Network for wildlife health surveillance in Europe

Diagnosis Card

Toxoplasmosis

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Etiology

The protozoan species *Toxoplasma gondii*: Four main groups of strains described (I, II, III, others); most strains found in the European wild fauna belong to type II group (group of low virulence in mice).

Affected species (wildlife, domestic animals, humans)

Any mammal or bird species may be affected as an intermediate host, while members of the Felid family are the only definitive hosts. In European wildlife, fatal cases have been found in hares, squirrels, foxes and various marine species. Among zoo species, new world monkeys and marsupials are particularly susceptible. Among domestic animals, sheep, goats, pigs and horses are the species most often infected. In humans, depending on the area, between 20% and 50% of adults are chronically infected.

Epidemiological characteristics and disease course

Complex life cycle with sexual multiplication in felids, an environmental phase and asexual multiplication in intermediate hosts. In definitive hosts, infection is generally acquired by predation. Infection entails an intestinal coccidiosis and results in the production of environmentally resistant oocysts. Intermediate hosts may be infected by ingesting oocysts, through vertical transmission or by carnivorism. In intermediate hosts, *T. gondii* tachyzoites disseminate in many organs and form bradyzoits, mostly in muscles and brain. Transmission to humans results either from the consumption of undercooked meat (including from game species or after manipulation of carcasses) or contact with oocysts in soil, water or contaminated vegetables.

Clinical signs

Most chronic infections in intermediate hosts are asymptomatic or entail mild symptoms. Severe cases of abortion, encephalitis, ocular lesions, pneumonia or disseminated forms are observed occasionally in many species, and frequently in the few highly susceptible species like hares.

Gross lesions

Highly variable depending on the organ concerned: most frequent lesions include encephalitis, pneumonia, myocarditis, hepatitis, visceral forms and concurrent infections.

Histological lesions

Local necrosis, vasculitis and perivasculitis, gliosis, presence of tachyzoites.

Differential diagnosis

At direct observation: *Neospora caninum* and *Sarcocystis neurona.*

Criteria for diagnosis

Diagnosis of acute or clinical infection:

- Immunochemical staining of sections of formalin-fixed, paraffin-embedded tissue samples
- In cats: coproscopic examination for excretion of oocysts

Diagnosis of chronic or latent infection:

- Serology
- PCR, bioassay and isolation of *T. gondii*

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Pros (P) and cons (C) given for each method:

(a) **Direct methods**:

- Bioassays of tissues in mice: (P) sensitive and highly specific method allowing for the isolation and typing of the strain; (C) requires fresh samples of muscles (usually heart or tongue) or brain kept in antibiotics solution; up to 25 g can be treated within 7 days after sampling; expensive and time-consuming.
- PCR: (P): highly specific; (C) not sensitive when few bradyzoits are present
- LAMP: (Loop-mediated isothermal amplification): (P) highly specific and most sensitive direct method; (C) limited availability to date
- Direct observation and immunohistochemical staining: Coproscopic search for oocysts in cats: (C) low sensitivity, moderate specificity (infection by *Hammondia hammondi* produces similar oocysts), only detects oocyst excretion.

Assay	**Principle**	**Pros**	**Cons**
Indirect hemagglutination test (IHAT)	Agglutination of red blood cells covered with Ag	- simple to use	- low Se in congenital and acute infections - low Sp
Modified Agglutination Test (MAT) (Toxo-screen®)	Agglutination of killed tachyzoites	- easy to use, no need of specific reagent, commercial kit available - highest Se and Sp among IHAT, MAT, LAT and ELISA in swine - validated in swine and chicken, used in numerous wild species	- low Se at the beginning of infection
Latex agglutination test (LAT) (ToxoTest®, Pastorex Toxo®)	Agglutination of latex particles covered with Ag	- easy to use, no need of specifi reagent, commercial kits available	- low Se in livestock
Indirect immunofluorescent antibody test (IFAT)	Fluorescence of conjugate Abs on killed tachyzoites-Abs complex	- high Se	- needs specific conjugate and a fluorescence microscope - low Sp, cross-reacts with rheumatoid factors and antinuclear antibodies
ELISA (*Toxoplasma* ELISA IgG®)	Coloration by an enzymatic reagent fixed on a conjugate Ab on killed tachyzoites-Abs complex	- May be standardized and automatized, commercial kits available - quantifies IgGs - high Se and Sp in some species	- needs specific conjugates, not validated for many wild species

(b) **antibody response: mosts tests are designed to detect IgG response**.The most often used are:

The Sabin-Feldman Dye test (DT), Complement-fixation test (CFT) and microprecipitation in Agar (MPA) are now less used in animals because of the high technical expertise required (DT, need to use live virulent tachyzoites) or lack of standardization (CFT, MPA).

APHAEA protocol (for harmonization at large scale)

Direct diagnosis: PCR or Real Time PCR is recommended for direct diagnosis on tissues, vectors or other suspected samples. It is fast, sensitive and performed in many laboratories.

Indirect Diagnosis: MAT is fast and sensitive and is performed in many laboratories.

Laboratories that can be contacted for diagnostic support

Laboratoire de Parasitologie-Mycologie, Hopital Maison-Blanche, Reims, France;daubert@chu-reims.fr

ANSES, Maisons-Alfort Lab for Animal Health; http://resapath.anses.fr/index.htm

Recommended literature

Dubey JP. 2010. Toxoplasmosis of animals and humans, Second edition. *CRC Press*, 313 pp.

EFSA 2007. Surveillance and monitoring of *Toxoplasma* in humans, food and animals. *The EFSA Journal* 583:1-64. http://www.efsa.europa.eu/en/efsajournal/doc/583.pdf.

Jokelainen P. 2012. Endemic *Toxoplasma gondii* genotype II causes fatal Infections in animal hosts in Europe – lessons learnt. In: *Toxoplasmosis, recent advances*, InTech, http://dx.doi.org/10.5772/49984