Toxoplasmosis

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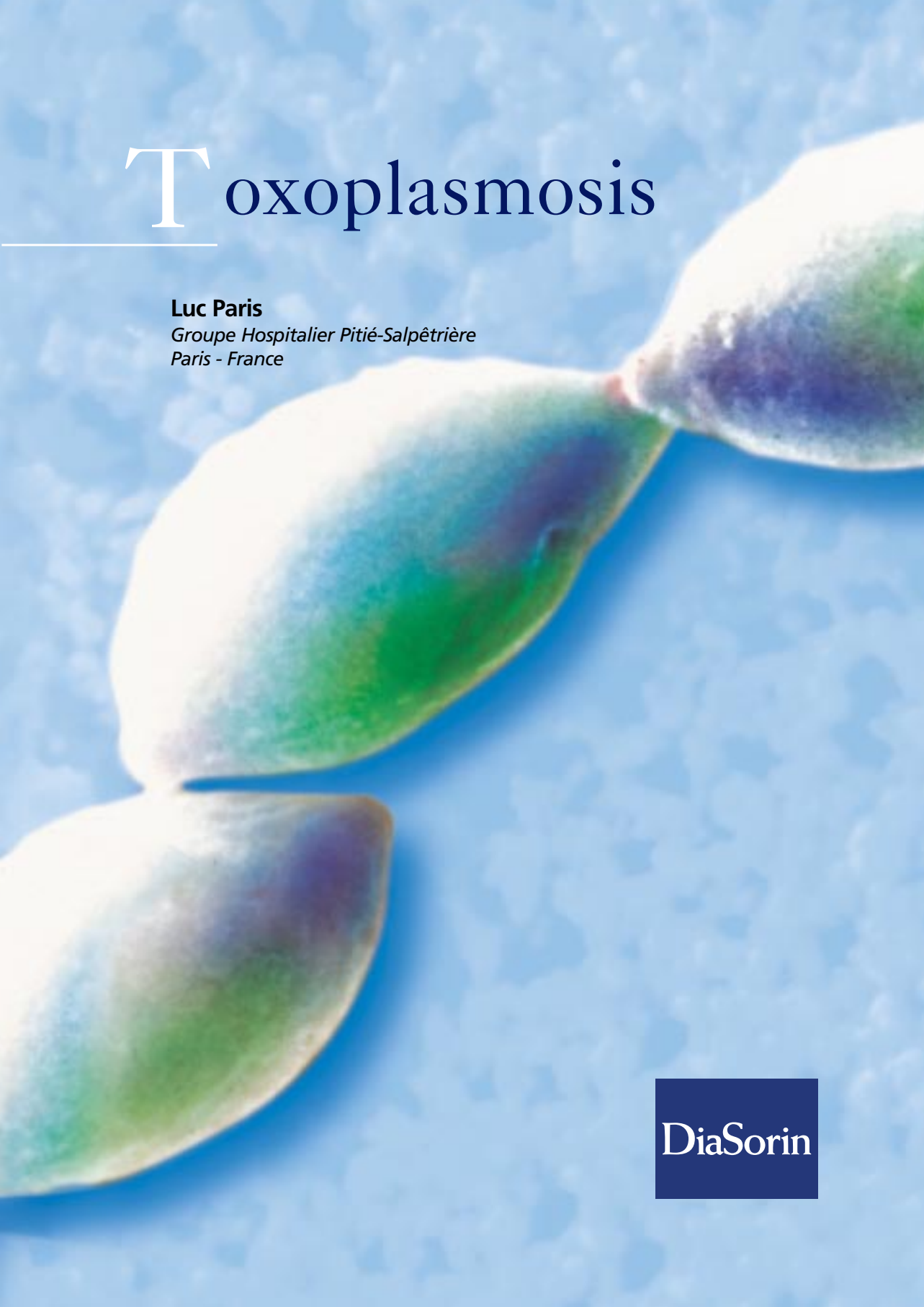

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The parasite and the disease

Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii*. It is generally benign but is potentially dangerous in the foetus and in immunosuppressed patients.

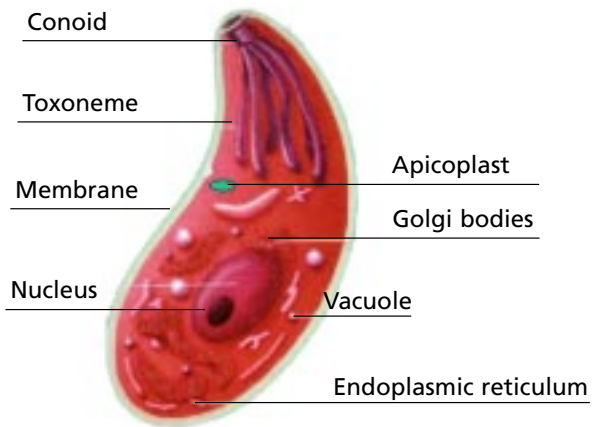
The parasite

Toxoplasma gondii (Nicolle and Manceaux, 1909) is an Apicomplexa protozoan belonging to the eucoccidida order and has three infectious stages:

- **The tachyzoite** is crescent shaped 6 to 8 µm long and 3 to 4 µm wide, with a rounded posterior end and a pointed anterior end. The cytoplasm contains the organelles normally present in eukaryotes (nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria) and a plastid organelle, the apicoplast, which derives from an ancestral chloroplast originating from endosymbiosis with a microscopic algae; the apical complex characteristic of the Apicomplexa phylum is located at the anterior end of the parasite. The tachyzoite is the actively multiplying intracellular form and is able to enter any type of cell.

- **The bradyzoite** is the intra-tissue resistance form and develops as a result of transformation of the tachyzoite stage. Apart from a few ultrastructural details, the major differences are in the metabolism and parasitophore vacuole. The membrane and matrix between the parasites thickens forming the toxoplasmic cyst, the rounded 5 to 100 µm diameter intracellular part which may

Structure of tachyzoite of *Toxoplasma gondii*



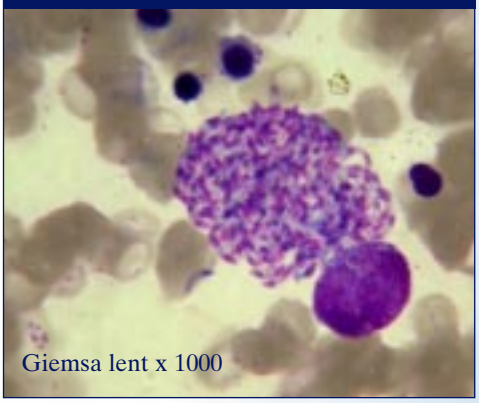
contain up to a thousand bradyzoites, which have an extremely slow metabolism and are inaccessible to the host's immune defences and to current anti-toxoplasmosis treatments. The cysts lie mostly within neurones, astrocytes, muscle cells and retinal cells.

- **The sporozoite** is the result of sexual reproduction which takes place in the intestinal epithelial cells of felines. It is not morphologically different from other infectious stages and is contained in the spore-forming oocysts. These cysts are not directly infective and may survive on the ground for more than one year in a humid climate.

