra lutris nereis*) and other marine mammals. Toxoplasma isolates have been obtained from a harbor seal, a California sea lion, and 52 California sea otters since 1998.

Based on multi-locus PCR-RFLP and DNA sequencing at polymorphic genes (B1, BSR4, SAG1, ROP1, BAG1, GRA6 and GRA7), two distinct lineages have so far been identified: type II and a new type, called type X, that possessed distinct alleles from archetypal strains at all eight polymorphic loci examined.

The majority (72%) of marine mammal *Toxoplasma* infections were of type X, with the remainder being infected with type II strains. No type I or type III genotypes were identified. Type X strains have also been identified infecting a variety of terrestrial animals in the US, including humans. Phylogenetic analyses separated the type X /Toxoplasma/ isolates from types I, II and III. When assayed through mice, a number of type X strains possessed differing degrees of virulence.

The genetic basis for the altered virulence patterns among type X strains is currently being assessed and will be presented.

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New World *Toxoplasma* and Old World *Toxoplasma*: Do they have a clinical relevance?

Gómez-Marín J.E.[1]

A seminal work by Lehman et al. [1] has contributed to our best knowledge of *Toxoplasma gondii* genetic divergence, but also, unexpectedly, to shed some light on the origin of the species. It should be remembered that the original description of the three clonal lineages as the main genetic intraspecies variability [2], led many parasitologists to try to determine if there was any clinical correlation.

For many years the most cited paper was the description of clonal lineages and their frequency in different clinical forms by using strains isolated mostly from North America [3]. It gave the wrong impression that there was a correlation between type I and congenital infection, and type II and reactivation in immunosupressed patients. Subsequent work has shown that in fact the three clonal lineages classified by using the SAG2 genotyping, can be found indistinctly in congenital or in immunosupressed patients [4-7].

The use of multilocus and microsatellite analysis and the greater scope of the geographical origin of samples analyzed by SAG2 genotyping, finally suggested that there was a geographical restriction in genotype distribution [6, 8-10]. Thus, there is a clear predominance of type I and III in South America and of the type II in Europe and North America. More recently, the serogenotyping of serum samples from Europe and South America confirmed this impression [11]. Serogenotyping has the advantage that classification is not limited by the capacity of the strain to be isolated or by the bias of strain preference to growth in specific cell lines or in mice.

South American origin of *Toxoplasma* species

A complete picture has now emerged from the data of the study of Lehman *et al.* [1]. This study in 271 strains isolated from free range chickens from four continents (Australia strains were not included) conclusively showed that the origin of the species was in South America. This is easily understood if we take into account that by the mid-Cretaceous period, about 100 million years ago, South America had separated from Africa. During most of the following 100 million years, South America was an island-continent, and it has ben linked physically to Central and North America only since three million years ago.

The most plausible hypothesis about the origin of *Toxoplasma* indicates that the beginning of the species was from a coccidian (maybe obtained from birds) that evolved in a large variety of unique small felids, and that it continues being endemic in South America, but not in other continents.

The parasite was scarcely transmitted during millions of years, which explains its homogenous genetic structure and that only recently it reached a global distribution with the introduction of the domestic cat.

The origin of the Euroasian *Toxoplasma* (clonal type II) was probably a first exotic migratory event and then the European conquest of the New World and the transatlantic commercial exchange gave as a result the present global strain distribution, including the emergence of recombinant lineage type III.

Evidence for more severe forms of human toxoplasmosis in South America

Another unexpected finding has been obtained during the SYROCOT international collaborative study [12].

This systematic review based on individual data metanalysis aimed to determine if there was any benefit for the prenatal treatment of *Toxoplasma* infection. For this analysis, 25 cohorts of infected mothers identified during prenatal screening were selected. No clear evidence was found that prenatal treatment significantly reduced the risk of clinical manifestations in infected live born infants but the risk of ocular lesions was much higher in the South American cohorts (47%, 18/38) than in European ones (14%, 79/550). Another finding was that the risk of transmission decreased significantly with higher latitude (OR = 0.71 for 5° higher, 95% CI [0.53; 0.96], p = 0.03).

