Toxoplasmosis: aspects concerning the epidemiology and the paraimmune prophylaxis

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Paraimmunity, as a reactive phenomenon of the host’s organism, induced by various heterogeneous antigens – due to some common antigenic components – appears alongside specific immunity. In bacterial and viral immunology paraimmunity is better known and studied than in parasitic immunology. It is known that, generally speaking, paraimmunity appears simultaneously with specific immunity, induced by the intervention of certain antigens, but it is smaller in intensity than the latter.

The intensity and duration of the paraimmunological response can be modelled by physical or chemical stimuli, which can be used as adjuvant – sodium hydroxide, Freund adjuvant, etc. But it is also conditioned by other factors: stressors, re-infestations with the same parasites, phylogenetic relations of the parasites, irradiations, and so on. Paraimmune mechanisms are based on the stimulation of phagocyte elements hypertrophy of the reticulo-histiocytary system and the mobilization of other elements belonging to the defensive systems. The intervention of the paraspecific antibodies is conditioned by the presence of common fractions of the heterogenous antigens (Suteu, 2005).

Paraimmunity is an inter-specific and intergenic phenomenon, but it also occurs in the presence of certain aggressors remote-related from a phylogenetic point of view, such as viruses and bacteria. Thus, it is known that BCG (Bacillus Calmett Gueren) vaccination of humans and animals, with extracts of Corynebacterium parvum or E. coli increases the resistance of organisms to infestation with Plasmodium spp. Our objective relates to the valorization of weak-pathogenetic parasites or avirulent, which induce paraimmune phenomena towards other species of parasites – very aggressive, with serious morbidity. This vast field refers to paraimmunological relations among protozoa as well as among helminths and arthropodes (Harder and co., 1992).

Toxoplasmosis is produced by Toxoplasma gondii, which is able to enter and multiply in numerous cells, but which has serious effects on the cells of the central nervous system, retina, cord and lung, for which Toxoplasma has a special tropism. The seriousness of the infection of a pregnant woman who has no protection (immunity) – and for whom the parasite can be the source of very serious fetus diseases – and of immune-deficient patients (who may witness a re-activation of the unapparent forms containing intratissular cysts that may be a source for cerebral abscess) is well known. The contamination can occur in two ways: either by ingesting oocysts from cat feces scattered on the ground or by ingesting intratissular cysts with food, as these can be found in the muscles of various species of animals (Dupouy-Camet and Ancelle, 2002; Skierve and co., 1998). Sometimes, the source of infection for humans can be un-boiled milk. This has been proven in the case of goat milk, but not in the case of cow milk. Milk contains tachyzoites, a part of which are not destroyed by the gastric juice because of its rapid passage through this digestive compartment (Cosoroaba, 2005).

Even if congenital transmission and ingestion of infected meat partially account for the infection with T. gondii, these cannot account the great number of cases of toxoplasmosis in vegetarians and herbivores.

The mystery of this was elucidated when they found a form of resistance of the T. gondii in feline feces and when, in 1970, the coccidian
phase of the biological cycle of the parasite was discovered (Dubey and co., 1988, 1996, 2005).

*Toxoplasma gondii* has not been cultivated on a-cellular media. *T. gondii* can be cultivated on laboratory animals, chicken embryos and cell cultures. The mice, the hamsters, the guinea pigs and the rabbits are susceptible species, but the mice are more frequently used, because they are more receptive and they are not naturally infected when they are bred in laboratory with dry food from trade, uncontaminated with cat feces.

The tachyzoites of certain serovars of *T. gondii* grow in the peritoneal cavity of mice, sometimes causing ascitis, and they develop in the greatest part of the other tissues. Virulent serovars generally cause diseases in mice and sometimes death, in about 1 – 2 weeks. Most serovars do not kill mice.

