

## **Surveillance and monitoring of *Toxoplasma* in humans, food and animals<sup>1</sup>**

### **Scientific Opinion of the Panel on Biological Hazards**

**(Question No EFSA-Q-2007-038)**

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#### **SUMMARY**

Toxoplasmosis has the highest human incidence amongst the parasitic zoonoses. However, toxoplasmosis is considered to be an under-detected and underreported disease in the European Union. Toxoplasmosis and agents thereof have to be reported by Member States according to their epidemiological situation (Directive 2003/99/EC). Despite this fact, no representative data are available for *Toxoplasma* in the EU, neither for humans nor for animals or food.

To improve data collection and to better evaluate the disease burden of toxoplasmosis, the Biological Hazard Panel is asked to give recommendations for optimising the surveillance and monitoring in humans, animals and foodstuffs and to give advice regarding methods for detection and identification of *Toxoplasma* from food and animals.

The assessment incorporates general aspects of *Toxoplasma* and human toxoplasmosis such as the life cycle of agent, infection sources and risk factors, clinical aspects in humans as well as disease burden. Furthermore, existing surveillance and monitoring systems for humans, animals used for human consumption and foodstuffs and currently used methods for the direct or indirect detection of *Toxoplasma* in respective matrices are reviewed.

The Scientific Panel on Biological Hazards has reached the following conclusions:

1. The analytical methods to be used to detect and identify *Toxoplasma* in food and animals need to be characterised in terms of sensitivity, specificity and other performance parameters associated with the reliability and consistency of such methodologies. In order for such characteristics to be attained, there is an absolute requirement for reference materials and reagents. As a first step, identification and long term availability of such materials and reagents would be a matter for CRL to undertake, after which field trials

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could be initiated to establish the suitability of current and future tests/assays for the above purpose.

2. Once such standardised methods are available, the Panel on Biological Hazards recommends that *Toxoplasma* monitoring should start on pre-harvest sector in sheep, goats, pigs and game.

**Key Words:** *Toxoplasma*, toxoplasmosis, surveillance, monitoring, human, animal, food, detection methods

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## BACKGROUND AS PROVIDED BY EFSA

The Directive 2003/99/EC<sup>2</sup> lays down the Community system for monitoring and collection of information on zoonoses, which obligates the Member States to collect relevant and where applicable comparable data of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks. In addition, the Member States shall assess trends and sources of these agents and outbreaks in their territory, and transmit to the European Commission, a report covering the data collected every year. The European Food Safety Authority (EFSA) is assigned the tasks of examining the data collected and preparing the Community Summary Report.

Data collected in the framework of Directive 2003/99/EC relate to the occurrence of zoonotic agents isolated from animals, food and feed as well as to antimicrobial resistance in these agents. The information concerning zoonoses cases in humans and related antimicrobial resistance is derived from the structures and/or authorities referred to in Article 1 of Council Decision No 2119/98/EC<sup>3</sup>.

EFSA published its second Community Summary Report<sup>4</sup> on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005 on 14 December 2006. For the first time, the European Centre for Disease Prevention and Control (ECDC) provided the data on cases of zoonoses in humans and also the analysis of these data in this report. The data used for analysis derived from several disease networks; the Basic Surveillance Network (BSN) and two Dedicated Surveillance Networks (DSN); Enter-Net and Euro-TB.

No information on human cases of *Toxoplasma* infections was available for 2005. Data on *Toxoplasma* in food was also sparse. Member States reported some *Toxoplasma* findings from various animals species, including sheep and goats, dogs and cats cattle, fur animals and wildlife. Many samples were based on clinical suspicion, and therefore the results do not reflect the general prevalence in animal populations and cannot readily be compared between Member States (the Community Summary Report 2005).

In 2004, the EU reported incidence of human toxoplasmosis was 0.6 cases per 100,000 population. The majority of cases were laboratory-confirmed clinical cases. Very few MS have

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<sup>2</sup> Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003 p. 31)

<sup>3</sup> Decision No 2119/89/EC of the European Parliament and of the Council setting up a network for the epidemiological surveillance and control of communicable diseases in the Community (OJ L 268, 3.10.1998, p.1)

<sup>4</sup> The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005, *The EFSA Journal* (2006), 94

a routine surveillance for toxoplasmosis in pregnant women or newborns (the Community Summary Report 2004<sup>5</sup>).

The Scientific Panels on Biological Hazards and Animal Health and Welfare concluded in their review the Community Summary Report in 2004<sup>6</sup> that parasites, including *Toxoplasma gondii*, were reported less frequently in humans, and have caused fewer outbreaks, than bacteria and viruses in the EU in 2004. However, in many instances, their impact (severe illness, disability, death, and costs related to diagnostic procedures, hospitalization and treatment) on vulnerable groups of the population, and often in immunocompetent persons, has probably been considerable. Despite having the highest reported human incidence amongst the parasitic zoonoses recorded in the Community Summary Report, toxoplasmosis is particularly considered to be an under-detected and underreported disease. The introduction of an improved surveillance and reporting system for toxoplasmosis was considered to be crucial for assessing its true disease burden.

#### **TERMS OF REFERENCE AS PROVIDED BY EFSA**

The Biological Hazard Panel is asked to:

- recommend optimal surveillance and monitoring methods in humans, animal populations and foodstuffs that are most optimal from the public health point of view. These recommendations may refer to, among other things, relevant human populations, animal species and food categories to be covered, the stages of food chain to be sampled as well as the type of sample to be collected;
- give advice regarding the analytical methods to be used to detect and identify *Toxoplasma* from food and animals.

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<sup>5</sup> The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, and Antimicrobial Resistance in the European Union in 2004, *The EFSA Journal* (2006), 310

<sup>6</sup> Opinion of the Scientific Panel on Biological Hazards and of the Scientific Panel on Animal Health and Welfare on “Review of the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004”, *The EFSA Journal* (2006) 403, 1-62

## ASSESSMENT

### 1. Introduction

Infections by the protozoan parasite *Toxoplasma gondii* are widespread in humans and many other species of warm-blooded animals (i.e. mammals and birds). *Toxoplasma gondii* is the only species in the genus and, hence, in this document is simply referred to as “*Toxoplasma*”.

Although most infections in humans are asymptomatic, severe complications may occur after congenital *Toxoplasma* infection such as abortion, stillbirth, mortality and hydrocephalus in newborns or retinochoroidal lesions leading to chronic ocular disease as well as lymphadenopathy, retinitis or encephalitis in immunocompromised persons due to postnatally acquired infection (Hill and Dubey, 2002). Moreover, recent outbreaks reported in the scientific literature suggest that immunocompetent individuals may develop clinical toxoplasmosis more often than previously thought (Burnett *et al.*, 1998; EFSA, 2006).

The multiple routes of transmission complicate the understanding and the ability to estimate the magnitude of the contribution of contaminated water and food to the overall burden of toxoplasmosis. There is a widespread distribution of *Toxoplasma* infection in a variety of livestock, wild animals and pets. Ingestion of environmentally robust stages (sporozoites in oocysts) or eating raw or undercooked meat or meat products containing tissue stages (tachyzoites or bradyzoites in tissue cysts) are the main transmission routes for *Toxoplasma* to humans. Transplacental infection by tachyzoites is an important mode of transmission in livestock and humans. Rain and surface water may transport infective oocysts into drinking water supplies, recreational sites, including fresh and marine waters, and irrigation waters, which, in turn, can contaminate the food supply through agricultural and food industry or household practices, from the farm to the fork. In a European multicentre study conducted in several regions of France, Italy, Belgium, Denmark and Sweden, between 30% and 63% of infections in the different centres were attributed to consumption of undercooked or cured meat products and 6% to 17% to soil contact (Cook *et al.*, 2000).

Although toxoplasmosis is the most reported parasitic zoonosis in humans in the EU, the system for routine monitoring or reporting is considered to be inadequate, thus the incidence of human disease and parasite occurrence in animals and food is undoubtedly underestimated. Data on the frequency, severity and duration of symptoms of human toxoplasmosis are crucial to improve the determination of the burden of disease which could lead to more adequate prevention strategies. Therefore, the scope of this report is to assess public health risks in regard to human toxoplasmosis and to give recommendations for an improved surveillance and reporting system in humans, animals and foodstuffs under the following conditions:

- The opinion is addressed to humans suffering from congenital toxoplasmosis and to vulnerable groups such as immunocompromised persons and to immunocompetent individuals who may acquire the infection and develop disease under certain circumstances. Recent strategies for surveillance and monitoring of *Toxoplasma* infections in humans are analysed with regard to their ability to produce appropriate data that can be used for evaluation of disease burden.
- Taking into consideration that food-producing animals may play an important role as infection sources for humans, currently available data on the occurrence of *Toxoplasma* infections in livestock and game are presented. Furthermore, post-harvest aspects such as *Toxoplasma* monitoring and sampling strategies in meat, milk and processed products are

addressed. The role of water that may contain infective *Toxoplasma* oocysts and thereby contaminate fruit or vegetables during growth/production is also considered.

- The availability of diagnostic methods for detection of *Toxoplasma* in humans, animals and food and their level of standardisation and harmonisation within and among MS is discussed. This includes methods for typing *Toxoplasma* isolates with respect to molecular epidemiology and virulence.