Life cycle and transmission

T. gondii is a widely distributed parasite, both in geographical and zoological terms, as all warm blooded animals, both mammals and birds, are potential intermediate hosts. The definitive hosts are felines and tropical neofelines, principally the cat in temperate areas. A unique feature of toxoplasmosis is the possibility of it undergoing an incomplete life cycle which does not involve the definitive host, as the parasite can pass from one intermediate host to another through the ingestion of cysts contained in the flesh of carnivorous or herbivorous animals. This effect, associated with the possibility of self-fertilisation in the cat (between male and female gametes of identical genotype), may partly explain the low genetic polymorphism of this parasite, most of the isolates of which are grouped into 3 principal multilocus genotypes (I, II and III), which are equivalent to clonal lines. This is surprising in principle for an organism which reproduces sexually. It is the type II genotype which usually causes human toxoplasmosis.

Toxoplasma cyst in the bone marrow



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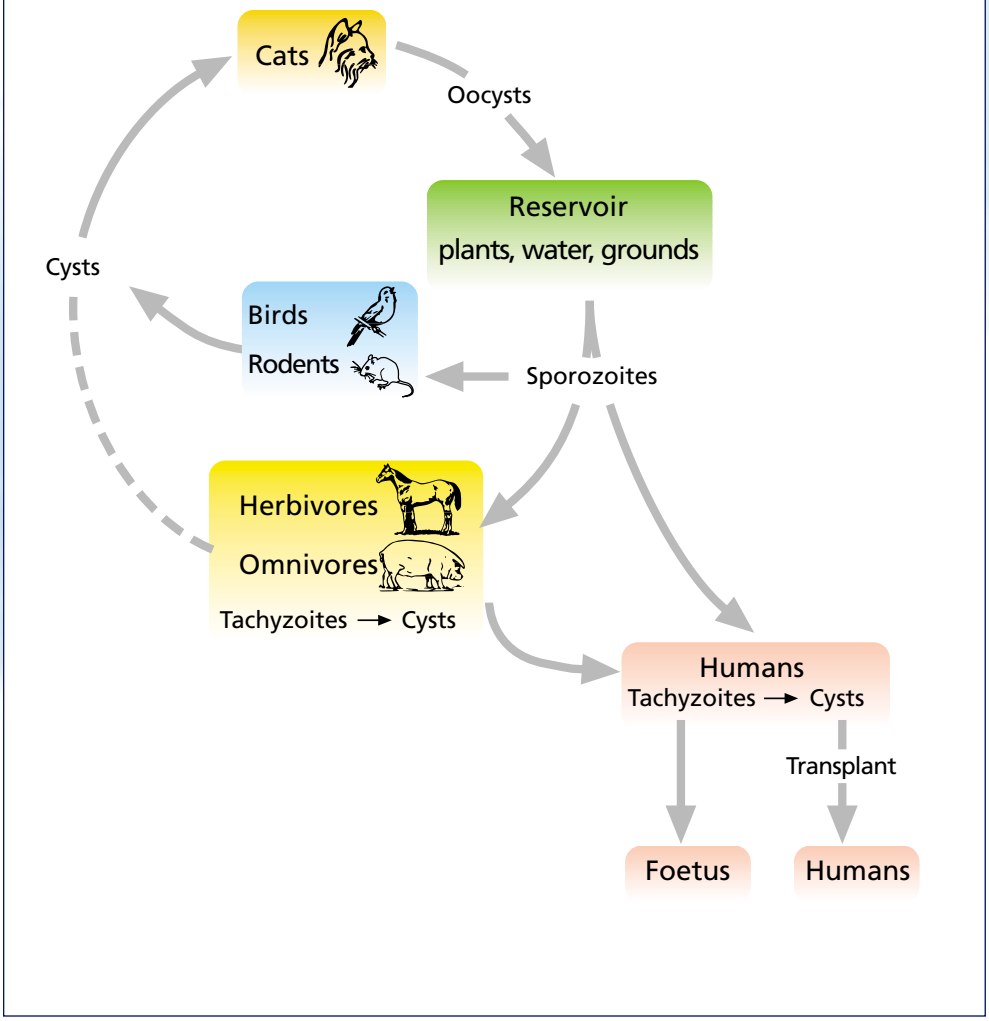
Human beings can be infected by one of the three following pathways:

- **Transmission through ingestion of oocysts:** this is essentially indirect transmission through the consumption of poorly washed raw fruits and vegetables or contaminated drinking water, contact with the ground (gardening) or animals. Recent studies have shown that dog fur is

Life cycle of *Toxoplasma gondii*



far dirtier than cat fur.

- **Transmission by cysts:** infection occurs through the consumption of smoked, marinated or inadequately cooked meats, as the cysts are only destroyed by cooking meat at 67°C or by freezing at less than -12°C for at least 3 days.

Prophylaxis also involves ensuring cleanliness of the refrigerator, working areas and cooking utensils. The cysts are also responsible for transmission in organ transplantation from a donor who is seropositive for toxoplasmosis to a recipient who was negative before

transplantation: the main whole organ usually involved in this type of transmission is the heart.

- **Transmission by tachyzoites:** the tachyzoite is fragile and easily destroyed in the external environment and by gastric juices. It is responsible for placental transmission, which causes congenital toxoplasmosis. The tachyzoite is also responsible for the rare cases of transfusion transmission which may occur if the donor was in the full parasitaemic phase of toxoplasmosis. Finally, the tachyzoite is also involved in accidental transmission in analytical laboratory technicians from a needlestick injury when handling a laboratory maintained strain.

In practice, in France, the major risk factor for acquiring toxoplasmosis in seronegative pregnant women (who undergo systematic, compulsory serological monitoring) is eating a meal outside of the home on a daily basis (a situation which does not allow for careful monitoring) that raw vegetables have been washed and meats cooked. The presence of a cat within the family does not emerge as a risk factor; several explanations may be offered for this paradoxical observation:

- The only cats at risk are young animals who hunt for their food. A pet cat which hardly ever leaves the house and is fed with commercial foods is not a "danger" of toxoplasmosis.
- In addition, cats only excrete oocysts for a few weeks during their life during the primary infection. In addition the oocysts must remain in the external environment for a certain length of time in order to be capable of infection.
- Finally, most pregnant women are aware of the association of cats and toxoplasmosis and take considerable precautions with respect to these animals.

Clinical manifestations

Acquired post-natal toxoplasmosis in the immunocompetent person

This is asymptomatic in more than 80% of cases, and a past history of toxoplasmosis is only evidenced as a result of systematic investigations.

The symptomatic forms associate fever, lymphadenopathy, and asthenia. The patient presents with a spike of fever (38-38.5°C) lasting a few days or weeks and which disappears spontaneously. The lymphadenopathy tends to be mostly cervical and of low volume, although other lymph node areas may be affected. The asthenia may be profound and may last for several months. The outcome is normally uncomplicated and recovery occurs spontaneously without treatment.