Tachyzoites of *Toxoplasma gondii* multiply on several cellular lines. Tissue cysts are obtained by injecting mice with tachyzoites, bradizoites and oocysts. The cysts become visible 8 weeks after the infection.

Intraepithelial phases of *T. gondii* have not yet been cultivated in vitro.

Oocysts can be obtained by feeding indemn cats with cysts obtained from infected mice (Dubey, 2005).

Approximately half a billion people throughout the globe display anti-*T. gondii* antibodies. Only a small percentage of the population (less than 1%) has congenital toxoplasmosis.

Toxoplasmosis is a major zoonosis with serious implications for human health, responsible for miscarriages and sterility in women.

In 1992 there were 3000 cases of cerebral toxoplasmosis in humans recorded throughout the world, and that was considered to be an epidemics. It is estimated that there had been over 20 million cases of corioretinitis contracted in the uteri, prior to the implementation of the program of prophylaxis. This prevention would require an annual sum of 75 million Euro. In the USA, the (medical and social) cost of congenital toxoplasmosis was estimated to be of about 0.4 – 8.8 billion dollars in 1992, and the cost of the treatment of each of the 4179 annual cases of toxoplasmosis is of 10.000$ per patient (Dupouy-Camet and Ancelle, 2002).

If the cat is responsible for the contamination of the environment through its feces, numerous investigations have demonstrated that toxoplasmosis can also be contracted through meat and milk, especially if it is goat or sheep milk or meat.

Consumers of raw sheep meat and beef have turned out to be serum-positive, in comparison to those who eat cooked meat. Jeannel and co. have demonstrated that pregnant women are more prone to developing toxoplasmosis, the risk in this case being 2.5 times bigger than in the case of consuming raw meat.

A national investigation performed in 1995 demonstrated a serological prevalence for toxoplasmosis, in pregnant women from France (53%) and an incidence of the seroconversion of 1.4%.

The serological prevalence of the toxoplasmosis in the animals from the slaughter houses is variable depending on region and species, but these studies must imperatively be brought up to date. In France, it has been found that 15-72% of the sheep, 18-36% of the swine, 10-29% of the equine were carrying of the antibodies and the seroprevalence in cattle was reduced (Dupouy-Camet and Ancelle, 2002).

In Switzerland, the parasite was detected by the method PCR in 1-3% of the cattle and 6% of the tested sheep.

The infection with *T. gondii* is common in sheep. According to a report done as a result of the analysis of 9654 animals, Fayer (1981) calculated that the average of the world prevalence of the antibodies anti-*Toxoplasma* is 31% with the variance between 0-96%.

Rynievicz and co. (2003) have made a study on the occurrence of toxoplasmosis in milk goat in Poland, in which they have shown that 46.6% of the 52 2-6 year-old goats tested have been found to be serum positive for *Toxoplasma gondii*.

In Romania, where goats are mainly bred in a household system, co-habituating with other animals, especially cats, the importance of
Toxoplasmosis has increased significantly (Stefan, 2000). 96% of the sheep in three farms from Romania have tested positive (Sharma, 1980, quoted by Cosoroaba, 2005).

The disease is widely spread with pigs 931% in Holland in 1995), sheep (below 1% in Germany in 1995), goat (47% in Holland – 1998; 63% in Germany – 1995). A 43% level of parasitism has been found in cats (in France – 1998) – and a 9% in Italy (1996). Cats represent the final host of the parasite (Suteu, 2004).

Toxoplasmosis is widespread in animals whose meat is destined to human consumption. Worldwide, the species most affected are sheep, swine and rabbits. Toxoplasmosis is frequently found in wild animals, especially deer and bears.

Tissue cysts are destroyed at temperatures of above 67°C and below -13°C, as well as by their exposure to gamma radiation of above 0.5 kGy.

Human cooking habits also play an important part in developing the infection with *T. gondii*. In France for example, the infection rate is particularly high. The high levels of contamination in South and Central America could be accounted by the high degree of contamination of the environment with oocysts.