## 2. *Toxoplasma gondii* and human toxoplasmosis

### 2.1. Life cycle and hosts

*Toxoplasma* is a ubiquitous parasite that occurs in most areas of the world. It is capable of infecting an unusually wide range of hosts and many different host cells. The life cycle of *Toxoplasma* includes asexual multiplication in the intermediate host (mammals and birds) and sexual reproduction in the definitive host (felines). All hosts, including humans, can be infected by three different life cycle stages i.e. tachyzoites, bradyzoites contained in tissue cysts and sporozoites contained in sporulated oocysts (see chapter 2.2)

The prevalence of *Toxoplasma* infections is not confined to the presence of a certain host species as horizontal transmissions may occur from definitive to intermediate hosts (via oocysts), from intermediate to definitive hosts (via tissue cysts), as well as between definitive (via oocysts) and between intermediate hosts (via tissue cysts or tachyzoites). In addition, transplacental vertical transmission of tachyzoites may occur in some intermediate host species, including humans, after primary infection with the parasite (Figure 1), and in some animal species, i.e. mice, rats, and sheep, serial transplacental infection of subsequent generations has been proposed as another route of vertical transmission (Williams et al., 2005).

In intermediate hosts, *Toxoplasma* undergoes two phases of asexual development. In the first phase, tachyzoites multiply rapidly in many different types of host cells. Tachyzoites of the last generation initiate the second phase of development which results in the formation of tissue cysts containing bradyzoites, which multiply only infrequently (Evans, 1992; Dubey, 1993; Dubey et al., 1998). The organotropism of tissue cysts varies in different intermediate host species (see chapter 2.2). In many hosts, tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the CNS, the eye as well as skeletal and cardiac muscles. However, to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys (Dubey, 1993; Dubey, 1998; Dubey et al., 1998). Tissue cysts are the terminal life-cycle stage in the intermediate host and are immediately infective. In some intermediate host species, including most livestock, they may persist for the life of the host.

In humans, initial infection and acute disease are characterised by the presence of first-replicating tachyzoites. Shortly after infection (>2 days) tachyzoites begin to differentiate into bradyzoites. The intracellular bradyzoites multiply slowly within tissue cysts. The tissue cyst wall protects the parasites from destruction by T-cells. In humans, tissue cysts, are long-lived or even life-long. The persistence of cysts guarantees continuous stimulation of the host immune response and is generally not associated with disease (latent infection). Reactivation of intracellular bradyzoites and bradyzoite-tachyzoite interconversion is probably preceded by the death of the host cell and/or tissue cyst rupture (McLeod and Roberts 2002). Most reactivations do not lead to overt inflammation except when occurring in the retina.

If tissue cysts are ingested by a definitive host, the bradyzoites initiate another asexual phase of proliferation in epithelial cells of the small intestine. The terminal stages of this multiplication initiate the sexual phase of the life cycle (gamogony) which is followed by the formation of oocysts. Oocysts are released into the intestinal lumen and passed into the environment with the faeces. Sporogony which leads to the development of infective oocysts (containing two sporocysts, each with four sporozoites) occurs in the environment (see chapter 2.2). Almost all felines that acquire a primary *Toxoplasma* infection via tissue cysts subsequently shed oocysts in their faeces. By contrast, only about 50% of felines shed new oocysts after primary infection with oocysts of *Toxoplasma*, while secondary infections with any stage of *Toxoplasma* only results in formation of oocysts if felines are immunosuppressed.

*Image pending copyright*

**Figure 1.**Life cycle of *Toxoplasma gondii*



## 2.2. Infection sources and risk factors

When incidences of prenatal infections are compared with seroprevalences in women of childbearing age, it would appear that only a small percentage of infections with *Toxoplasma* in adult human populations are acquired vertically. This raises the question of how humans acquire the infection postnatally. Not all possible routes of infection are epidemiologically important, and sources of infection may vary greatly among different ethnic groups and geographical locations.

In general, it is believed that the majority of horizontal transmissions to humans are caused either

- by ingestion of tissue cysts in infected meat, meat-derived products or offal (viscera) *or*
- by ingestion of soil, water or food contaminated with sporulated oocysts derived from the environment or (less frequently) directly from feline faeces.

However, the relative importance and frequency of horizontal transmissions via tissue cysts versus oocysts in a given population is unknown.

### *Tachyzoites*

Tachyzoites of *Toxoplasma* have been detected in body fluids, including saliva, sputum, urine, tears, semen and milk of several intermediate hosts, including sheep, goats, and cows (Tenter et al., 2000), and an early study reported that *Toxoplasma* tachyzoites may be isolated from raw chicken eggs laid by hens with experimentally induced infection (Jacobs and Melton 1966).

While the role of semen as a potential source of *Toxoplasma* infection has not yet been investigated (Costa, 2007; Lopez, 2007), feeding animals with milk or milk products containing tachyzoites may transmit the infection. For example, it has recently been shown that feeding of unpasteurised goat whey was associated with *Toxoplasma* infection of slaughter pigs.

Infrequently, consumption of unpasteurised goat's milk has also been associated with acquired clinical toxoplasmosis in humans (Tenter et al., 2000). While tachyzoites are sensitive to proteolytic enzymes and are usually destroyed by gastric digestion, it has been suggested that, on rare occasions, they may enter the host by penetration of mucosal tissue and thereby gain access to the host's circulation or lymphatic system before reaching the stomach (Riemann et al., 1975; Sacks et al., 1982; Johnson, 1997). In addition, tachyzoites may occasionally survive for a short period of time (up to 2 h) in acid pepsin solutions (Dubey, 1998). In adult humans, solid meals may raise the stomach pH up to 5 for several hours so that tachyzoites may be deposited into the small intestine, and infants who are more susceptible to toxoplasmosis than adults have a lower concentration of proteolytic enzymes in their gastrointestinal tract. This may explain one report of toxoplasmosis in a breast-fed infant whose mother acquired a primary infection with *Toxoplasma* (Bonametti et al., 1997), and it can not be excluded that other postnatally acquired human infections are also caused by ingestion of tachyzoites. For example, a study assessing risk factors associated with primary *Toxoplasma* infections in women of childbearing age suggested that in Poland, drinking milk is a potential risk factor for horizontal transmission to humans (Paul, 1998).

In the past, it has often been thought that the risk of acquiring an infection with *Toxoplasma* by drinking cow's milk, if any, is minimal, but it can not be excluded that any type of milk is a potential source of infection if consumed raw. For these reasons, it is advisable that milk and eggs should be pasteurised or boiled before human consumption, as these procedures will inevitably kill any potentially present tachyzoites.

### *Tissue cysts*

Tissue cysts of *Toxoplasma* contained in meat, meat-derived products or offal (visceral organs) may be important sources of infection for humans. Some authors assume that about 50% of all human toxoplasmosis cases are related to foodborne infection (Slifko et al., 2000), and retrospective epidemiological analyses of human toxoplasmosis outbreaks suggest that many were associated with consumption of raw or undercooked meat or other edible parts of animals (Tenter et al., 2000). Such analyses also highlight that the risk of acquiring a *Toxoplasma* infection via food varies with cultural and eating habits in different human populations. However, data derived from outbreaks of acute toxoplasmosis are usually linked to an occasional point source of infection and, hence, do not necessarily reflect the major, epidemiologically important sources of infection for the whole population. It should be kept in mind that most *Toxoplasma* infections in immunocompetent humans are asymptomatic and, thus, will not be recorded unless systematic screening programs are carried out in the population under study.

Although comprehensive case-control studies have recently been aimed at identifying the different sources of *Toxoplasma* infections in different human populations, they have been based mostly on data derived from women of childbearing age (Buffolano et al., 1996; Kapperud et al., 1996; Baril et al., 1999; Cook et al., 2000). A European multicentre study, which included selected cities in Belgium, Denmark, Italy, Norway, Switzerland, and the United Kingdom, identified the consumption of undercooked meat, contact with soil, and travel outside Europe and North America as strong risk factors for acquiring a *Toxoplasma* infection, with 30-63% of infections in the various regions being attributed to consumption of undercooked or cured meat products (Cook et al., 2000). Likewise, consumption of raw pork and tasting of raw meat during meal preparation were the principal risk factors for acquiring a *Toxoplasma* infection in a similar population in Poland (Paul, 1998). Frequent consumption of meat or consumption of undercooked meat has also been associated with seroconversion or seropositivity for *Toxoplasma* in case-control studies on healthy adults in France and Yugoslavia (Bobic et al., 1998; Baril et al., 1999).

However, while consumption of raw or undercooked meat was consistently identified as a risk factor in all of these studies, the relative importance of the risk factor and the type of meat associated with it varied among different countries (Cook et al., 2000). For example, in France and Norway consumption of undercooked lamb was a stronger risk factor than consumption of undercooked pork (Kapperud et al., 1996; Baril et al., 1999), whereas in Poland consumption of undercooked pork was the principal risk factor identified in the study (Paul, 1998). The consumption of beef has been identified as a risk factor in some European countries (Cook et al., 2000). These findings may reflect differences in eating habits of consumers or different prevalences of infection in meat-producing animals in these regions. Thus, in Norway up to 18% of sheep, but only 3% of slaughter pigs are infected with *Toxoplasma* (Skjerve et al., 1996, 1998), whereas 36% of slaughter pigs are infected in Poland (Bartoszcze et al., 1991).