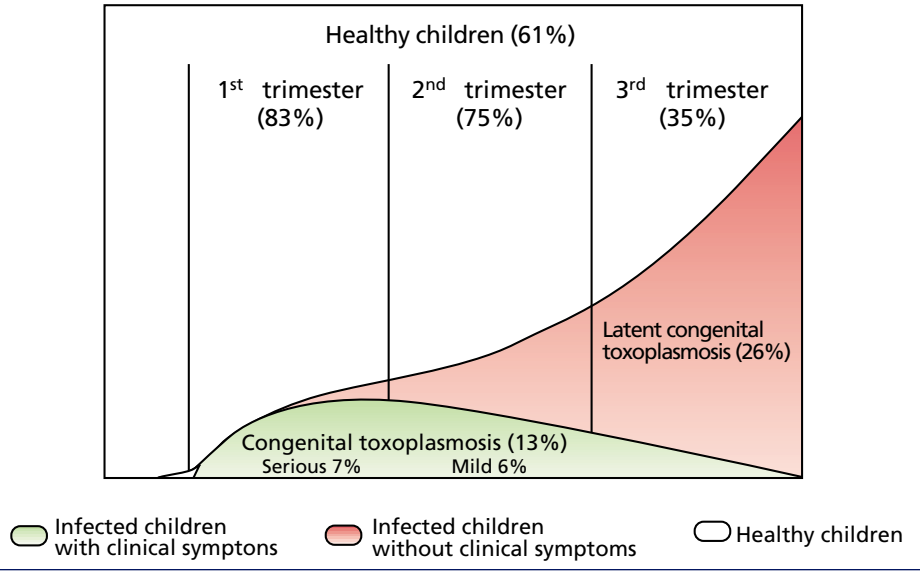
More serious forms of acquired toxoplasmosis have been reported in recent years in immunocompetent people, in particular with ocular, neurological and even other tissues as in the immunosuppressed, which have in some cases led to the patient dying. In all of the cases

in which the strains of parasites involved have been studied, gene typing has demonstrated an unusual frequency of type I or atypical recombinant genotypes. Most of the cases described in France originate from French Guyana where the risk factor is the consumption of wild game meat.

Congenital toxoplasmosis

This occurs as a result of infection of the foetus during pregnancy. The most common cause is the development of a primary infection in the pregnant woman, although transmission may also occur during a recurrence of parasitaemia in an immunosuppressed pregnant woman (reactivation toxoplasmosis). The risk of vertical transmission increases with term inversely to the severity of foetal disease, which decreases. There is also a risk of transmission if infection occurs less than 2 months before conception, the period during which parasitaemia may persist. Finally, vertical transmission has been very rarely described in reinfections. This occurrence is sufficiently rare that it does not invalidate the general rule that considers an immunocompetent protected woman with serology indicative of old toxoplasmosis at the start of the pregnancy protected: it is simply necessary to undertake a toxoplasmosis assessment of the new-born child if the child presents evidence of neonatal in-

Risk of transmission and severity of congenital toxoplasmosis depending on the term of pregnancy



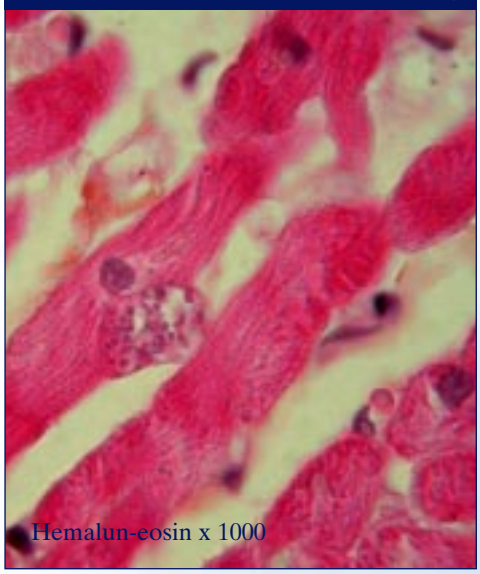
fection of undetermined origin.
Congenital toxoplasmosis may be responsible for miscarriage. Three clinical situations are conventionally described in a pregnancy that proceeds to term:

- Severe congenital toxoplasmosis is an encephalo-meningo-myelitis which is seen from birth onwards and represents infection at the beginning of the pregnancy. Two clinical forms are classically described, the first associates macrocephaly with hydrocephaly, intra-cranial calcifications and ocular involvement in the form of pigmented chorioretinitis, and the second presents a serious neonatal infection with a poor prognosis. These serious forms are now rarely seen because of modern methods of management of seroconversion in pregnant women.

- Benign congenital toxoplasmosis (reduced or delayed), which develops due to infection later on in the pregnancy is diagnosed from birth onwards or during early childhood. The symptoms leading to the clinical diagnosis are delayed psychomotor development, progressive development of hydrocephalus, the onset of seizures and pigmented chorioretinitis.

- Neonates with latent congenital toxoplasmosis are clinically normal at birth and are diagnosed only on a laboratory basis. This form represents approximately 80% of congenital toxoplasmoses in France. Early treatment of these cases avoids their possible secondary progression to a delayed form of the disease.

Toxoplasma in a cardiac biopsy



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Toxoplasmosis in the immunosuppressed

This is a serious and invariably fatal disease without treatment, except for the isolated ocular forms which may lead to blindness. The classical descriptions distinguish localised from disseminated forms although in reality they are often less distinct.

- **Localised toxoplasmosis**
  The organ most frequently infected is the brain: the clinical sign is an ab-

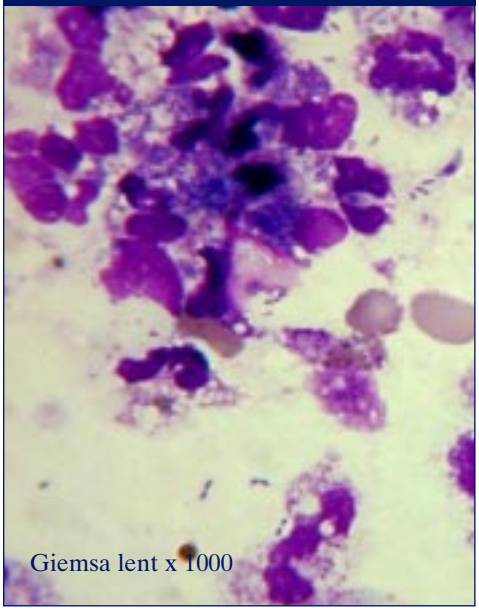
scess. The symptoms manifested include fever, headaches, and a localised defect related to the site of the abscess(es). Diffuse encephalitis or inaugural epilepsy attacks have been also described.

The second most affected organ is the eye. The patient complains of reduced visual acuity, the impression of "floaters" and redness of the eyes. The diagnosis is ophthalmological. Cerebral involvement is also present in 40% of cases during HIV infection.

Pulmonary toxoplasmosis is characterised by febrile pneumonia, causing dyspnoea and suggests pneumocystis. The correct diagnosis is carried out by examination of the bronchiolo-alveolar lavage material, provided that the laboratory practitioner is not limited to silver staining which does not allow for the identification of toxoplasmosis.

The *T. gondii* tachyzoite may enter any type of cell and literature is rich with cases reported in various sites, the diagnosis being made histologically.

Tachyzoites in bronchiolo-alveolar lavage fluid



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- **Disseminated toxoplasmosis**

  In this case the main problem is isolated fever with possible involvement of secondary organs. In most cases the diagnosis is based on identification of the parasite by staining, animal inoculation or molecular biology.