This results can now be understood at the light of our knowledge of a predominance of type I strains in South America. Type I strains are more virulent in mice than any other genotype and has been shown to have more capacity of migration through the extracellular matrix [13]. Type I strains also have higher number of transcripts for P30 protein [14], that is important for adherence during the invasion process. It seems that virulence in type I strains can not be attributed to a unique virulence factor or pathogenicity islands of genes but rather that is a multilocus trait [15].

For many years we observed that there were differences in the humoral immune response between European and South American patients with many clinical forms of toxoplasmosis. When comparing the frequency of immunoglobulins during recurrences of ocular toxoplasmosis by using the same methods Colombian patients seem to have more serum specific IgA [16] than it is reported in Dutch patients [17] (table 1).

Tabla 1

Frequency of serum specific anti *Toxoplasma* IgM and IgA immunoglobulins in Colombian and Dutch patients with recurrence of ocular toxoplasmosis

	IgM	IgA
Colombian patients (n=7)	28%	14%
Dutch patients (n=42)	12%	2%

Data taken from references [16] and [17].

Another report comparing Brazilian and Swiss patients also found differences in the humoral immune response [18]. Also, in Colombian HIV infected patients [19] with and without cerebral symptoms, serum specific IgM and IgA are more frequent than in European patients [20] (table 2).

Tabla 2

Frequency of specific anti *Toxoplasma* IgM and IgA immunoglobulins in Colombian and French HIV infected patients

	IgM	IgA
HIV Colombian patients with cerebral toxoplasmosis	53%	84%
HIV French patients with cerebral toxoplasmosis	12%	48%
Colombian patients without cerebral toxoplasmosis	12%	24%
French patients without cerebral toxoplasmosis	5%	12%

Data taken from references [19] and [20].

Also, there is a higher frequency of acquired ocular toxoplasmosis in South America. Studies in Brazil have found rates of ocular toxoplasmosis in the general population of 17% [21] and in Colombia of 6% [22]. These figures exceed largely the expected frequency by congenital infections.

The most severe cases of toxoplasmosis in the world in immunocompetent patients have been described in French Guiana, located on the northern region of the South American Amazonian forest [23]. In Colombia similar cases have been described in patients coming from the Amazonian region [24]. It has been postulated that this wild strains are more virulent given their unusual contact with humans, and therefore our immune system would not be prepared to their adequate control.

Prospects for research in South America

This new data is invaluable to guide future research in this field. One question to be solved is whether there is retinogenic strain. Strain characterization studies obtained from the eye have shown the presence of atypical genetic polymorphisms. In both, a first study of 12 samples from North America [24] and a Brazilian study with 11 clinical samples [24], results indicated a tendency in the majority of ocular strains to have a genetic divergence from the typical clonal lineages. The limitation to study these strains is that eye sampling could be a procedure limited to a few number of cases. Serogenotyping could be a possibility, and also the use of non invasive methods (i.e., fluorescent angiography) looking for biomarkers.

In conclusion, we think that the distinction between New World *Toxoplasma* and Old World *Toxoplasma* could help determining clinical prognosis during pregnancy, and maybe also in ocular presentations. A practical consequence might be that clinical trials to test new drugs should be done preferably in South America. To prove any positive effect in New World toxoplasmosis would need smaller samples that would be needed in Europe.

The III International Congress of Congenital Toxoplasmosis (ICCT) will be held in Armenia, Colombia, from 13 to 16 may 2007 (http://www.acin.org/montenegro/) and this will be a great opportunity to discuss this topic at the "birthplace of *Toxoplasma*".

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Future of vaccination in toxoplasmosis

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Protective immunity against *Toxoplasma gondii* is mainly T cell mediated. There is little antigenic variability between different strains of T. gondii, and natural infection induces at least a partly protective cross immunity when challenging with a heterologous strain.

It is possible to induce protective immunity in animals with a live, attenuated T. gondii strain, but immunity is not long lasting, probably because the strain does not produce tissue cysts, side effects are common and the vaccine is expensive.