It is important to note that the organotropism of *Toxoplasma* and the number of tissue cysts produced in a certain organ vary with the intermediate host species. In livestock, tissue cysts of *Toxoplasma* are most frequently observed in various tissues of infected pigs, sheep, and goats, less frequently in infected poultry, rabbits, and horses. Tissue cysts of *Toxoplasma* in venison and other meat of wild animals, including hares, wild boars, deer and other cervids, kangaroos, and bears are other potential sources of infection for humans (Tenter et al., 2000). Professional

groups such as abattoir workers, butchers, and hunters may also become infected during evisceration and handling of meat (Frenkel, 1990; Buzby and Roberts, 1997).

*Toxoplasma* bradyzoites are more resistant to digestive enzymes (i.e. pepsin and trypsin) than tachyzoites. Therefore, ingestion of viable tissue cysts by a non-immune host will usually result in an infection with *Toxoplasma*. Although tissue cysts are less resistant to environmental conditions than oocysts, they are relatively resistant to changes in temperature and remain infectious in refrigerated (1 to 4°C) carcasses or minced meat for up to 3 weeks, i.e. probably as long as the meat remains suitable for human consumption (Tenter et al., 2000). Although most tissue cysts are killed at temperatures of -12°C or lower, occasionally some tissue cysts may survive deep-freezing, and it has even been suggested that some strains of *Toxoplasma* may be resistant to freezing (Grossklaus and Baumgarten, 1968; Kotula et al., 1991; Kuticic and Wikerhauser, 1996; Dubey, 2000).

Some studies have suggested that tissue cysts are killed by commercial procedures of curing with salt, sucrose, or low temperature smoking (Dubey, 1997; Lundén and Uggla, 1992). However, the survival time of tissue cysts varies greatly with the concentration of the salt solution and the temperature of storage. Under laboratory conditions, tissue cysts were killed in 6% NaCl solution at all temperatures examined (4 to 20°C), but survived in aqueous solutions with lower concentration of salt for several weeks (Dubey, 1997). It has also been shown that salting does not necessarily kill tissue cysts in home-made pork sausages (Jamra et al., 1991; Navarro et al., 1992). In one study, *Toxoplasma* tissue cysts were killed by 3% table salt after 3-7 days (Jamra et al., 1991). This is much longer than the usual storage time for pork sausages (what depends on the kind of preparation) and, thus, salting alone is probably not sufficient to prevent transmission to humans via tissue cysts.

Heating to 67°C or higher is considered sufficient to immediately kill tissue cysts (Dubey et al., 1990; Dubey, 2000). Survival of tissue cysts at lower temperatures depends on the duration of cooking. For example, under laboratory conditions tissue cysts remained viable at 60°C for about 4 min and at 50°C for about 10 min (Dubey et al., 1990). It is important to note that cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *Toxoplasma* in all parts of the meat. Some tissue cysts will remain infective if cooking procedures are used in which the meat is heated unevenly, for example microwave cooking (Lundén and Uggla, 1992).

To prevent foodborne transmission of *Toxoplasma* to humans, meat and other edible parts of animals should not be consumed raw or undercooked, i.e. they should be cooked thoroughly (at 67°C or higher) before consumption. Although freezing alone is not a reliable means of rendering all tissue cysts non-infective, deep-freezing meat (-12°C or lower) before cooking can reduce the risk of infection. In addition, meat should not be tasted during preparation or cooking (Paul, 1998; Cook et al., 2000). It is also essential that preventive measures to reduce the risk of horizontal transmission of *Toxoplasma* to humans via tissue cysts include a high standard of kitchen hygiene. Thus, in a case-control study in Norway, washing kitchen knives infrequently after preparation of raw meat was independently associated with an increased risk of primary infection during pregnancy (Kapperud et al., 1996). Both tissue cysts and tachyzoites are killed by detergents and, thus, hands and all kitchen utensils used for the preparation of uncooked meat or other food from animals should be cleaned thoroughly with hot water and soap (Dubey, 2000).

Tissue cysts are also killed by gamma irradiation at a dose of 1.0 kGy (Dubey, 2000), but irradiation of meat has not been approved in the EU.

Recently, high pressure processing at 300 MPa or higher has been shown to inactivate tissue cysts of *Toxoplasma* under laboratory conditions (Lindsay et al., 2006).

### ***Sporulated oocysts***

Oocysts passed in the faeces of felines are unsporulated and, thus, are not immediately infective. Therefore, direct contact with cats usually does not result in a *Toxoplasma* infection. With appropriate preventive measures the risk of acquiring an infection from a pet cat can be controlled by its owner (Tenter et al., 2000). Accordingly, in a case-control study in Norway, as well as a European multicentre case-control study on primary *Toxoplasma* infections in pregnant women, neither direct daily contact with cats nor living in a household or neighbourhood with cats were associated with acquiring the infection (Kapperud et al., 1996; Cook et al., 2000).

By contrast, sporulated oocysts present in the environment may be a potential source of infection for humans and other intermediate hosts. While consumption of undercooked meat was identified as the principle risk factor in several recent case-control studies on *Toxoplasma* infections in humans (Buffolano et al., 1996; Kapperud et al., 1996; Paul, 1998; Roghmann et al., 1999; Cook et al., 2000), this finding does not explain the high rate of seropositivity (24-47%) in some populations of vegetarians (Hall et al., 1999; Roghmann et al., 1999). However, a few other risk factors identified in those studies point to the importance of oocysts in the transmission of *Toxoplasma* infections to humans. For example, contact with soil was identified as a strong risk factor in a European multicentre case-control study, and 6-17% of primary infections in humans were attributed to this factor (Cook et al., 2000), while in a case-control study in Norway, eating unwashed raw vegetables or fruits was associated with an increased risk of primary infection during pregnancy (Kapperud et al., 1996). In addition, *Toxoplasma* oocysts in sand pits used as childrens' playgrounds may be a source of infection. Geophagia was strongly associated with an outbreak of acute toxoplasmosis in six of 11 pre-school-aged children of an extended family who played in the same sandy yard of their grandmother's house, which was also visited by cats (Stagno et al., 1980).

Contamination of the environment with *Toxoplasma* oocysts may be due to infected domestic cats or wild felines. After primary infection, a single cat may shed more than 100 million oocysts into the environment (Tenter et al., 2000). Under environmental conditions with sufficient aeration, humidity, and warm temperature, oocysts may sporulate and become infective in less than 1 day. Depending on the *Toxoplasma* strain, ingestion of as few as 10 sporulated oocysts may cause an infection in intermediate hosts, such as pigs (Dubey et al., 1996).

Soil contamination with sporulated *Toxoplasma* oocysts depends on the presence of cats roaming on the land, the rate of infection of these cats, and climate conditions. Although oocysts can resist harsh conditions it can be expected that high local temperatures in Southern European countries and extremely low temperatures in Scandinavian countries may influence their sporulation rate as well as survival. Sporulated oocysts of *Toxoplasma* are very resistant to environmental conditions. They survive short periods of cold and dehydration, and remain infective in moist soil or sand for up to 18 months (Frenkel, 2000). Under laboratory conditions, sporulated oocysts survived storage at 4°C for up to 54 months and freezing at -10°C for 106 days. However, they are killed within 1-2 min by heating to 55-60°C (Dubey, 1998). Sporulated oocysts are highly impermeable and, therefore, very resistant to disinfectants (Tenter et al., 2000).

Oocysts are distributed in the environment through wind, rain and surface water, or harvested feeds. In addition, oocysts may be spread via earthworms, coprophagous invertebrates or manure (Dubey and Beattie, 1988; Hiepe and Buchwalder, 1991). Soil contaminated with oocysts can be taken up by pastoral animals, such as sheep and goats, during grazing. Poultry having outdoor access will also take up considerable amounts of soil and can thus become infected with *Toxoplasma*. Therefore, free-ranging chickens are now used as sentinel animals to isolate and characterise *Toxoplasma* strains throughout the world (Lehman et al., 2006).

Oocysts can also contaminate plant products that are later used as animal feed (for instance to pigs in certain production systems). Hay, straw, and grain that had been contaminated with cat faeces have been identified as sources of infection for livestock (Dubey and Beattie 1988; Buxton 1990). No data are available on the level of contamination of hay or silage with *Toxoplasma* oocysts and as yet the survival of oocysts during storage of these feedstuffs has not been addressed. Hay and silage can also become contaminated at a later stage depending upon whether cats have access to the storage sites and whether they defecate on these stored feedstuffs. Hay and silage may also contain (*Toxoplasma* tissue cyst carrying) rodents which may be consumed by livestock when fed to them. Many farm animals are fed concentrates that are composed of various feed materials. In feed factories the ingredients are mixed and pelleted. Some feeds are heated to eliminate bacterial contamination but often temperatures do not exceed 50°C, which may not be sufficient to kill *Toxoplasma* oocysts. No reports are available concerning the detection of *Toxoplasma* in pelleted feedstuffs.