Epidemiology

Toxoplasmosis is a cosmopolitan parasitosis: its prevalence increases with age and varies between regions, depending on the climate, environmental conditions, dietary habits and level of hygiene. Overall, infection occurs earlier on in less environmentally favourable areas and in developing countries than in wealthier populations which have a higher level of hygiene.

In the developed countries, Europe and North America, infection is mostly due to consumption of contaminated meat. Its prevalence is low, generally less than 25% in countries in which meat is eaten well cooked (United

Kingdom, Scandinavia). The figures are higher in France and in Germany, in the region of 40 to 60%, because of the habits of eating rare or smoked meats. Prevalences in Italy and Spain are between these two (20 to 50%). Regional differences exist and have been well studied in France, figures range from 38% in cold winter mountain areas (Vosges, Jura, Massif Central, Alps) to 68% in the south and humid regions of the North-West. The prevalence is less than 25% in North America.

Prevalence is extremely low in South-East Asia and Japan, less than 10%, and in the region of 20 to 30% in the Indian sub-continent and the Middle East.

Infection in tropical African and American countries is mostly due to ingestion of oocysts. Prevalence is low in areas where the climate is warm and dry, a climate which is unfavourable for oocyte survival on the ground and is higher, up to 80% in some cases, in humid regions.

New habits of consumption (freezing meat, improved hygiene in rearing houses, use of commercial foods for animals) are tending to reduce the prevalence in developed countries.

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Laboratory diagnosis

The laboratory diagnosis of toxoplasmosis may be undertaken using serology or by testing for the parasite itself. Very schematically, serology is used for the diagnosis in immunocompetent people, including pregnant women, and direct testing for the diagnosis in the foetus and in the immunosuppressed. Both approaches are necessary and complimentary in the neonate at the boundary between anti and neonatal.

Identification of the parasite

This must be used in circumstances in which serology does not contribute to the diagnosis of infection of the foetus (antenatal diagnosis) and of reactivation toxoplasmosis in the immunosuppressed. This test also forms part of the neonatal diagnosis (examination of the placenta).

Direct testing

This is difficult, demanding and very often negative. It must, however, always be attempted on different biological samples sent to the laboratory: peripheral blood, bone marrow, cerebrospinal fluid, bronchiolo-alveolar lavage (BAL), and various biopsies, particularly brain biopsies. The classical May-Grünwald-Giemsa stain allows for the identification of the parasites, theoretically intracellular but often released onto the slides because of damage to the cells caused by the preparatory stages prior to staining the slides. We should point out here that performing solely silver stains on BAL in order to diagnose pneumocystis or other fungal infection may mask a pulmonary toxoplasmosis which may have damaging consequences. Sensitivity of the test may be increased using fluorescein labeled monoclonal antibodies.

Indirect testing

- **Animal inoculation**

  This old technique is irreplaceable to isolate toxoplasma strains. The biological material is injected intraperitoneally in mice which are seronegative for toxoplasmosis. In most cases the mouse develops chronic toxoplasmosis, the diagnosis of which is confirmed by serological monitoring in the animal. The disadvantage of this method is the long response time (the serological controls are performed on the mice 15 days and then 3 and 6 weeks after inoculation in our laboratory). In the case of acute toxoplasmosis in animals, the parasite is found in the ascitic fluid or in the brain.

- **Cell culture**

  Most teams have abandoned this diagnostic method in favour of PCR. The cell culture model, however, is still of great use, particularly in pharmacological studies.

- **Molecular biology**
  Molecular biology techniques, in particular PCR, are now used routinely for the diagnosis of toxoplasmosis. They are included on the list of medical laboratory procedures for antenatal diagnosis. Most teams are moving towards real time PCR methods on Light Cycler or Taqman automated instruments which are faster, less subject to contamination and which provide objective readings. The most widely used targets at present are the B1 gene and ribosomal DNA although other sequences currently being evaluated appear to increase the sensitivity of the method.

Serological diagnosis methods

Serological screening is based on concomitant testing for IgG and IgM. Testing for IgA (and secondarily IgE) is only performed after if necessary.

Methods using a cellular antigen

- **Dye test.** The lysis test or dye test of Sabin and Feldman (an adaptation of this method developed by Desmonts is used in France) remains the reference method to test for IgG. The results are expressed in international units per millilitre (IU/mL) compared to a standard serum. This method is scarcely used other than in a few reference laboratories as it requires the maintenance of a strain of living toxoplasma and a source of complement from fresh antibody free human serum.

- **Indirect immunofluorescence (IIF)**
  Using formol treated trophozoites is still widely used in specialist Parasitology laboratories. When testing for IgG the result is expressed in IU/mL. When testing for IgM (Remington test) the result is expressed as the reciprocal of the last positive dilution. This is an excellent method which does, however, require appropriate equipment (fluorescence microscope). Reading is occasionally tricky and must be performed by a trained technician. The presence of antinuclear antibodies is a source of false positive IgG and rheumatoid factor is a source of false positive IgM. A change in the IgM curve by IIF occurs over approximately 3 months, although the method is far less sensitive than immunoadsorption techniques and it unfortunately produces a number of false negatives.

- **Agglutination techniques**

1. The "classical" direct agglutination method (Fulton method) is hardly used any more. It offers poor sensitivity for the detection of residual antibodies but on the other hand is excellent at the very beginning of seroconversion, with the large difference between titres before and after

2-mercapto-ethanol (2 ME) allowing for the presence of IgM to be confirmed. Unfortunately the method is equally sensitive for natural IgM and a difference of at least three dilutions is required before suspecting the presence of immune IgM.

2. On the other hand, high sensitivity agglutination (HSAg), the commercial version which uses antigens which have been trypsinised, is only performed after treatment of the serum with 2 ME, and is very sensitive. It is an excellent method to discriminate positive from negative sera. A system of conversion is used to express the titration in IU/mL.

3. A second variation (with methanol treatment of antigens), so-called acute stage specific agglutination (AC agglutination) only identifies IgG antibodies produced at the beginning of the infection (first 6 to 12 months). It is very useful in dating the infection, although is not however, available commercially and antigen preparation can be difficult.

4. ISAgA (immuno-sorbent agglutination assay) is a semi-quantitative method which is both sensitive and specific, and can be used for the detection of IgM, IgA and IgE. It is the reference method for the testing of IgM. There is no commercial kit to test for IgE.

Methods using a soluble antigen

The complement binding method is entirely obsolete and has been withdrawn from the French market for several years. A latex method exists which is rapid and simple to perform but which is only of use in screening as it only detects total antibodies; false negatives may also occur through the zone effect.

- **Haemagglutination**

  The antigen is bound to sheep red blood cells and the technique can be performed on serum whether or not treated with 2 ME, allowing for an estimate of the presence of IgM. It is a good screening method.

- **Immuno-enzymatic methods**

  ELISA methods, either on microtitre plates or on closed automated instruments, are increasingly being used because of their clear ergonomic advantages: objective reading, measurement of large batches, automation and adaptation to multichannel analysers.

  Commercial kits are available for IgG, IgM and IgA testing.

  The optical density/titre relationship for IgG titration is not linear if the IgG titre is very high, therefore required additional dilutions add a degree of imprecision to the final result.