Protein subunit vaccines or vaccines using recombinant proteins have not been very successful, and since the development of plasmid vaccines and live, attenuated virus vectors, most work is concentrating on these two delivery systems. Natural infections are through the gastrointestinal tract and mucosal immunity is important. Mucosal application of plasmid constructs and viral vector constructs as well as special adjuvants like cholera toxin have been found to induce mucosal immunity. One group is working on antigen delivery using attenuated *Salmonella* bacteria.

Much work has focussed on the selection of antigens. The surface antigen 1, SAG1, is a major vaccine candidate, because it is immunodominant at least in the humoral immune response, and other antigens, which have been shown to induce a high degree of protection, include the ROP and MIC antigen families.

It was shown earlier that immunization can protect mice against death after challenge with a virulent strain, but the important endpoint, at least from a veterinary view is protection against formation of tissue cysts, and reduction in tissue cysts burden is a much used endpoint in immunization studies.

In human, protection against maternal-fetal transmission in pregnant women and prevention of pathology in congenitally infected children is important endpoints, and studies need to be done in proper animal models to predict this outcome in humans.

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SYROCOT CONCLUSIONS

GILBERT R.[1]

Despite three decades of prenatal screening for con genital toxoplasmosis uncertainty remains about the effectiveness of prenatal anti-toxoplasma treatment.

Methods: We conducted a systematic review of cohort studies based on universal screening for congenital toxoplasmosis. We did a meta-analysis using individual patient data to examine the effect of the timing and type of prenatal treatment (spiramycin or a pyrimethamine-sulphonamide combination) on mother to child transmission of infection and clinical manifestations in infancy.

Analyses were adjusted for gestational age at maternal seroconversion and characteristics of the study center.

Results: We included 25 cohorts in the review but confined the meta-analysis to 22 European cohorts. In 1,438 treated mothers identified by prenatal screening, we found weak evidence for a higher risk of mother-to-child transmission when prenatal treatment was started later after seroconversion (Odds ratio [OR]: 1.07 per week; 95% confidence interval [CI]: 1.02 - 1.11).

Among 550 infected infants identified by prenatal or neonatal screening, we found no evidence that prenatal treatment significantly reduced the risk of clinical manifestations in infected live born infants (OR for treated vs. not treated: 1.11; 95% CI: 0.61 - 2.02).

Gestational age at seroconversion was strongly associated with mother-to-child transmission (OR: 1.15; 95% CI: 1.12 - 1.17) and with the risk of intracranial lesions (OR: 0.91; 95% CI: 0.87 - 0.95) but marginally with eye lesions (OR: 0.97; 95% CI: 0.93 - 1.00).

Conclusions: There is no clear evidence of a beneficial effect of prenatal treatment for congenital toxoplasmosis. Further evidence from observational studies is unlikely to change these results and would not reduce biases due to confounding. Only a large randomized controlled clinical trial would provide clinicians and their patients with a valid evidence of the potential benefit of any prenatal treatment in this indication.

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EFFECT OF PRENATAL TREATMENT ON LONG TERM OCULAR DISEASE AND NEUROLOGICAL IMPAIRMENT - RESULTS FROM EMSCOT

GILBERT R.[1]

Information is lacking on the effects of congenital toxoplasmosis on development, behavior, and impairment in later childhood, as well as on parental concerns and anxiety. This information is important for counselling parents about the prognosis for an infected child and for policy decisions on screening.

Methods: We prospectively studied a cohort of children identified by screening for toxoplasmosis in pregnant women or neonates between 1996 and 2000 in ten European centers. At 3 years of age, parents of children with and without congenital toxoplasmosis were surveyed about their child's development, behavior, and impairment, and about parental concerns and anxiety, using a postal questionnaire.

Results: Parents of 178/223 (80%) infected, and 527/821 (64%) uninfected children responded. We found no evidence that impaired development or behavior were more common in infected children, or that any potential effect of congenital toxoplasmosis was masked by prenatal treatment. Parents of infected children were significantly more anxious and reported more visual problems in their children.