A growing body of evidence points towards water as an important source of *Toxoplasma* infection. Surface water is expected to be variably infected with *Toxoplasma* oocysts. Oocysts are considered to be transferred into the surface water following heavy rainfall. They can remain viable for long periods of time in water and can resist freezing and moderately high water temperatures (Dubey, 1998). Environmental factors (climate) play an indirect role in allowing the more or less prolonged survival of oocysts in the environment. *Toxoplasma* prevalence is higher in moist and hot countries than in dry or cold countries. Under laboratory conditions, oocysts kept at 35°C were infective for 32 days, at 40°C for 9 days, at 45°C for 1 day, and at 50°C for 1 h. At 55°C and 60°C oocysts were rendered non-infective in 2 and 1 min, respectively (Dubey, 1998).

The largest and best documented outbreak of acute toxoplasmosis in humans to date occurred in 110 individuals in Vancouver, Canada, in 1995. Comprehensive, retrospective epidemiological studies provided strong evidence that this outbreak was caused by contamination of municipal drinking water with oocysts (Bell et al., 1995; Mullens, 1996; Bowie et al., 1997; Aramini et al., 1998; Aramini et al., 1999). More recently, waterborne human toxoplasmosis was suspected and the consumption of untreated water was considered as a risk factor in Brazil (Bahia-Oliveira et al., 2003). Waterborne transmission to humans was also identified in a study from Turkey (Ertug et al., 2005). In areas where humans use untreated surface water for consumption, and probably also in areas where there is contact with fresh water, for instance for recreation, oocysts transmitted through water may be an important source of infection. This risk factor has not been evaluated in the EU, but may play a role in European countries where water management is not adequate.

Contaminated water and soil may act as vehicles for the transfer of oocysts to vegetables and fruit for human consumption, although there are very few data available to confirm this.

### 2.3. Clinical aspects and treatment of *Toxoplasma* infection in humans

For an opportunistic parasite such as *Toxoplasma*, expression of disease is mainly dependant of host immunity and genetic background. It is usual to consider that *Toxoplasma* infection is an asymptomatic or mild disease except in immunocompromised patients or in congenitally infected children. However, a part of the variation in disease manifestation may be related to the parasite itself: inoculum dose, infecting stage, route of infection, and parasite genotype. In Europe and USA, the population genetic structure of *Toxoplasma* is highly clonal with three predominant types (I, II, and III), whereas in other parts of the world (notably South America and Africa) atypical or different genotypes seem to be predominant. Type II strains, which are avirulent in mice, have been identified as the cause of more than 70% of human cases of toxoplasmosis in the United States and Europe as shown primarily in France (Howe and Sibley, 1995; Ajzenberg et al., 2002). Type I, recombinant and atypical strains have been associated with a higher frequency of ocular toxoplasmosis (Grigg et al., 2001) and severe toxoplasmosis in immunocompetent patients (Carme et al. 2002, Ajzenberg et al., 2004).

The main drugs used for treating toxoplasmosis (sulfonamides and pyrimethamine) and the synergy between the two drugs have been known for nearly sixty years (Eyles and Coleman 1953). Spiramycine was shown to be effective against *Toxoplasma* (Beverly 1958; Garin and Eyles 1958). These drugs are still the main choice for the treatment of *Toxoplasma* infections in pregnancy, in infants with congenital toxoplasmosis, in immunocompromised patients, and in cases of ocular toxoplasmosis. They are not able to eradicate *Toxoplasma* tissue cysts.

#### *Immunocompetent individuals*

Infection with *Toxoplasma* rarely causes severe disease in an immunocompetent individual. Acquired *Toxoplasma* infection is asymptomatic in approximately 80% of individuals. A considerable number of cases with mild symptoms are either ignored by the patients or the non-specific symptoms are not attributed to a *Toxoplasma* infection. Usually, clinical symptoms occur 10-14 days after infection and consist primarily of mild, local to generalized, self-limiting lymphadenopathy. Lymphadenopathy is seen in 3-20% of the acutely infected people (prevalence in MS unknown) and may persist for weeks or months. Chronic active toxoplasmosis is rare and suspected when clinical symptoms and high antibody concentrations persist for several months or even years. Life-threatening cases of pneumonia due to *Toxoplasma* infection, have recently been described in immunocompetent individuals from South America and may be associated with genetically atypical and highly virulent strains of the parasite (Ajzenberg et al., 2004; Carme et al., 2002, Leal et al., 2007). Acquired ocular toxoplasmosis may have been underestimated (see further).

In the absence of clinical symptoms, immunocompetent patients are not treated. In those presenting with mild clinical symptoms, the possible benefit of a treatment with spiramycin, sulfadiazine-pyrimethamine or cotrimoxazole on the duration of symptoms is not demonstrated. Severe toxoplasmosis is preferably treated with sulfadiazine-pyrimethamine.

#### *Immunocompromised patients*

The majority of *Toxoplasma* infections in immunocompromised hosts are reactivations of previous infections or reactivation of tissue cysts in transplanted organs and in bone marrow

transplants, whereas primary infection is a rare event (Mele et al., 2002). The clinical presentation ranges from asymptomatic reactivation to severe disseminated disease with encephalitis, meningoencephalitis or, more commonly, tumor lesions with a mass effect. Motor syndrome, consciousness disturbances, seizures and focal signs are common manifestations that are clinically indistinguishable from other CNS complications. Other organs may be involved, notably lungs, and eyes. A disseminated disease with detection of *Toxoplasma* in blood and bone marrow may occur. The reactivated disease may be fatal in immunocompromised individuals if not recognized and treated early.

*HIV-infected patients:* In AIDS patients with CD4-T cells numbers < 100/μl *Toxoplasma*-encephalitis is the most common clinical sign. In the pre-HAART era up to 30% of *Toxoplasma* seropositive HIV-infected patients developed encephalitis when their immunosuppression progressed, depending on the prevalence of *Toxoplasma* infection in the community. Trimethoprim-sulfonamide prophylaxis for *Pneumocystis jiroveci* also reduced the risk of *Toxoplasma* encephalitis in HIV-infected patients (Schurmann et al., 2002).

*Organ transplants:* In patients receiving a cardiac transplant, prophylaxis for six weeks with pyrimethamine reduced infection (Wreghitt et al., 1992). *Toxoplasma* infection has also been described after kidney transplants.

*Bone marrow transplants (BMT):* An early review of 55 patients with allogenic BMT with *Toxoplasma* infection found that only 4% survived (Chandrasekar et al., 1997). The European Group for Blood and Bone Marrow Transplantation reported on 106 allogenic stem cell transplants of which 55% of the donors were *Toxoplasma* IgG positive.

In immunocompromised patients, the reference treatment regimen for *Toxoplasma*-encephalitis is the combination of pyrimethamine with sulfadiazine, for at least 4 weeks.

### ***Prenatal Toxoplasma infection and congenital toxoplasmosis (CT)***

Prenatal infection results from a primary infection of the mother during pregnancy. The overall risk of a materno-fetal transmission of the parasite is estimated to be approximately 30% and there is an increasing risk for infection parallel to the increasing time of gestation (Dunn et al., 1999; SYROCOT, 2007). The presentation of congenital toxoplasmosis varies widely from subclinical to severe cases, including fetal or neonatal death. Recent findings indicate that type II strains can cause both benign and complicated CT, whereas the severity of infection is primarily related to the period of maternal infection and to the parasite concentrations in the amniotic fluid (Romand et al., 2004). However, severe cases of congenital toxoplasmosis due to type I or to atypical genotypes have been observed after late maternal infection (Ajzenberg et al., 2002).

The CNS and the eyes are practically always infected, other organs like liver, spleen, kidneys and lungs may also be involved. Infection during early pregnancy may cause fetal death and abortion or severe damage like chorioretinitis, intracranial calcification, hydrocephalus or microcephalus. Infection during late pregnancy is often subclinical in the newborn with manifestations such as retinochoroiditis or, rarely, neurological disorders.

A changing pattern of the clinical manifestations of CT is reported from many specialized, European centres. Until the 1960s, CT was primarily recorded as a disease showing the classical triad of chorioretinitis, intracranial calcification, and hydrocephalus or abnormalities of the CNS. Most cases were recorded after the referral of complicated pregnancies or newborns with clinical manifestations (reviewed in Montoya et al., 2000). Today, severe

manifestations are found in approximately 5% of infected newborns (SYROCOT 2007), whereas the majority of prenatal infected children is born without congenital disease. Subclinically infected newborns are diagnosed on laboratory criteria after supervision within the frame of a newborn or a pregnancy screening program. They remain at risk of developing chorioretinitis during their life. Severe cases of CT with hydrocephalus and neurological signs have been reported to be rare in MS like Austria, Belgium, France, Germany, Italy, and in Scandinavia.

Generally, congenitally infected individuals are at risk of repeated disease reactivation due to the inability of the currently available treatments to eliminate tissue cysts.