  As testing for IgM and IgA is per-

formed after immunoadsorption, there is no competition with IgG. The result is expressed in the form of an index calculated with respect to the OD of the threshold value.
One of the major problems of these ELISA methods is the total absence of standardisation, as each manufacturer has different threshold values for IgG and specific index calculation systems for the other isotypes. It is perhaps useful to remember that, in general, in serology terms, results obtained from two consecutive sera from the same patient can only be compared if they have been processed in the same laboratory by the same method during the same run.Trying to compare two results obtained by ELISA using two different reagents can only result in incorrect conclusions.

- **Measurement of IgG avidity:** some ELISA IgG kits and several automated multi-channel instruments can also be used to measure IgG avidity, in other words the strength of antigen-antibody binding: this increases with time during the weeks following primary infection and then stabilizes. It may be quantified from the ratio of the optical density (OD) obtained using the method after washing with a dissociating agent (in practice a urea solution) to the OD obtained using the technique with the normal wash buffer (without dissociating agent) on the same serum. There is controversy in Europe as to how this method should be interpreted. The widely accepted opinion in France is that avidity is used as an exclusion method: if the IgG avidity is high, the infection is considered to be old, the exclusion period varying depending on the kit. If the avidity index is low or intermediate, no conclusion may be drawn as some patients retain a low avidity index with old infection. In these cases, other isotypes must be tested and a second serum taken at a 15 to 20 day interval, should always be tested in parallel. Other authors consider that low avidity is sufficient evidence to conclude that the toxoplasmosis is acute; commercial kits are being developed on this basis. When considering a method which has recently been introduced for the laboratory diagnosis of toxoplasmosis, it is wise to remain cautious. Experience obtained by French authors on the subject of toxoplasmosis is such that their opinions should be taken seriously (because of the very strict legislation which is unique in the world, and which requires screening for any organ, cell or tissue donation, pre-marriage screening and screening of pregnant women, together with monthly systematic follow-up until childbirth in seronegative women). Finally, the avidity index cannot be interpreted if the patient has received anti-toxoplasmosis treatment.

- **Immuno-blotting (Western blot) and ELIFA (enzyme linked immunofiltration assay):** the major benefit of these methods, the former having the advantage over the latter of being commercially available, is the comparison of several sera from the same patient or of a mother-child pair. The development or increase in a precipitating system in the child helps to provide information supporting the diagnosis of congenital toxoplasmosis where conventional techniques are occasionally deficient.

Interpretation of results

Testing for the toxoplasma

Identification of *T. gondii* on a stained film allows the diagnosis to be confirmed. Unfortunately this situation is rare. Isolation of the parasite following inoculation in the mouse is of the same value. We should note here that the presence of toxoplasma in the placenta is a sufficient criterion to confirm the diagnosis of congenital toxoplasmosis, although there are 4 known exceptions to this rule associated with type I toxoplasma strains (as a large majority of congenital toxoplasmoses are type II). Finally, an isolated PCR result must always be placed in the clinical context but if the method appears to be valid (positive control, negative control, amplification control, exclusion of possible contamination) the diagnosis must be made.

Serology

The general rules for interpretation of serology remain valid. A few points however should be stressed. For IgG low levels must be interpreted with great caution. For the same patient, particularly when using ELISA techniques, repeated blood sampling or the same sample tested on several occasions may lie alternatively above and below the threshold value: the application of two methods based on different principles are recommended in order to confirm the specificity of the detected antibodies. In case of doubt in pregnant women, the best thing to do with a patient who has borderline levels very close to the threshold is to monitor them in the same way one would a seronegative woman.

Current methods for the detection of IgM are very sensitive to persistent IgM. IgM is found by ISAgA in more than 50% of patients 18 months after seroconversion, and with some ELISA reagents the figures are in the region of 30% over the same period. Commercial reagents which are relatively insensitive to residual IgM are also relatively insensitive to IgM at the start of seroconversion. This is worrying as the main aim is the earliest possible diagnosis of seroconversions in pregnant women who represent by far the greatest number of cases in terms of toxoplasmosis serology. The concomitant presence of IgG

and IgM is therefore not a sufficient criterion (or even necessary, as very rare cases of seroconversion without IgM exist) to conclude that recent infection, has taken place and it is at most a warning sign which should lead to further investigations to date the infection more precisely. As the AC agglutination method does not have a commercial application, it is by studying IgG avidity and testing for other isotypes, principally IgA which provides the conclusion. In particular in the pregnant woman, the first serology measurement must have been performed during the first trimester in order to date the infection satisfactorily from the time of conception: however highly performing current toxoplasmosis serology methods have no means of excluding peri-conception seroconversion if the serology sample taken at the end of pregnancy demonstrates the presence of IgG and IgM. Regardless of serological status, testing a second serum taken at an interval of 2 to 3 weeks is essential in order to offer a definitive conclusion. Finally, we should remember that it is the appearance of IgG which allows for the conclusion of definite seroconversion to be made when monitoring a patient who has been negative up to this point. The development of a positive IgM reaction is not sufficient for the diagnosis, as this may be associated with non-specific intercurrent reactions. With some methods, particularly by ELISA, it is not unusual to see IgM reactivity develop during pregnancy without IgG appearing, the serology spontaneously becoming entirely negative after childbirth. These patients should be considered as being seronegative. Interpretation of IgA must always be prudent. This isotype appears very early, at the same time or immediately after IgM, and theoretically disappears over 6 to 7 months. Seroconversions without IgA, however, exist and may persist beyond the usual periods (we have seen a case of persistence for 15 months after seroconversion). Finally, in case of seroconversion in an immunosuppressed patient, for example post-transplantation rejection, the time of appearance of antibodies may be longer than in immunocompetent people (because of interference of anti-rejection treatments in antibody kinetics) and the direct diagnosis may precede the development of positive serology.

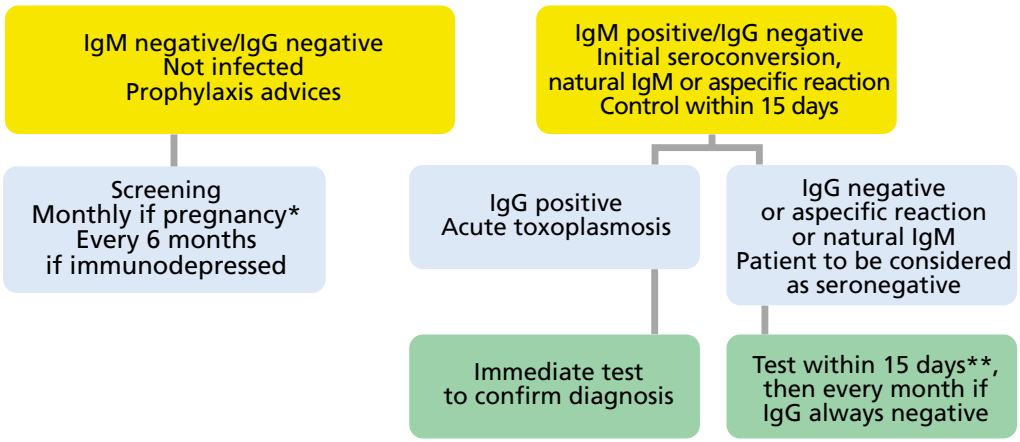
The process for serological diagnosis in the immunocompetent patient is described in the following flow chart.