Conclusions: On average, children aged three to four years with congenital toxoplasmosis identified by screening and treated during infancy in this European setting had risks of abnormal development and behavior similar to uninfected children. Parental anxiety about infected children needs to be addressed by clinicians. Future studies with longer follow up and clinician-administered assessments may be better able to detect any subtle differences in child outcomes.

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Laboratory diagnosis of ocular toxoplasmosis

Kijlstra A.[1]

Typical retinal scars with satellite lesions are indicative of ocular toxoplasmosis. The diagnosis is, therefore, based primarily on the findings of an ophthalmologic examination. In certain cases the fundus may be obscured due to the intraocular inflammation. In these cases the suspected clinical diagnosis of ocular toxoplasmosis can be confirmed by the analysis of intraocular parasite-specific antibody production. Intraocular synthesis of immunoglobulin G antibodies against microorganisms is considered as an indirect proof of uveoretinal infection.

To prove the validity of this hypothesis we determined local anti-viral and anti-Toxoplasma antibody production in a large group of uveitis patients and controls. Paired serum and aqueous or vitreous samples were tested for total IgG levels and antibodies to herpes simplex virus, varicella-zoster virus, cytomegalovirus and toxoplasma using commercial-ly available test kits. Local toxoplasma antibody testing in toxoplasma chorioretinitis had a sensitivity of 74.0% and a specificity of 100%.

The polymerase chain reaction (PCR) can be used to detect T. gondii DNA in ocular fluid samples, although PCR is mainly useful in the acute stage of inflammation in immunocompetent individuals. PCR assay of aqueous humor is helpful in the etiologic diagnosis of HIV-infected patients since antibody production can be disturbed.

Intraocular fluid sampling also allows research into the role of cytokines in the pathogenesis of ocular toxoplasmosis. Cytokine analysis may increase the knowledge concerning the pathogenesis of ocular toxoplasmosis but is not suitable as a diagnostic tool.

Analysis of the specificity of intraocular T cells obtained from patients with ocular toxoplasmosis has revealed that the intraocular inflammatory response is due to an antiparasitic reaction and that autoimmunity does not seem to be involved. The technical aspects of this latter approach make it less suitable for diagnostic purposes.

Taken together, our studies from the past decades have shown that the analysis of intraocular antibody production is still the most important tool in the etiological diagnosis of *Toxoplasma* uveitis.

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Ocular toxoplasmosis in Colombia

de la Torre A.[1]

Despite the fact that in tropical countries ophthalmologists know that they have to deal daily with ocular toxoplasmosis as one of the most frequent causes of intraocular inflammation, there was not any previous report about the clinical characteristics and epidemiology of this entity in Colombia. So, we decided to study the real impact of this disease in our country and carried out a study describing pattern distribution and clinical features of uveitis in two ophthalmological referral centers in Bogotá, comparing similarities and differences between Colombia and other countries (de la Torre A, *et al.* Clinical patterns of uveitis in two referral centers in Colombia, submitted to Ocul Immunol Inflamm., March 2007).

We reviewed retrospectively their clinical records between 1996 and 2006, and analyzed sex, age at presentation, classification, antecedents, affected eye, diagnosis, and complications comparing them with published data from other countries. We found 747 patients with some type of uveitis. Infectious diseases were the most frequent cause (46.9%) -toxoplasmosis being the most common (36.9%)--, followed by idiopathic (32.1%), trauma (16.8%), non-infectious

diseases (9.1%), and toxocariasis (4.4%). Thus parasitic infections play an important role as a cause of uveitis in Colombia, and it may be related with the high frequency of posterior uveitis.

In another study we screened a young adult population by fundoscopy to determine the prevalence of chorioretinal scars, which turned out to be 6% (de la Torre A, *et al*. Am J Ophthalmol. 2007;143:354-6). Many important findings came out of these results. A significant proportion of the population had chorioretinal scars without being aware of their condition, which means that *Toxoplasma* retinal lesions are more common than expected. It is noteworthy that three of our subjects had significant visual defects in one eye but had not visited the ophtalmologist; contralateral eye compensation may explain the reason for this type of behavior. Also, there is the possibility of recurrent inflammatory activity in these lesions or the appearance of new lesions that could significantly affect visual acuity.