In pregnant women who presumably acquired toxoplasmosis during pregnancy, the benefit of a treatment with the aim to reduce the risk of materno-fetal transmission of *Toxoplasma* and potential fetal lesions has not been demonstrated. Spiramycin is usually proposed. However, a large European multicentre study found no evidence that spiramycine treatment could reduce parasite transmission to the fetus (Gilbert and Gras, 2003). When evidence for parasite or parasite DNA is found in amniotic fluid (France), or a pregnancy with proven or suspected infection passes the 16<sup>th</sup> week (Germany, Austria), spiramycin treatment is switched to a combination of pyrimethamine plus sulfadiazine, administered with folinic acid. Postnatal treatment of congenital toxoplasmosis relies on administration of the same compounds during 2-6 months (subclinical infection) to 1 or 2 years (clinical infection) according to centres and countries.

### ***Ocular disease***

Ocular toxoplasmosis leads to permanent loss of vision in affected eyes in nearly 25% of patients (Bosch-Driessen et al., 2002). Ocular disease occurs in a large percentage of congenitally infected patients, but the classical explanation that most cases were due to congenital infection has recently been challenged (Glasner et al., 1992, Gilbert and Stanford 2000). It has been suggested that at least two thirds of ocular toxoplasmosis is caused by postnatal infections. No diagnostic test can discriminate between congenital or postnatal acquired ocular disease and no obvious differences have been ascribed in the clinical ophthalmological picture between the two aetiologies. The appearance of ocular lesions varies with duration of active retinal infection and intensity of inflammation. The predominant picture is necrotizing retinitis, satellite of an existing scar, whose situation in the fundus of the eye, number, size, aspect and evolution are variable and determine the outcome of the disease. Lesions may occur uni-or bilateral. Reactivation is a common event occurring in approximately 80% of cases (Bosch-Driessen, 2002). Ocular toxoplasmosis presented as anterior uveitis, scleritis or papillitis is rare.

Studies from Brazil, the United States and Poland suggest that human ocular toxoplasmosis may be associated predominantly with type I strains or with strains from a mixture of type I and III alleles.

In ocular toxoplasmosis, the treatment with a combination of pyrimethamine with sulfadiazine is the most currently used and is often combined with corticosteroid according to the degree of inflammation. Several other drug regimens using single drug treatment such as atovaquone or clindamycin or drug combinations (macrolides or clindamycine with pyimethamine or cotrimoxazole) have been proposed (Holland and Lewis, 2002).



The efficacy of treatment in immunocompetent patients has been questioned and early uncontrolled studies did not show a difference in the duration of inflammation or the number of recurrences between treated and untreated eyes (Rothova *et al.* 1993). A meta-analysis of clinical trials has pointed out that there is a lack of evidence supporting the routine use of antibiotic treatment for ocular toxoplasmosis (Stanford *et al.*, 2003). There is evidence showing that long-term treatment may reduce recurrence in patients with chronic relapsing ocular toxoplasmosis but these initial findings need to be confirmed by placebo controlled trials (Silveira *et al.* 2002).

#### 2.4. Disease burden

The incidence of toxoplasmosis is difficult to establish since the infection is usually asymptomatic and many of the symptomatic cases (15-20% of infections) are not recognized as being caused by this parasite. The seroprevalence in Europe in the early 1990s varied between 8.1 in the UK to 77.4% in Yugoslavia. This already suggests that there is also a large variety in the annual increase in seroprevalence. In The Netherlands, the incidence has been estimated as 1.4 % per year, whereas in France a figure of 2.66% has been calculated from data collected on pregnant women (Kortbeek, *et al.*, 2004; Berger *et al.*, 2007).

Congenital toxoplasmosis has traditionally been regarded as the most serious outcome of *Toxoplasma* infection and has an incidence of between 1-15 per 10,000 live births (reviewed by the EUROTOXO group, 2006). The disease burden is related to the incidence of various clinical manifestations including stillbirth (3%), hydrocephalus (1.9%), intracranial calcifications (11.4%), CNS abnormalities (2.7%), chorioretinitis (14%) and neonatal death (0.75%) (Havelaar, *et al.*, 2007). The incidence of chorioretinitis later in life is estimated as 16.9% (Havelaar, *et al.*, 2007; Binquet *et al.*, 2003).

Ocular toxoplasmosis is the most frequent cause of infectious posterior uveitis in the world. The incidence of uveitis following acquired toxoplasmosis is not well known. During the recent outbreak in Canada it was calculated that between 0.3 and 0.7% of infected individuals developed ocular toxoplasmosis (Burnett, *et al.*, 1998). The incidence of eye disease following acquired infection has been estimated to be as high as 25% in certain regions of Brazil (Glasner, *et al.*, 1992). An incidence rate of 0.4 per 100,000 has been established for patients born in Britain cumulating in a life time risk of 18 per 100,000 (Gilbert, *et al.*, 1999). Most of the cases described in the British study were acquired cases of ocular toxoplasmosis and comparison of these data with seroprevalence data from the UK indicated that the risk of ocular toxoplasmosis following acquired infection is much lower than the 0.3% estimate generated in the study from Vancouver (Burnett, *et al.*, 1998). The discrepancy with the Vancouver data may be due to the parasite load or strain involved. The Vancouver outbreak, was not caused by a genotype II organism and the morbidity was therefore probably higher than we would see in Europe with predominantly *Toxoplasma* genotype II.

More data on the prevalence and incidence of ocular toxoplasmosis in Europe is badly needed.

Toxoplasmosis in immunocompromised patients such as HIV infected individuals was an important cause of life threatening encephalitis in the period that AIDS emerged up to the late 1990s. With the advent of antiviral drugs, the incidence of toxoplasmosis in AIDS patients has rapidly dropped (see AFSSA report 2005). In 1992, in France, there were 800 AIDS patients with toxoplasmosis whereas 200 cases were reported in 2002 ( AFSSA 2005).

Disease burden of toxoplasmosis has been investigated by a number of groups using different approaches and include numbers of symptomatic cases, hospitalizations and deaths.

Mead et al. (1999) used an illness attribution approach and showed that *Toxoplasma* plays a much more important role than previously anticipated. In that study, *Toxoplasma* ranked fourth in hospitalizations and third concerning deaths when compared to other foodborne pathogens. A recent French study confirmed these findings showing that toxoplasmosis was the third cause of death due to foodborne infection (35 cases per year), preceded by *Salmonella* (92-535 cases) and *Listeria* (78 cases) (Vaillant, et al., 2005). Both studies dealt with data collected from the late 1990s whereby the contribution of AIDS cases with toxoplasmosis was high. Due to the introduction of effective antiviral therapy and other prophylactic treatments the incidence of *Toxoplasma* infections in AIDS patients has markedly decreased.

Number of illnesses, hospitalizations and deaths are however not an accurate estimate of disease burden. A more quantitative estimate of disease burden grouped together in one single figure is the so called Disability Adjusted Life Year (DALY) approach (Murray and Lopez, 1997).

#### **DALY = YLL + YLD**

YLL is defined as the number of years of life lost due to mortality and YLD is the number of years lived with a disability, weighted with a factor between 0 and 1 for the severity of the disability. The YLL due to a specific disease can be calculated by summing up of all deaths due to that specific disease, whereby each case is multiplied by the expected individual life span at the age of death. YLD is calculated by accumulation over all cases and all health outcomes of the product of the duration of the illness and the severity weight of a specific disease.

Data concerning incidence of illness and death are derived from regional or national clinical, epidemiological and surveillance studies. In case these are not available, estimates can be made from studies from other similar countries. Data on severity weights of a particular disease manifestation are obtained from elicitation of special panels, taken from the general population or medical experts.

The DALY approach was recently used to compare the disease burden of a number of well known foodborne pathogens, including *Toxoplasma* (Havelaar, et al., 2007, Kemmeren, et al., 2006a). When comparing the DALY's of toxoplasmosis (both acquired and congenital) with other foodborne pathogens (Figure 2) it is clear that *Toxoplasma* could cause the highest disease burden amongst the food pathogens evaluated (Kemmeren, et al., 2006b). There is a high degree of uncertainty around the most likely estimate for the DALYs for *Toxoplasma*, which is due to the uncertainty in the incidence data.

(See Appendix: Table 1, for consideration of the economic losses due to *Toxoplasma* infection).