Two distinct situations must be considered separately in the immunosuppressed patient.

1. Patients susceptible to a relapse of an old toxoplasmosis infection. Serology in these patients never provides confirmation that the acute clinical episode is indeed related to toxoplasmosis and only allows a possible diagnosis to be made. It is testing for the parasite or the effectiveness of a trial therapy

Toxoplasmosis

Interpretation algorithm



* The final control must be performed more than one week after childbirth in order not to miss seroconversion at the very end of pregnancy. In practice, a period of 2 to 4 weeks may be proposed.

** Depending on the subjects and reagents used, detection of IgG will be more or less delayed compared to the appearance of IgM. It is very rare to see a period of more than 3 weeks although we have a personal example in which this period was more than two months.

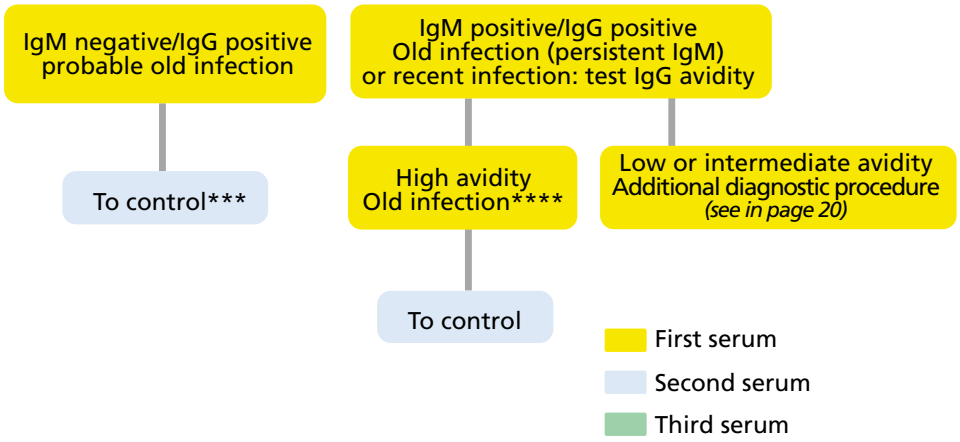
(justified by the presence of a cerebral abscess) which will confirm the diagnosis. The affected patients are those suffering from profound cellular immunity deficiency, particularly those with an HIV infection and CD4 count < 100/mm^3 and bone marrow transplantation patients who were seropositive for toxoplasmosis before transplantation.

2. Whole organ transplant patients, particularly heart transplant patients in cases of mismatch (seropositive donor for toxoplasmosis-negative recipient). These cases involve primary infection (infection by the transplant) and the serology is still contributory, although there is a delay in the appearance of antibodies which justifies direct testing in the event of suggestive clinical signs.

In contrast, in whole organ transplant patients who are seropositive for toxoplasmosis pre-transplantation, major

serology

in immunocompetent subjects



*** This control is justified for two main reasons: the very occasional cases of seroconversion without IgM and confirmation of the first result (tube labeling error).

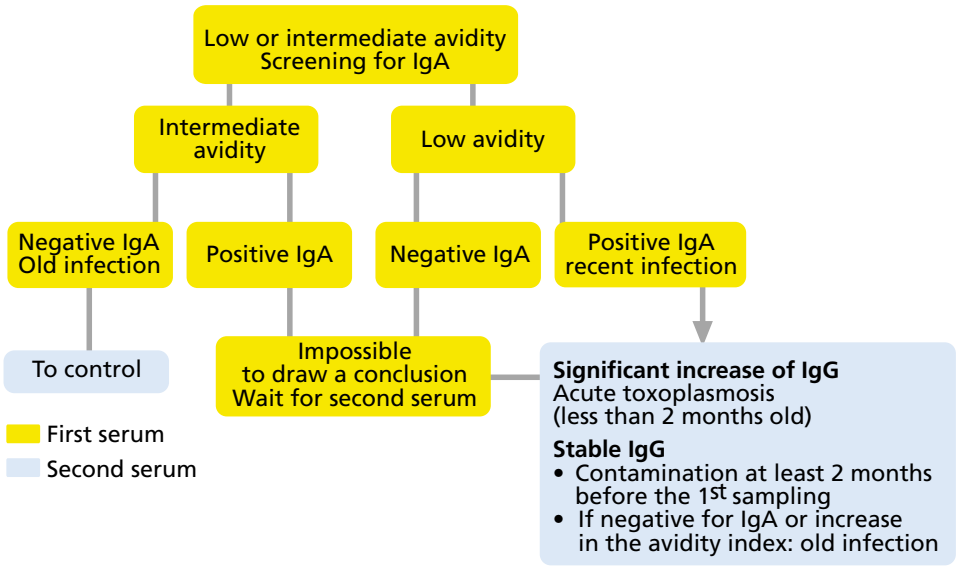
**** The exclusion period depends on the kits or methods used.

serological reactivation of IgG may occur post-transplantation, occasionally accompanied by the re-emergence of other isotypes, although usually without clinical consequence.

Antenatal diagnosis of congenital toxoplasmosis

The antenatal diagnosis is based on amniocentesis with inoculation of amniotic fluid into the mouse and on PCR. The diagnosis of congenital toxoplasmosis may be confirmed by a positive result from either of these two methods. Conversely, a negative result does not exclude foetal disease, as published data describe a false negative rate of approximately 35%. The laboratory methods for a neonatal diagnosis must therefore be used for all neonates whose mothers have a suspect serological history during pregnancy, with a negative or unperformed antenatal diagnosis. These methods involve testing for the parasite

Routine protocol used in the parasitology laboratory of "Groupe Hospitalier Pitié-Salpêtrière", Paris - France