A follow-up study would allow us to determine if population screening by fundoscopy for ocular toxoplasmosis is justifiable in highly endemic geographical areas. Another interesting finding was that its high prevalence indicated that the most likely origin of ocular infection was in the postnatal period.

Previous research in the same region (Quindío, Colombia) had shown that congenital infection occurs at a maximum of 5 cases per 1,000 live newborn children (0.5%); thus there is a significant excess of cases (at least 5.5% in young adult population) that could not be explained by congenital infection. This study found an unexpected high prevalence of chorioretinal scars in Armenia (Colombia); however, more studies are needed for a better understanding of the impact of *Toxoplasma* infection on visual health.

We also evaluated the clinical characteristics of patients with ocular toxoplasmosis in Armenia, Colombia (de la Torre A, *et al.* Clinical features of ocular toxoplasmosis in a Colombian cohort, preliminary report. Infectio 2006;10:105). We studied 44 patients (59 eyes) between September 2005 and November 2006 (20 months), 20 male (45.5%) and 24 female (54.5%), with a median age at the first episode of 24 years (P_{25}=10 - P_{75}=33). Twenty cases had active lesions (45.5%) and bilateral involvement was found in 17 cases (38.6%). The number of lesions ranged between 1 and 10 with a 2.1 average; lesion size ranged between 0.5 to 7 disc diameters with a mode of 1; 8 inactive cases (33.3%) had legal blindness (visual acuity less than 20/200). The most frequent complications were posterior sinequiae, cataract, and strabismus. We found 2 cases (11.8%) with recurrent lesions and positive IgM.

As far as we know, this is the first report in Colombia about clinical characteristics of patients with ocular toxoplasmosis. We found a high percentage of inactive cases with legal blindness, mainly affecting young population. There was macular compromise in 67.8% of the cases. Consequently, we would like to emphasize the need to create primary prevention programs in this particular area.

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NEW BIZARRE FEATURES IN OCULAR CONGENITAL TOXOPLASMOSIS

RODRÍGUEZ A.[1]

Toxoplasmosis is a protozoan infection that can invade practically every structure in the body and may cause pathological changes that vary to some extent with the tissue involved. Worldwide interest in human toxoplasmosis is responsible for advances in pathophysiology, reports on new features, in technological documentation and in management.

Methodology: Retrospective, observational, descriptive study of new clinical ocular pictures associated with ocular congenital toxoplasmosis that were subjected to documentation, follow up and management. Entities to be discussed are: vitreomacular traction syndrome, parasitic choroidal macular neovascular membranes, and focal vascular occlusions in retinochoroiditis scars.

Results: This is not and statistical report but a description of cases with scars of toxoplasmic retinochoroiditis presenting clinical features related to the diseases to be discussed.

Conclusions: Toxoplasmosis can affect most ocular structures, preferentially the retina and can be associated with other diseases.

- Progress in evaluation has allowed documentation of clinical features associated to scars of toxoplasmic retinochoroiditis
- Fluorescein angiography and optical coherence tomography are important tools in evaluation.
- Follow-up is important in decision-making.
- Adequate management, medical or surgical, is important to prevent complications and to obtain success in anatomical and functional results.

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Treatment evidence: What is needed?

Stanford M. 1

Toxoplasmic retinochoroiditis is a leading cause of ocular morbidity in tropical countries. Despite the use of a number of well proven antibiotics that are active against the parasite, the damage done and recurrence of disease does not seem to be affected by their use.

An ideal antibiotic for use against toxoplasmic retinochoroiditis should have the following characteristics:

- It should be effective.
- It should be parasitocidal.
- It should be active against both bradyzoites and tachyzoites.
- It should penetrate cysts.
- It should be distributed to the back of the eye.
- It should cause little adverse effects.

Unfortunately, none of the current agents in use has this profile.