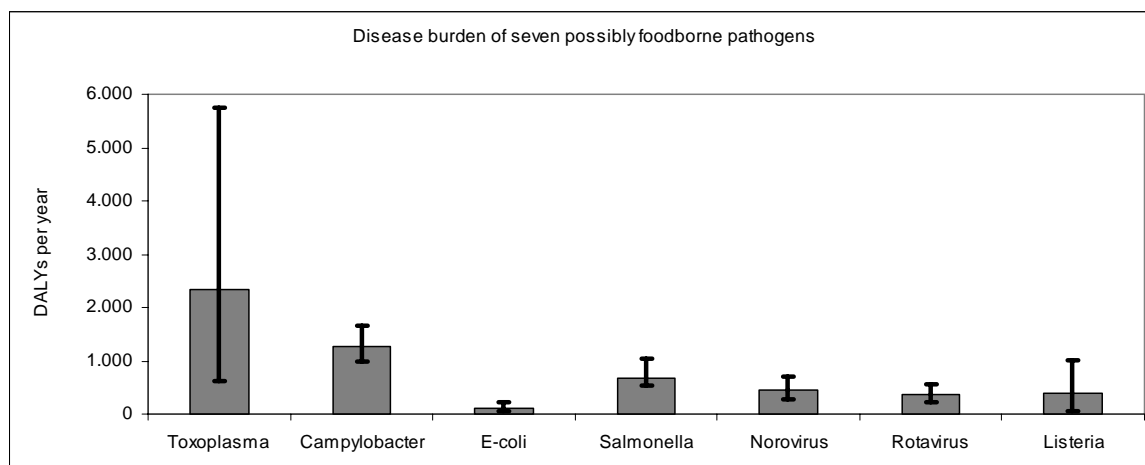


Figure 2. Disease burden of toxoplasmosis (acquired and congenital cases) compared with seven pathogens in the Netherlands (Kemmeren, et al., 2006b)

### 3. Surveillance and monitoring in humans

#### 3.1. Legislation, notification, experiences from Member States

In Europe, there is a high heterogeneity between countries in regard to the epidemiological situation of congenital toxoplasmosis. A survey with up-to-date and detailed information on systems implemented for the surveillance of toxoplasmosis is provided by the EUROTOXO group (2006). Out of 28 countries, which participated in the survey, 11 countries have surveillance systems which only report symptomatic toxoplasmosis (Bulgaria, Cyprus, Czech Republic, Estonia, Ireland, Latvia, Lithuania, Malta, Poland, Slovakia and regional United Kingdom, Scotland), 2 countries reported congenital toxoplasmosis (regional Italy, national Germany), one reports symptomatic and congenital toxoplasmosis (Denmark) and 14 countries have no surveillance system for toxoplasmosis at all. Following the Euortoxo report, France now also reports congenital toxoplasmosis. A few examples for surveillance and monitoring of *Toxoplasma* in humans shall reflect the diversity of systems between MS:

In France, it is mandatory since 1978, to screen women who get married for *Toxoplasma* antibodies and, since 1985, to screen and treat *Toxoplasma* infection during pregnancy (Ambroise-Thomas et al., 2001). When a previously sero-negative woman or a woman with an unknown serological status becomes pregnant, testing is conducted at her first prenatal examination (usually at 2 or 3 months of pregnancy) and every month during pregnancy, until delivery. In addition, women should be educated about primary prevention methods during pregnancy, using a leaflet describing hygienic measures given with the laboratory results. This program is free of charge for individuals for screening and health care induced.

When a primary *Toxoplasma* infection of the pregnant woman is detected, treatment for the woman is initiated with spiramycin as soon as possible. *In utero* diagnosis is performed through amniocentesis to detect infected fetuses and through ultrasound examinations to detect fetus lesions. If infection in the fetus is confirmed, a pregnancy termination is proposed only in cases of fetus lesions. In the case of an infected fetus without detectable fetal lesions, spiramycin is changed for pyrimethamine and sulfadiazine or sulfadoxine.

In 2006, a National Reference Centre for Toxoplasmosis was created to better estimate the burden of congenital toxoplasmosis in France and since 2007, notification of all cases of

congenital toxoplasmosis (including abortion or fetal deaths) is made to the Institut National de Veille Sanitaire (InVS) by reference laboratories in charge of the diagnosis (22 laboratories with national agreement for antenatal diagnosis). The following items are used to define a case of congenital toxoplasmosis in children or fetuses from mothers who seroconverted during pregnancy:

- detection of *Toxoplasma* in tissues (placenta, abortion products) or biological fluids (amniotic fluid, CSF, cord blood or ascetic fluid) by PCR and/or mouse inoculation
- detection of a *Toxoplasma* specific immune response in newborn or child
  - o IgM or IgA antibodies during the first week of life, or neosynthesis of IgG detected by Western blot,
  - o or rise or stability of specific IgG antibodies after the first month of life,
  - o or specific IgG antibodies persisting at one year of life.

In Germany only the disease in the newborn (congenital toxoplasmosis) is notifiable since 2001 according to the national legislation (IfSG, § 7 Abs. 3). Notification is anonymous to the Federal Health Institute (Robert Koch-Institut, RKI). Diagnosis is based on clinical and/or laboratory results. The level of reporting (109 notified cases during the period 2001-2005) is considered to be too low, since there were between 80 up to 180 notified cases a year during the period from 1986-1990 when notification was based on the Bundesseuchengesetz. In two federal states of Germany, notification is also for “toxoplasmosis” (Saxonia) or primary infection during pregnancy (Berlin). The diagnosis and management of *Toxoplasma*-infections in pregnant women and newborns follows the recommendations published under the patronage of the former Federal Health Office, now the Robert Koch-Institut. The last recommendation, which had been published in 2001, was accepted by the chamber of physicians as a guideline. The recommendation is to test every woman at the beginning of pregnancy and seronegative women once per trimester. Serotesting is either required by the pregnant woman herself or recommended by the gynaecologist. The costs for testing for toxoplasmosis are covered by most of the Health Insurances only if a specific risk factor had been identified or a disease attributable to toxoplasmosis had been diagnosed. Today, the costs for pregnancy screening are increasingly covered by private individuals (IGeL= individuelle Gesundheitsleistung). In the former German Democratic Republic (DDR) a national surveillance system for toxoplasmosis was implemented in 1978 and ended 1990 after the reunification of the two German states.

Austria implemented a so called “obligatory” pregnancy screening in 1975. Screening for toxoplasmosis is free of charge. It is carried out as a part of routine obstetrical care once during the first, second and third trimester. Seronegative women get advice how to prevent *Toxoplasma* infection, seroconverters or women with suspected acute primary infection are treated immediately and the newborns are followed up until prenatal infection is excluded or confirmed. It includes almost 100% of the pregnant women due to a bonus system (pregnant women received money after birth when all requirements of the mother-child passport had been fulfilled). The mother-child passport system is considered as very effective but does not prevent prenatal infection in every case.

Denmark had a neonatal screening programme for congenital toxoplasmosis from January 1999 until August 2007. The programme was based on detection of *Toxoplasma*-specific IgM antibodies eluted from the PKU-filter paper (Lebech et al., 1999). The results of the first four years have been published by Schmidt et al. (2006b). The incidence of congenital toxoplasmosis found by the programme was 2.1 per 10,000 live-born children. The programme

was terminated by the National Board of Health because most children were asymptomatic or had only small peripheral eye lesions with no influence on visual acuity, and because new evidence (SYROCOT) did not support any effect of postnatal treatment on long-term outcome.

Portugal started implementing a legally mandatory national program aimed at preventing congenital toxoplasmosis in 1984<sup>7</sup>. Routine surveillance of toxoplasmosis in pregnant women was extended to include pre-conceptional screening in 1998<sup>8</sup>. At present, mass screening for toxoplasmosis requires serological testing (IgM and IgG) before or as early as possible in pregnancy, and for all women identified as seronegative, a three-monthly testing throughout their pregnancy<sup>9</sup> is performed. The Ministry of Health provides specific recommendations for primary prevention in non-immunized pregnant women intended for health education through health professionals and public health services<sup>9</sup>. Although this allows a general perspective of the prevalence in the Portuguese population, the true burden of the infection may be underestimated, as toxoplasmosis still remains a non-reportable disease. The absence of an efficient network between health professionals, laboratories and national health authorities, severely impairs effective collection and comparison of data. Nevertheless, available information on human toxoplasmosis obtained by different institutions shows that *Toxoplasma* infection has an undeniable impact in terms of severe illness, death, and costs related to diagnostic procedures, hospitalization and treatment. In 2003 prevalence data from a seroepidemiological study showed a 35% infection rate in the south compared to 65% in the north of Portugal (Ângelo, 2003). Results from a survey involving 7,362 parturient women from 48 hospitals indicate that prevention of congenital toxoplasmosis is still deficient: 18% of the women with negative serology did not take the necessary precautions (“eating well-cooked meat”, “avoiding contact with cats” and “eating well washed salads”) while 62.1% of pregnant women with positive serology had taken unnecessary precautions (Machado et al., 2006a). Regarding self-perceptive knowledge on toxoplasmosis, 77.3% of the women had already heard about toxoplasmosis. Age was a very important factor too, as about 50% of women <20 years had never heard of toxoplasmosis before (Machado et al., 2006b). In immunocompromised individuals, a study involving 63 HIV infected patients showed a 57% infection rate. Five of these patients developed *Toxoplasma*-encephalitis. The results obtained during the course of this work suggest that in Portugal, 7.3% of HIV infected individuals in the ARC and AIDS stadium are in risk of developing toxoplasmic encephalitis with CD4 cell count <200 cells/ $\mu$ l (Ângelo, 2000). *Toxoplasma*-encephalitis has also been reported in transplant patients treated with immunosuppressive drugs: one case described in 1991 in a bone marrow transplantation patient (Abecassis, 1991) and another case in 1994, after renal transplantation (Da Cunha et al., 1994).

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<sup>7</sup> Norma de Serviço CS/SMA/2/84 from 9/10/1984: *Saúde Materna e Vigilância Pré-Natal* (Maternal Health and Pre-Natal Surveillance).