A cat can excrete millions of oocysts after ingesting even a single infected mouse. Oocysts are resistant to most environmental conditions and can survive months or even years. Congenital toxoplasmosis appears in cats, and kittens infected intrauterine can also eliminate oocysts.

Theoretically, toxoplasmosis can be transmitted sexually, through saliva, through milk and eggs.

Human infections have been recorded that were due to consumption of un-boiled milk.

Transmission of toxoplasmosis through organ transplanting has become more relevant: either through the implant of an organ or of the bone marrow from an infected donor to a non-immune or an immune-deficient patient, or through the reactivation of a latent infection of the immune-deficient patient (Dubey, 2005).

Benign congenital toxoplasmosis can be detected in humans at birth, through micro-ophtalmopaty, strabismus, corioretinitis, convulsions, transitory icterus, hepato-megaly. In its latent congenital form positive serology must be monitored (Cretu and Cilievici, 2004).

In sheep with natural infections clinical signs appear after 3-5 days of incubation and consisted of fever, respiratory insufficiency, ataxias, paralysis, muscular trembling, mass abortions or death of the foetus (Elias and Budin, 1973, quoted by Dida, 1996). There can also be placentitis and fetal encephalitis. It is possible to deliver on time, but still-born (Dulceanu and co., 2000).

*Toxoplasma gondii* can be isolated by inoculating lab animals or cell cultures with secretions, excretions, vital fluids and tissues taken through ante-mortem biopsies, or tissues with macroscopic lesions collected post-mortem from humans or animals infected with toxoplasmosis.

There are numerous serological tests to detect antibodies; these include Sabin-Feldman colouring tests, indirect hemaglutine test, indirect immune-fluorescent test (IFI), TAD, ELISA test, TAIA. IFI, TAIA and ELISA tests have been modified to detect IgM antibodies. IgM antibodies appear earlier than IgG antibodies, but they also disappear earlier.

Steriu and co. (2003), investigating through serologic tests a number of 759 persons with ocular pathology, have highlighted infections with toxoplasmosis in 49,3 – 55, 8% of the patients (Steriu, 2003).

It can also be diagnosed by highlighting the presence of *T. gondii* in tissues collected through biopsy and necropsy. They are stabilized with methyl acid and coloured with Giemsa. Toxoplasmosis formations that have been well preserved have the form of a scythe and can well be coloured with any of the Romanovsky colour-dyers.

Electronic microscopy can support the diagnosis (Cozma, 1996). Tachyzoites of *Toxoplasma gondii* are always located in vacuoles and display rhoptries in the form of honey comb. Tissue cysts are free of septum, and the cystic wall is thin and without the plasmalema of the host cell. The immune-histochemical colouring of the
parasites with serum anti-\textit{T. gondii} can also help the diagnosis process (Dubey, 2005).

Medical prophylaxis can be achieved experimentally by vaccinating cats and/or intermediate hosts.

As the cat is the main source of toxoplasmosis, its vaccination could constitute the basis of the prophylaxis. Vaccination with heterolog antigens has been attempted (\textit{Hammondia hammondi} (Cosoroaba, 2005). Other experiments included T263, a mutant of \textit{T. gondii} obtained through repeated passages on mice. This produces cysts which, being intact determine, after oral ingestion (cat), the appearance of antibodies and the development of an immune response that allows the production of post-vaccine schizogonies, but not the a-sexuate reproduction, as the administered \textit{Toxoplasma} is mono-gametal. Cats thus vaccinated do not disseminate oocysts. The length of vaccine immunity is not very well known, but it does not cover the lifespan of a cat’s life (Euzeby, 1998).

In order to prevent abortion in sheep, a vaccine prepared from Toxovax has been used with some success. Unfortunately, such vaccines have proved to be unsuitable for humans.