Rothova *et al.* (1983) compared the use of 3 different treatment regimes, all of which included the use of systemic corticosteroids, against nothing (for peripheral lesions) in Holland. She showed no difference in the outcome of any regime (including nothing). The principal messages of her study were that:

- The duration of an attack depended on the size of the active retinitis at presentation.
- Treatment did not reduce the length of an attack.
- Treatment was no more effective in recurrent disease as opposed to in those who presented for the first time.
- Treatment did not alter the eventual visual prognosis.
- There was a 50% recurrence rate in all groups at 3 years.

What's the current evidence?

In a systematic review of the use of antibiotics in toxoplasmic retinochoroiditis (Stanford *et al.,* 2003), only 3 placebo randomised control trials were found that addressed this problem. All were methodologically weak and compared very few patients and none reported the effect of treatment on visual acuity. Perkins found no effect of daraprim *versus* a dummy tablet although it was not clear whether his patients actually had uveitis due to active toxoplasma. Akers found no effect of a combination of clindamycin and spiramycin *versus* placebo but only included 10 patients in each arm of the study. Silveira and colleagues (Silveira *et al.*, 2002) examined the effect of twice weekly Bactrim® on the recurrence rate of patients at high risk of recurrence over 20 months. They showed a reduction in the relapse rate in the treated group (OR=0.28, 95% CI 0.1-0.78). However, this was not a masked study and there was a high loss to follow up in both arms of the study. It is possible that long term prophylaxis may alter the recurrence rate in this situation but further trials are required. An increase in adverse effects was reported in two studies. The systematic review concluded that there was no evidence for an effect of antibiotics in toxoplasma retinochoroiditis either for primary (visual outcome, recurrence rate) or secondary (duration of attack, size of resultant chorioretinal scar) outcomes. Despite the lack of evidence a recent survey of members of the American Uveitis Society showed that nine different antibiotics were in use in 24 possible combinations. Nine other randomised head-to-head comparisons of different antibiotics have been published but since none of these included a placebo arm it is impossible to tell if the treatments actually affected the natural history of the infection. The role of corticosteroids is also unclear. Antibiotics are effective for toxoplasmic retinochoroiditis in the immunocompromised and maintenance treatment may be required long-term (Holland, 2004).

Why don't the drugs work?

It is well known that these drugs are effective *in vitro*, in experimental models and in immunosuppressed patients. Why then should they have apparently no effect in recurrent disease? One possibility comes from evidence that stage conversion in active infection in a primed immune system can occur quite quickly. It is therefore conceivable that by the time the patient presents with symptoms a significant number of tachyzoites will have reverted to bradyzoites and/or encysted. The duration of disease (e.g., symptoms) would then be determined by the duration of the intraocular inflammatory response.

What is needed?

The obvious answer it to perform a prospective, double blinded, placebo controlled, randomised trial for lesions appearing anywhere in the fundus with sufficient power to disprove the null hypothesis. Such a study would need to examine objective end points such as visual acuity and field, time to 'hardening' of lesions and the recurrence rate at 5 years. To be of sufficient power, at least 125 patients would need to be entered into each arm. It is unlikely that such a trial will ever be

performed given the expense, the lack of pharmaceutical company interest, the difficulty of enrolling at least 300 patients within a practical time and the likely high loss to follow up over the period of the trial.

Are there other ways of reducing the likely burden of visual morbidity?

– Starting treatment at the start of symptoms (in practice giving patients with recurrent disease a course of antibiotics to take as soon as symptoms start)
– Developing antibiotics that are effective against bradyzoites and treating for longer
– Reducing the acquisition of disease in childhood through improved public health measures.

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TOXOPLASMA NEWBORN SCREENING PROGRAMS IN DEVELOPING COUNTRIES

GÓMEZ-MARÍN J.E.[1]

Newborn screening programs for congenital toxoplasmosis are valid alternatives for regions or countries where prenatal programs are too expensive in relation to available public health funds. Some of the advantages of newborn screening programs are:

i) They are less expensive.

ii) Around 60% of infected children are asymptomatic; thus, it is possible to treat them before damage manifestations.

iii) Some mothers do not go to prenatal control (in Colombia 10% of pregnant women), and the newborn detection program is the only possibility to make the diagnosis.