<sup>8</sup> Circular Normativa N° 2/DSMIA from 18/03/98: *Prestação de Cuidados Pré-concepcionais* (Pre-Conceptional Health Care), repealed by Circular Normativa N° 2/DSMIA from 16/01/06

<sup>9</sup> *Orientações Técnicas* (Technical guidelines) from the *Direcção Geral de Saúde* – Ministry of Health (2000).

### 3.2. Detection methods in humans

In general, diagnosis of toxoplasmosis can be achieved by serological methods or by direct detection of the parasite, its proteins or its nuclein acid. Different diagnostic strategies exist for the detection of an acute or chronic *Toxoplasma* infection in the immunocompetent, immunocompromised, pregnant or newborn organism (reviewed also by the EUROTOXO, April 2006).

#### *Serological methods*

The demonstration of specific antibodies in human serum and other body fluids proves infection with the parasite. Highly sensitive in-house tests like the Sabin Feldman dye test (SFDT), which is still the “gold standard”, and the agglutination test remain available in a few reference laboratories, whereas the indirect immunofluorescence test (IFT) is still in use in many centres (sensitivity close to the SFDT). In many MS the serological diagnosis relies on quality controlled, CE-marked and automated in vitro-diagnostics. The test systems allow a quantitative and qualitative determination of IgG, IgM, and IgA antibodies. Extended external quality controls (QC) contribute to the surveillance of the CE-marked *Toxoplasma* in vitro diagnostics. The QCs are organized primarily by academic experts from national authorities in France (National Agency for Quality Control in Parasitology), Germany ([www.instand.ev.de](http://www.instand.ev.de)) and the UK ([www.ukneqas.org.uk](http://www.ukneqas.org.uk)).

In the immunocompetent adult no single assay can separate the different stages of primary *Toxoplasma* infection (Roberts et al, 2001). The diagnostic performance is increased by a sequential use of different test systems. Initial IgM assay followed by an assay quantifying IgG antibodies and an assay measuring IgG avidity is recommended as a combined diagnosis in several MS. Further antibody isotype testing (IgA) and the use of follow-up samples may help to differentiate recent from past infections in unresolved cases.

A recent infection is practically ruled out by a negative result in a sensitive IgM assay. A positive IgM test result, however, does not indicate acute infection, since specific IgM antibodies tend to persist for months or even years after primary infection (Gras et al., 2004).

The IgG avidity has become an important tool for the differentiation of recent and latent infections. The current consensus is that measurement of high IgG avidity can rule out an acute infection. A high variability in the kinetics of the avidity index over time limits the diagnostic value of the test (Lappalainen and Hedman, 2004) which is of low diagnostic value in the newborn or immunocompromised individual.

Fetal or newborn infection is proven by the demonstration of actively synthesized IgM, IgA and/or IgG class antibodies. The IgM- and IgA-antibody assays applied must be of very high sensitivity like immunocapture assays (e.g. ISAGAs). The increased sensitivity of IgA assays over IgM assays is described for fetal and newborn samples (Robert-Gagneux et al., 1999, Pinon et al., 2001) but IgA assays are less specific (Gilbert et al., 2007). Early maternal infection reduces the sensitivity of IgM assays (Gilbert et al., 2007). A comparative immunological profile analysis of maternal and fetal IgG antibodies is usually performed after birth, the second and/or 6<sup>th</sup> week of life. It is supposed that only a combination of all available serological methods will allow detecting up to 96% of the prenatally infected and untreated infants within a postnatal period of at least 3 months (Pinon et al., 2001). Extended treatment within the pre- or postnatal period by use of pyrimethamine-sulfadiazine may alter the individual antibody formation.

Ocular toxoplasmosis can be diagnosed by performing paired serological tests for *Toxoplasma* antibodies in the eye and peripheral blood and thereby determining intraocular pathogen-specific antibody production, which is an indirect proof of intraocular *Toxoplasma* infection (Kijlstra et al. 1989).

Serotyping of *Toxoplasma* by peptide-based serological testing is a promising method for seroepidemiological studies.

### *Parasite detection*

The mouse inoculation test (bioassay) is going to be replaced by in vitro inoculation or nucleic acid technologies (NAT) in most diagnostic laboratories. Specialized laboratories continue to use this test not only because of its superior sensitivity but also for the isolation of *Toxoplasma* strains for molecular subtyping and associated epidemiological information.

Immunohistochemistry allows the microscopic detection of the parasite (all developmental stages) in human tissue by use of different direct or indirect immunostaining techniques with mono- or polyclonal anti-*Toxoplasma*-antibodies.

Nucleic acid technology (NAT) is increasingly used for the diagnosis and management of patients with acute *Toxoplasma* infection, primarily in immunocompromised individuals but also for congenital and ocular toxoplasmosis. *Toxoplasma* PCRs are based on in-house tests supplemented by few CE-marked assays with widely varying test protocols and sensitivity for clinical diagnosis (Bastien et al., 2007). Targets are for example the B1-, the SAG1-gene or the 529-bp repeat element. The detection limit of PCRs is indicated between 1 to  $\geq 10$  *Toxoplasma* genome equivalents for the different methods. PCR on amniotic fluid (AF) allows a prognostic marker for fetal infection when amniocentesis is performed at least 4 weeks after maternal infection. The parasite load in AF estimated using real-time PCR is less than 10 parasites/ml in 40 to 46% of samples and 11 to 100 parasites/ml in 30 to 40% of samples (Nowakowska et al., 2006). Recent results of an international QC study demonstrated a sensitivity of 63.2% when AF probes with a concentration of 10 *Toxoplasma* genome equivalents were tested (Kaiser et al, 2007). Due to the irregular distribution of *Toxoplasma* cysts in the placenta 100 to 200g of tissue is required to achieve a diagnostic sensitivity of 60 to 70% after prenatal infection; the sensitivity, however, is reduced to 25% after prenatal treatment with sulfadiazine and pyrimethamine (Bessieres, 2001). PCR has also been successfully used to diagnose ocular, cerebral and disseminated toxoplasmosis.

The general level of sensitivity is relatively high, and the rate of false-positive reactions is low as shown in national QC programs of France (Bastien et al., 2007) and Germany ([www.instand.ev.de](http://www.instand.ev.de)) and at the European level ([www.qcmd.org](http://www.qcmd.org)). The development of international reference material for the development and validation of PCRs was recently suggested (Kaiser et al, 2007).

## **4. Surveillance and monitoring in animals used for human consumption**

For the investigation of *Toxoplasma* infections both in farmed and in wild animals, serological surveys have usually been the method of choice for researchers, due to the ease of collection and testing of blood samples. Consequently, a huge mass of data regarding several animal species has accumulated through the years and is available to the scientific community. Epidemiological data on *Toxoplasma* infections in animals for human consumption are not regularly collected with the aim to compare them between countries. In addition, comparison of data with this aim needs standardised tests, procedures and sample sizes of the animal

populations under investigation. In 2003, the European Community agreed to collect information on zoonoses, whereby all MS are obliged to collect relevant data on zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks (Directive 2003/99/EC). According to the Directive, *Toxoplasma* data collection should be carried out depending on the epidemiological situation and therefore only 8 countries in 2005 reported data on farm animals. The numbers of animals tested are small and because of the lack of standardisation these results cannot be compared so far (Appendix: Table 2).

In this chapter, current information about the presence, prevalence or monitoring/surveillance data to detect *Toxoplasma* infections in animals produced for human consumption is summarised. Data referring to serological investigations of different species of farmed and wild animals are shown in Tables 3-10 in the Appendix. Data refer to the analysis of sera collected in different animal species and are presented in order to provide some baseline information as to the occurrence of toxoplasmosis in farmed and wild animals. However, it should be noted that data from different studies are not directly comparable due to variability in the sampling strategy, in the type of method employed for the testing, and in the protocol of analysis (e.g. cut-off value, reagents, conditions of the reaction). Therefore, caution must be used in comparing data obtained in the same species if tested with different methods. Because of the small sample sizes tested in most studies, data are not representative, and therefore the term “*Toxoplasma* seropositivity” is used rather than *Toxoplasma* seroprevalence.

Although the cat plays an important role in the life cycle of *Toxoplasma*, cats and other animals not meant for human consumption at least in Europe will not be discussed in this chapter.

#### 4.1. Livestock

Many studies in livestock have been published. In most of those studies serological assays have been used to determine the infection status. These studies were extensively reviewed by Tenter et al. (2000).

##### 4.1.1. Impact of farming systems on the risk of *Toxoplasma* infections

Recent data show that it is possible to significantly reduce the risk of *Toxoplasma* infection in livestock using intensive farm management with adequate measures of hygiene, confinement, and prevention. These measures include: (A) keeping meat-producing animals indoors throughout their life-time, (B) keeping the sheds free of rodents, birds, and insects, (C) feeding meat-producing animals on sterilised food, and (D) controlling access to sheds and feed stores, i.e. no pet animals should be allowed inside them.

On the contrary, production of free-ranging livestock will inevitably be associated with *Toxoplasma* infection. Animals kept on pastures with an increased pressure of infection due to contamination of the environment with oocysts, such as goats and sheep, show a high level of seropositives in many areas of the world, i.e. up to 75 % to 92 % respectively. Especially in sheep and goats, seropositivities, are high while they are distinctly lower and more varying in horses and poultry. This may reflect epidemiological factors such as different types of confinement, hygiene of stables, and different types of feed.