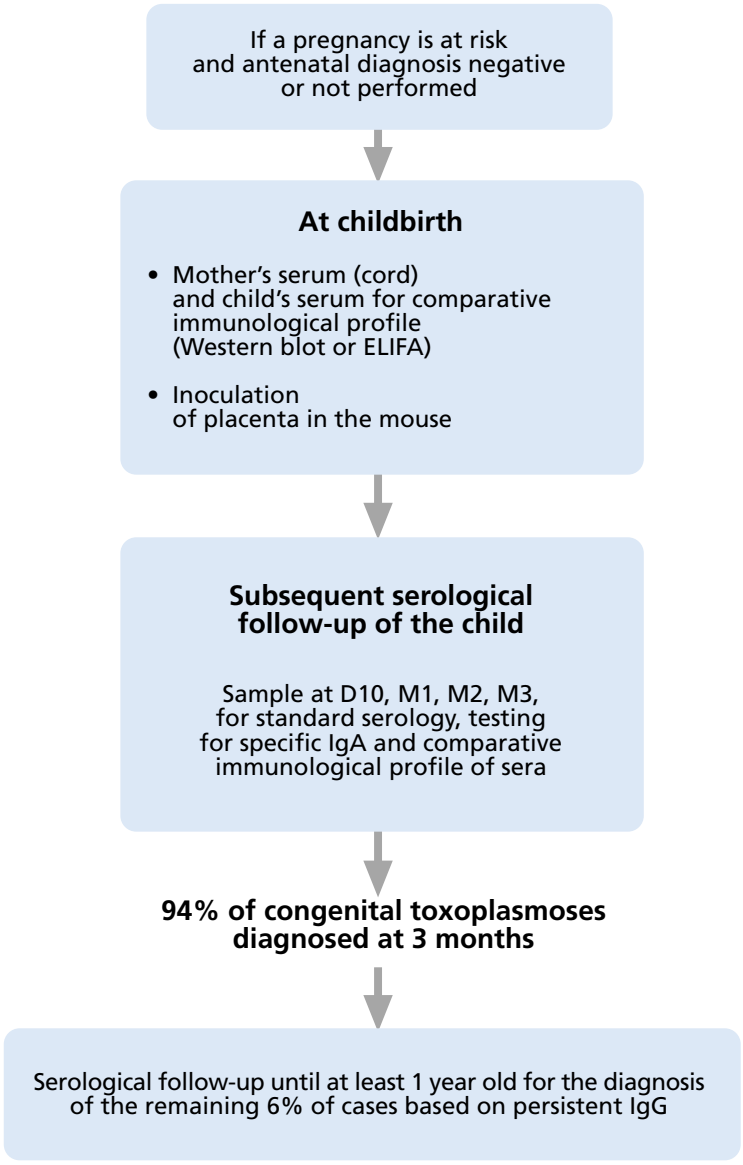


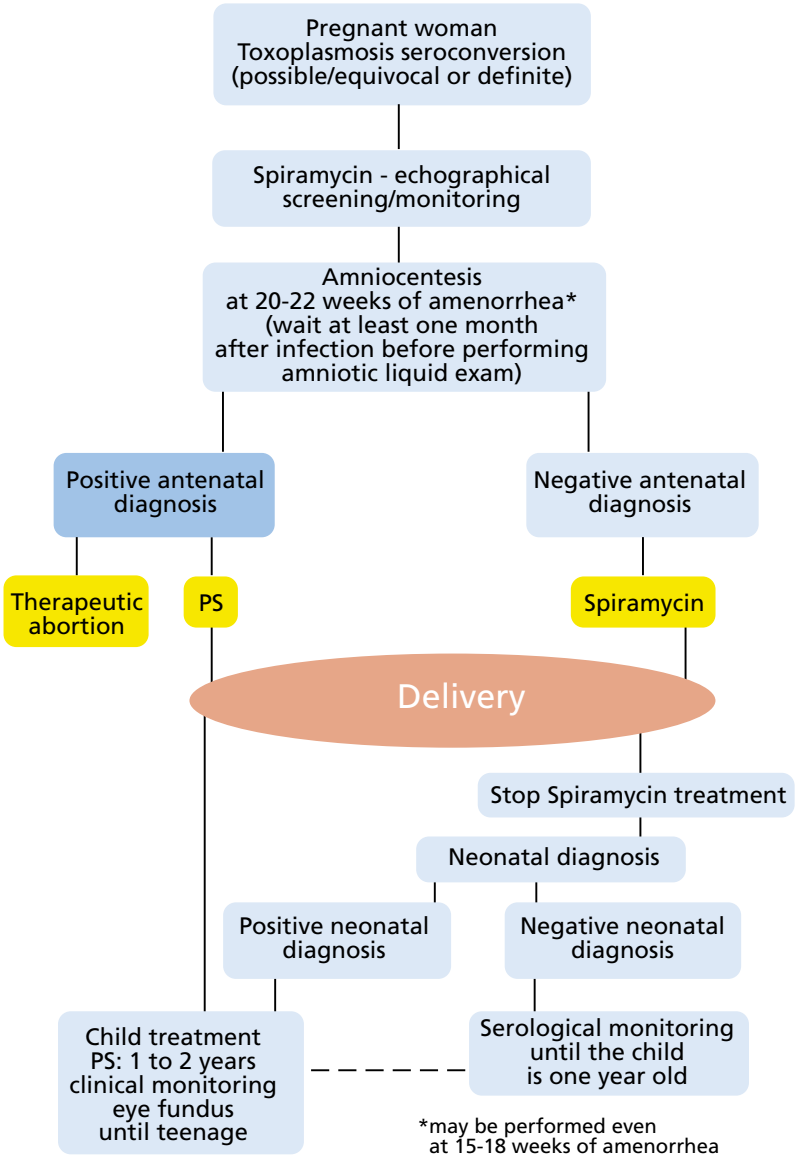
and serology.

Testing for the parasite is always performed indirectly by inoculation of a mouse or by PCR. The biological substances studied are the placenta, cord blood and CSF. The child's serology at birth is not truly contributory as detection of IgM or IgA may be due to passage of maternal blood into the child at the time of delivery. At this stage it is the comparative mother/child immunological profile which allows the diagnosis to be made through the presence of precipitant systems specific to the child. A few days after birth, the presence of specific IgM or IgA allows the diagnosis of congenital toxoplasmosis to be confirmed. Conversely, the absence of these isotypes does not exclude congenital toxoplasmosis. In approximately 6% of cases only serological follow-up beyond the third month after birth leads to the diagnosis because of persistent IgG, which normally disappears within a year. The sensitivity of methods for neonatal diagnosis is however decreased if the mother has received treatment with pyrimethamine and sulphonamides during the pregnancy. In addition, with such treatment, serology has been reported to become negative in children who genuinely suffer from congenital toxoplasmosis, although they usually become positive again when the treatment is discontinued.

The practical approach is summarised in the table (on the right).

Neonatal diagnosis of congenital toxoplasmosis





PS = Pyrimethamine Sulphonamides

Antenatal diagnosis = echography, amniocentesis for PCR detection and mouse inoculation

Neonatal diagnosis = clinical symptoms, serology (IgM, IgA), placental inoculation in mouse, mother and child comparative immunological profile (WB, ELIFA)

Management of toxoplasma seroconversion in a pregnant woman

The diagnostic and therapeutic management of toxoplasma seroconversion in a pregnant woman, as managed in France, is summarised in the algorithm (on the left). Some teams do not perform amniocentesis in the case of very late seroconversion during pregnancy and treat the mother from the outset with pyrimethamine and sulphonamides because of the very high risk of vertical transmission close to term. This approach, which is defendable, does on the other hand, seriously hinder the possibilities of neonatal diagnosis. Different choices have been made in other countries: for example German authors recommend a course of pyrimethamine and sulphonamides for one month in cases of seroconversion in a pregnant woman before carrying out an amniocentesis. This treatment is then continued only if the antenatal diagnosis is positive.

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Practical examples of the management of serological diagnosis in immunocompetent pregnant women

In all of the examples below, the results of IgG titration and avidity and the results of IgM testing have been obtained with the LIAISON® automated analyser (DiaSorin, Saluggia, Italy). The criteria for interpretation of the results are those proposed by the manufacturer:

- IgG or IgM < 6; negative
- IgG or IgM > 8; positive
- IgG or IgM ≥ 6 and ≤ 8; equivocal
- IgG avidity < 0.2; low
- IgG avidity > 0.25; high
- IgG avidity ≥ 0.2 and ≤ 0.25; intermediate

IgM specificity was confirmed by a commercial Toxo ISAgA method and assayed using specific IgA and IgE was an in-house immunoadsorption-agglutination method. The interpretation criteria are the same for all three isotypes: 0 to 5 negative, 6 to 8 equivocal, 9 to 12 positive.