In our experience with two newborn programs in Colombia, sampling was a significant problem which was addressed differently when the program was carried out at a third level reference hospital or at community hospitals. In reference hospitals the best option was to obtain the sample at birth from the umbilical cord. On the other hand, in community hospitals the best compliance and follow-up of children was obtained when the sample was taken at the first medical visit of the child after birth.

In order to implement this strategy we need to determine the efficacy of treatment, and whether it should be given during 3 months or 1 year. Also, a large multicentric study is necessary to determine the impact of follow-up strategies and the best strategy for blood sampling, umbilical cord blood or at the first visit of newborn. Of outmost concern is follow-up loss of patients (41% in our case), which makes compliance a major goal for this program.

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OCULAR PROGNOSIS IN CHILDREN WITH CONGENITAL TOXOPLASMOSIS

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In an attempt to understand and interpret recent data on the effect of pre- and postnatal treatment, we tried to compare the data of our cohort of 430 children with computed tomography to those published by other groups. 30.2% (130) of our patients developed ocular toxoplasmosis lesions during a median follow-up of 12 years comparing well to recently published data with ranges from 19-39% after follow up times of 1-4.5 years.

Results: Only two children of our own series or 1.6% of all children showed a bilateral affection of their visual function. These results were achieved in our as well as in utmost of other recently published cohorts with a combination of *in utero* - and postpartal treatment throughout the first year of life and are markedly better than reported for historical cases who were untreated or treated for shorter periods.

This view is also supported by the results of the SYROCOT metaanalysis which reports a weak evidence for an association between early prenatal treatment and reduced risk of congenital toxoplasmosis. Nevertheless, treatment efficacy is still a concern, since there is no obvious effect of antenatal tratement on the incidence of organ manifestations, and progression of eye lesions may be observed in a number of eyes despite therapy during the first year of life.

Conclusion: In the absence of controlled studies we have to assume that there exists a small but eventually relevant benefit of pre- and postnatal treatment. Although not all children do well with treatment, the outcomes of recently published cohorts indicate the importance of diagnosis and treatment of infants with congenital toxoplasmosis until the results of larger randomised controlled clinical trials provide valid evidence of the potential benefit of pre- and postnatal treatment.

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Pharmacokinetics of postnatal treatment of congenital toxoplasmosis

Peyron F.[1]

Congenital toxoplasmosis is a chronic infection which essentially causes ocular lesions that can recur spontaneously throughout life. Significant malformations are rare and are generally detected before birth. The systematic treatment given to an infected newborn is calculated to reduce the ocular and neurological sequelae of the illness. At present, two combined medications are used for the postnatal treatment of congenital toxoplasmosis: pyrimethamine and/or pyrimethamine and sulfadiazine. Spiramycine is no longer used for the treatment of this disease in neonates. There is no consensus on the treatment of congenital toxoplasmosis and no study has been carried out to evaluate the efficacy of any treatment. Moreover, few data are available on the pharmacokinetics of these medications in children who suffer from the disease.

Pyrimethamine

Three studies on the pharmacokinetic characteristics of this drug in congenitally infected children, using different doses and therapeutic regimens, have been reported in the literature. The half-life of pyrimethamine is between 2.67 and 5.5 days. Large interindividual variations have been observed for the other pharmacokinetic parameters. Concentrations in the cerebrospinal fluid, and the cerebral ventricles were 10% and 25% of those observed in serum, respectively.

Sulfadoxine

Six pharmacokinetic studies in children have been identified. A dose of 25 mg/kg every 10 days was generally used. The half-life was between 1.5 and 22.4 days, with an average of 6 days. Other pharmacokinetic parameters varied considerably. The intracerebral concentration of the drug was not reported in children. In adults with no meningeal inflammation, the concentration of sulfadoxine was of an order of 12.5 to 20% of that observed in serum and increased in cases of meningitis.