Recent progress in reversed genetics has allowed to clearly separate the role of antigens from the surface of the tachyzoites in the process of invasion and the conclusion drawn is that these antigens are important in the preparation of the vaccine Cosoroaba, 2005; Sommer and co., 1991).

The aim of anti-toxoplasmatic vaccination include: the reduction of the affection of the fetus, reduction of the number of tissue cysts in animals and prevention of the development of oocysts in cats.

Nowadays, there are no sub unitary vaccines or efficient inactivate for the immunization against \textit{T. gondii}, but studies are being carried out in several laboratories.

Prevention of the excretion of oocysts by cats is the key to the prevention of the spreading of the infections with \textit{T. gondii} (Frenkel, 1990; Frenkel and co., 1991).

The aims of vaccinating farm animals are:

1. reduction of the abortions with toxoplasmic etiology, mainly in sheep and goat;
2. reduction of the risk of contamination of humans, as a consequence of the consumption of infected milk and meat (and implicitly of the risk of fetal infections).

In order to achieve these objectives the focus is on un-persistent stems of \textit{T. gondii}. In Europe and New Zealand, there is a vaccine against fetal loss due to toxoplasmosis, which contains tachyzoites (S48) that do not last in the tissues of sheep (Buxton, 1992).

These stems of \textit{T. gondii} (S48, RH, ts-4) do not induce excretion of oocysts in cats (Dubey, 2005; Chartier and co., 2001; Carther and co., 1988).

The phenomenon of paraimmunity is studied mainly in view of creating vaccines with protection effect in case of serious zoonosis. Dubey (1981) induces experimentally a state of anti-toxoplasmosis paraimmunity in sheep as a result of their vaccination against \textit{Hammondia hammondi}. The two parasites with ontogenic and fenotypic affinities, have GD cat and their oocysts are identical.

The vaccine experimentations on goats with \textit{H. heydorni} oocysts, parasite of the dog, were negative (Dubey, 1981).

A primary \textit{Cystoisospora felis} infection, followed by a first \textit{Toxoplasma gondii} infection confers strong immunity against toxoplasmosis and \textit{C. felis} re-infections don’t re-activate any more the toxoplasmic coccidiosis (Euzeby, 1998).

Studies have confirmed that there is common antigenity only between \textit{H. hammondi} and \textit{T. gondii}, and the former id non-pathogenic for humans and farm animals and would have the advantage of being used in preparing anti-toxoplasmosis vaccines.

The grave pathogenicity and the sickening with psycho and neuromotor complications in children, caused by toxoplasmosis, are known.

Serological cross responses between \textit{H. hammondi} and \textit{T. gondii} have also been noticed in pregnant sheep, after experimental infections. IFI tests have shown that the concentration of
anti-toxoplasmosis antibodies was lower in animals infected with *Hammondia hammondi* (1/16) than in those with *T. gondii* (Munday and Dubey, 1986, 1988). Similar results have been obtained in other species – guinea pigs, mice, marsupials – but serological cross responses against *Toxoplasma* is proved only through vaccination with *H. hammondi*, but not with *H. heydorni* (Radcliff and co., 1993).

**REZUMAT**

**Toxoplasmoza: aspecte privind epidemiologia și paraimunoprofilaxia**

Paraimunitatea, ca fenomen reactiv al organismelor gazdă, indus de diverse antigene heterogene – datorită unor componente antigenice comune – apare paralel cu imunitatea specifică.

Fenomenul de paraimunitate este cercetat în mod special prin prisma preparării unor vaccinuri cu efect protector în protozooze grave.

O infecție primară cu *Cystoisospora felis*, urmată de o primoinfecție cu *Toxoplasma gondii*, conferă o solidă imunitate contra toxoplasmei și reinfectiile cu *C. felis* nu mai reactivează coccidioza toxoplasmică.

Reacții serologice încrucișate între *H.hammondi* și *T.gondii* s-au observat și la oi gestante, după infecții experimentale.

**Bibliography**