Case No. 1

In a pregnant woman monitored monthly, negative results were obtained in three sera tested prior to serum No. 1.

Comments: The appearance of IgM on D23 indicates early seroconversion and a check up was performed 12 days later. A rise in the IgM index was seen although IgG was still not present. On the same day a test for IgA was negative. The test performed on D55, i.e. three weeks after the last positive IgM serum, detected specific IgG thereby confirming seroconversion. Measurement of the avidity index does not provide additional information and was performed systematically, the result obtained (low avidity) being expected.

	IgG IU/mL	IgM UA/mL	Avidity (index)
Serum 1	<3	<3	
Serum 2 D23	<3	8.1	
Serum 3 D35	<3	59	
Serum 4 D55	33.1	56.5	0.141

Case No. 2

This was the first serum taken from a woman who was 2 months pregnant with no known past serological history.

	IgG IU/mL	IgM UA/mL	Avidity
Serum 1	390	55.3	0.38
Serum 2 D21	372	57.9	

Comments: The avidity index observed as early as the first serum suggested that this was a case of old toxoplasmosis, despite the presence of IgM, the specificity of which was confirmed by ISAgA. Testing for specific IgA and IgE in the same serum was negative. This is also a factor supporting an old infection. The stability of the results at 3 weeks makes any further investigation unnecessary and confirms the initial conclusion, allowing the future mother to be reassured.

Case No. 3

As in the previous case, this was the first serum taken from a woman in the second month of her pregnancy.

	IgG IU/mL	IgM UA/mL	Avidity	ISAgA IgA
Serum 1	94.5	22.1	0.132	0
Serum 2 D15	76.2	23.2		

Comments: The first serum was positive for IgG and IgM, with low avidity. A test for specific IgA was performed and was negative. It is very difficult to offer a conclusion on this first serum. The serological situation was entirely stable on the second serum which, combined with the absence of specific IgA in the first serum, allows for the conclusion of an old toxoplasmosis.

Case No. 4

This series of serum came from a pregnant woman followed up monthly. All of the sera taken before serum number 1 were negative.

	IgG IU/mL	IgM UA/mL
Serum 1	<3	13.3
Serum 2 D15	<3	21.2
Serum 3 D28	<3	15.5
Serum 4 D42	<3	9.6
Serum 5 D70	<3	7.9
Serum 6 D90	<3	<3

The appearance of IgM can be seen in this series of results and is confirmed by ISAgA on the first three samples. The tests for IgG performed in parallel using a second method were all negative. The woman delivered on D70 and the post-partum serum control was negative. In these cases it is difficult to resist the temptation to prescribe treatment as soon as the first positive IgM serum is obtained. Prescription of spiramycin could interfere with antibody kinetics and in fact delay the diagnosis of any seroconversion, which can only be confirmed with the appearance of IgG.

Practical examples of the management of serological diagnosis in neonates

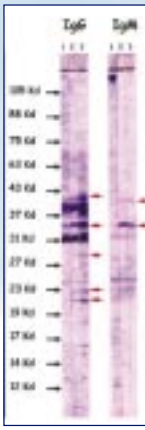
In the cases described below, IgG was titred by indirect immunofluorescence (laboratory antigen, threshold value 6 IU/L), and IgM tested by commercial Toxo ISAgA. The ISAgA IgA, IgE and Western Blots were carried out using in-house laboratory methods.

Case No. 5

The table summarises the serological findings in the mother at delivery and

	IgG IU/mL	IgM	IgA	IgE
Mother	50	12	12	3
Baby D1	100	12		
Baby D7	200	12		

in the child on D1 and D7.
In the seven day old baby the presence of IgM is sufficient evidence to confirm congenital toxoplasmosis. This diagnosis is confirmed by the positive result of placental inoculation in the mouse. The Western blot profile (see figure) confirms the presence of congenital toxoplasmosis as early as the initial sample because of additional bands observed in the child in the 6-44 Kd range for IgG and 13-44 Kd range for IgM.



Comparative immunoserological profiles by Western Blot

- 1 : mother's serum at D1
- 2 : child's serum at D1
- 3 : child's serum at D8
- Additional bands
  - IgG : 28, 23, 21, at D1
  - IgG : 41, 35, 28, 21, 12, at D8
  - IgM : 41 and 35

Case No. 6

This child's mother seroconverted in week 23 of the pregnancy. No antenatal diagnosis was performed and placental inoculation in the mouse was negative. The serological progression is shown below (D = day, M = month). The comparative immunological profile of the sera from D1 to M3 by Western Blot did not demonstrate the appearance of additional bands. The diagnosis was made on M4 due to inflection of the IgG curve, confirmed by the appearance of additional bands in the same serum on the Western Blot.
In the cases described below (with the kind permission of Dr. Valeria Meroni - University of Pavia), anti-Toxoplasma IgG and IgM were titred using LIAISON® assays while IgA tested by ELISA DiaSorin ETI-Toxo IgA. Western Blot bands were obtained with a commercial WB kit.

Case No. 7

A mother seroconverted during the 30th gestational week (M1, IgM were present but there was no evidence of IgG) and was treated with pyrimethamine, sulphonamides and rovamycin. IgM were present but there was no evidence of IgG.
Four months after delivery and interruption of therapy, the mother showed a marked increase in IgG titers.

	IgG IU/mL	IgM	ISAgA IgM	IgA	IgG Avidity
Mother M0	Neg	Neg	-	-	-
Mother M1	Neg	75	12+	Neg	-
Mother M2	Neg	26	12+	Neg	-
Delivery					
Mother M 4	Neg	16	12+	Neg	-
Mother M 5	10	8	12+	Neg	0.397 High
Mother M6	55	8	12+	Neg	0.379 High
Mother M7	500	7	12+	Neg	0.345 High
Baby D1	10	Neg	Neg	Neg	-
Baby D60	Neg	Neg	Neg	Neg	-
Baby D120	35	Neg	Neg	Neg	-
Baby D180	20	Neg	Neg	Neg	-

The high avidity results confirmed the seroconversion 4 months before, excluding the possibility of a false positive result in the early detection of IgM and of an acute infection after delivery. The delay in the appearance of IgG could be attributed to the early therapy.
The baby was born with an asymptomatic Toxoplasma infection (confirmed by the result in Western Blot) and was treated only at the time of antibody rebound at day 120.

Case No. 8

A mother seroconverted during the second trimester of pregnancy. She was treated with spiramycin. The PCR on amniotic fluid was negative.
The baby was completely asymptomatic. He was not treated but monitored until he turned 1 year old.
The IgG results were always negative as well as the IgG/IgM Western Blot analysis and confirmed the absence of infection.

	IgG IU/mL	IgM	ISAgA IgM	IgA	IgG Avidity
Baby D1	500	Neg	Neg	Neg	-
Baby D28	225	Neg	Neg	Neg	-
Baby D56	118	Neg	Neg	Neg	-
Baby D86	28	Neg	Neg	Neg	-
Baby D180	10	Neg	Neg	Neg	-
Baby D240	Neg	Neg	Neg	Neg	-
Baby D360	Neg	Neg	Neg	Neg	-

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