We studied the pharmacokinetic characteristics of Fansidar® (a combination of pyrimethamine and sulfadoxine) in 32 children who were treated during the first year of life. Doses of 1.25 mg/kg of pyrimethamine and 25 mg/kg of sulfadoxine were modified every 3 months according to weight gain. The drug was administered every 10 days. The parents also received explanations on how to administer the drug and a dose schedule to improve compliance with the treatment. Levels of the drugs in serum were evaluated every 3 months using high-performance liquid chromatography. Each child also received 50 mg of calcium folate each week. The half-times varied from 0.5 to 9.6 days for pyrimethamine and from 8.2 to 14.3 days for sulfadoxine, with no correlation between the two drugs. Other pharmacokinetic parameters showed considerable interindividual variations. The minimal and maximal concentrations varied from 0.01 to 0.25 µg/ml and 0.15 to 1.20 µg/ml for pyrimethamine and from 10 to 90 µg/ml and 50 to 200 µg/ml for sulfadoxine, respectively. Overall, the product was well tolerated; only seven children (10.8%) showed a haematological intolerance that could reasonably be ascribed to the drug.

Sulfadiazine

Two studies on the levels of sulfadiazine in children suffering from congenital toxoplasmosis have been reported, but only minimal concentrations were measured and pharmacokinetic constants were not addressed. Moreover, the doses administered were much higher than those used to treat other conditions.

In healthy children, the half-life of the drug was estimated at 21.3 hours, which is longer than that observed in adults. The maximal concentrations in children treated with 12.3-16.4 mg/kg of sulfadiazine were comparable with those observed in adults. In children that received 50-100 mg/kg of sulfadiazine, the minimal concentration 12 hours after administration of the drug was 35-123 µg/ml.

Feeding increased absorption of the drug by two to threefold. The concentration of sulfadiazine in the

cerebrospinal fluid was between 10% and 80% of that observed in serum.

In summary, the pharmacokinetic data on pyrimethamine and sulfoxadine in children who suffer from congenital toxoplasmosis show considerable interindividual variations. Information on the most appropriate therapeutic regimen is lacking, and no data are available on the doses and modes of administration. For sulfadiazine, the pharmacokinetic parameters of the drug at doses used to treat congenital toxoplasmosis are unknown.

Prospects

Should pharmacokinetic studies be carried out? This could be the case for sulfadiazine, for which such information is not available.

The real question is: What will we do with the results?

Effectively, if, from experimental data and pharmacokinetic studies, we could determine the optimal blood concentrations and the toxicity threshold, how could we evaluate the efficacy of the treatment of congenital toxoplasmosis?

The difficulty that we encounter is the lack of clinical and biological criteria.

Treatment at birth is generally continued for 1 year. On the basis of what physiopathological concept was this length of treatment determined?

Does the first year of life correspond to a period of parasitic replication in children who were contaminated *in utero*?

Does the treatment help the organism's defenses to control parasitic replication during a period of immune immaturity?

We know that retinal lesions can appear belatedly and their mechanism has not been clearly elucidated. Moreover, the efficacy of treatment on these lesions has not been demonstrated. In contrast to antenatal treatment, for which the efficacy of the medication on mother-to-child transmission is a criterion that can be determined, there are no pharmacodynamic markers of postnatal treatment of congenital toxoplasmosis. Our experience through the follow-up of more than 400 cases of congenital toxoplasmosis, treated before birth, has shown that the illness is very rarely apparent at birth. The detection of ocular sequelae, which affect only a minority of patients, necessitates a period of follow-up that is far longer than the duration of treatment generally prescribed. For these reasons, it is difficult to devise a pharmacodynamic study to determine the relationship between concentrations of the drug and the response to treatment. Postnatal treatment of congenital toxoplasmosis is long, constraining and runs a significant risk of side effects. From an ethical point of view, this situation is disastrous. To improve it, a better understanding of the physiopathology of the disease during the first year of life is needed:

Does parasitic replication occur in newborns?

When does the immune response in children become effective?

In the immediate future, in order to reduce these constraints, a maximal duration of treatment should be defined in order to provide a reasonable proposition.

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