#### 4.1.2. Sheep and goats

In sheep and goats, *Toxoplasma* infection is a major cause of abortion and stillbirth. Subclinical infections are also quite common in adult animals of affected flocks and herds. Seropositives reported in different countries vary widely. In a review by Tenter et al. (2000) of recent surveys carried out in Europe, values range from 4% to 92% in farmed sheep. Recent publications have confirmed that seropositives can be very high (Appendix: Table 3). Limited data available in slaughtered sheep report seropositive rates of 16-66% in Europe (Tenter et al., 2000; Dumètre et al., 2006). Seropositivity is logically found correlated with age, increasing from lambs (22%) to ewes (65.6%) (Dumetre et al, 2006). A nationwide survey on sheep was started in France in 2007, which should confirm those results at national level.

Similarly, seropositivities reported in Europe in farmed goats vary from 4% to 77%. No data have been published about slaughtered goats in Europe, but findings in goats of non-European countries range from 0% to 40% (data reviewed by Tenter et al., 2000) (Appendix: Table 4). Sheep, rather than pigs, are the main source of infected meat in Southern European countries and correlation between serological results and contamination with viable cysts has not been carried out quantitatively.

#### 4.1.3. Swine

In swine, toxoplasmosis is associated with reproductive disorders such as abortion and premature birth, and with pneumonia, myocarditis and encephalitis. However, most infections in pigs are subclinical or present with mild non-specific symptoms such as hyperthermia, anorexia and tachypnea.

Seropositivities in swine reared in Europe reviewed by Tenter et al. (2000) (Appendix: Table 5) and reported in the last 28 years vary from null to 64% for fattening/slaughter pigs and from 3 to 31% for sows. Several epidemiological studies conducted in The Netherlands show that the prevalence of antibodies specific for *Toxoplasma* antigens in industrially kept fattening pigs is declining over the years. In 1969, a seroprevalence of 54% for *Toxoplasma* antibodies was observed in fattening pigs, while in 1982 this was only 1.8%. This downward tendency has been observed worldwide and is presumed to be related to the indoor housing system of pigs, where contact with cats is prevented and vermin in the stables is under control (van Knapen et al., 1995). Recent studies on fattening pigs raised on farms using intensive management in the Netherlands, Austria, and Germany demonstrated that the prevalence of *Toxoplasma* infection in pigs has decreased significantly (i.e. to <1 %) over the last decade with changes in pig production and management. In several countries of the EU seropositivities in older pigs, such as sows, which are usually kept on farms with more extensive management and which are more frequently exposed to the environment than fattening pigs, also decreased distinctly. The change in the seroprevalences, as seen from data from different surveys tabled by Tenter et al. (2000), has been more relevant in fattening pigs (about 10-25 times reduction) than in sows (about 2-10 times reduction). However it is difficult to conclude a real decline of seroprevalence without representative data obtained from nationwide studies.

Recent trends in consumer habits indicate a shift towards consumption of "animal-friendly" or "organic" pigs, which include increased exposure of the pig to the environment. A study on seroprevalence of *Toxoplasma* antibodies conducted in 2001-2002 in The Netherlands indeed demonstrated a prevalence of 2.9% in pigs kept in animal-friendly housing systems, while 0%

of the indoor pigs were seropositive (Kijlstra et al., 2004). Moreover, a seroprevalence study in indoor, organic and free ranging pigs carried out in 2004 in the Netherlands showed an overall prevalence of antibodies specific for *Toxoplasma* of 2.6%. In this study the seroprevalence in intensively raised pigs was close to nil (0.38%), whereas in organic pigs was 2.74%, and in free-range pigs the prevalence was 5.62%. The risk of detecting *Toxoplasma* antibodies in a free-range farm is statistically higher (almost 16 times higher) than in an intensive farm (van der Giessen, 2007). Pigs reared in organic farms and free-ranging pigs have indeed increased opportunities of contact with *Toxoplasma* compared to animals reared in close confinement, as they may be more exposed to contact with soil contaminated with *Toxoplasma* oocysts or to ingestion of infected preys, like rodents harboring tissue cysts. In addition, farm management practices in organic farms such as feeding goat whey to pigs and allowing contact between pigs and cats may influence the prevalence of *Toxoplasma* infection in the herd (Meerburg et al., 2006).

#### 4.1.4. Cattle

Seropositivities in cattle to *Toxoplasma* reported in several surveys published since 1979 in Europe range from 2% to 92% (reviewed by Tenter et al., 2000) (Appendix: Table 6). Seroprevalence studies in the Netherlands carried out in 1995 ranged from 13 to 43% and recent studies in the Netherlands using the same serological assay in a random sample of cattle presented at 10 Dutch slaughterhouses in 2006 showed seroprevalences of about 30% (Opsteegh et al., in preparation). In another recent Dutch longitudinal study (2006/2007), preliminary results indicate high infection rates (20/25) in calves during their first grazing season indicating that calves become infected after exposure to *Toxoplasma* on pastures. Testing of follow-up samples will provide further insight into the development of the serological response during, for example, reinfection or gravidity. Despite a high seropositivity in cattle in some studies, *Toxoplasma* is considered to be far less infective to cattle, where clinical signs are usually not observed in naturally infected animals. Moreover, the parasite has been detected very rarely in tissues of an adult cow (Dubey, 1992), and in aborted fetuses (Canada et al., 2002). Isolation of infective tissue cysts from beef is rarely reported, and compared to the high seropositivity this probably means that positive serology is not related to infective tissue cysts, for example of 2094 samples of retail beef meat in US, no *Toxoplasma* infection was detected (Dubey et al., 2005b). In contrast, viable *Toxoplasma* tissue cysts are recovered from about 3% of seronegative pigs (Dubey et al., 1995a). For cattle, resistance to *Toxoplasma* infection and the ability to clear the infection has been suggested (Munday and Corbould, 1979). The relationship between a seropositive calf or cow and the presence of infective tissue cysts needs to be clarified, because although food habits differ between European countries, eating raw beef or beef products is common in many regions of Europe.

#### 4.1.5. Poultry

Several recent surveys in different non-European countries have shown that seropositivities to *Toxoplasma* in poultry can be moderate to high (Appendix: Table 7). Due to their habit of feeding close to the ground, poultry is indeed considered a good indicator of environmental contamination by *Toxoplasma* oocysts and to identify *Toxoplasma* strains throughout the world (Lehman et al., 2006). As for European countries, *Toxoplasma* has been detected in hens in Croatia (Kuticic and Wikerhauser, 2000). In addition, seropositivity to *Toxoplasma* and

isolation of the parasite has been recently reported in free-range chickens reared in Austria (Dubey et al., 2005a) and in Portugal (Dubey et al., 2006). In broilers however, no recent data are available.

#### 4.1.6. Other livestock

Several surveys have also reported the finding of antibodies to *Toxoplasma* in horses. Seropositivities reviewed by Tenter et al. (2000) (Appendix: Table 8) ranged from <1% to 8% in EU, up to 32% in non-EU countries. Few surveys in farmed rabbits reported seropositivities between 6% and 53% in Europe (Appendix: Table 9).

## 4.2. Game (Including farmed game)

*Toxoplasma* has been detected in European game, such as different species of cervids and wild boars (Appendix: Table 10).

In general, the highest values of seropositivity and antibody titers to *Toxoplasma* have been found in game of mountainous areas with shade and relatively high humidity (Gauss et al., 2006), which are environmental conditions that favour the survival of *Toxoplasma* oocysts.

Both farmed and wild cervids living close to human settlements, may be exposed to oocysts shed from domestic cats, either directly or via contaminated water. Cervids may be also be infected following ingestion of *Toxoplasma* oocysts shed by wild felids, which in Europe are represented by the lynx and the wild cat. In this regard, the wild cat population is still considerably small all over Europe compared to the domestic cat population, but it has been increasing in the last few years in some areas (Gauss et al., 2006). The impact of this increase has not been evaluated to date.

In Europe, roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) should be considered since those are cervid species that inhabit suburban areas close to human settlements, including gardens and pastures. Roe deer appear to be particularly susceptible to *Toxoplasma* infection, as antibodies are detected more often in this species compared to other cervids, and the percentage of seropositivity often increases with the age of the sampled animals (Gauss et al., 2006). On the other hand, according to Vikøren et al. (2004) and to experimental data (Williamson & Williams, 1980), red deer seem to acquire the infection only temporarily, and get rid of it in early adulthood. Consequently, it has been suggested to restrict the testing for *Toxoplasma* to only young adult red deer. However, this issue is still controversial and has not been confirmed in other studies (Reichel, 1999; Gauss et al., 2006). Limited data of seropositivity to *Toxoplasma* have been also published for farmed red deer (Williamson & Williams, 1980; Reichel, 1999).

Other European cervid species that have to be considered as possible carriers of *Toxoplasma* are moose and reindeer. As moose feed on the ground to a lesser extent than other deer, they are thought to be less exposed to *Toxoplasma* oocysts shed in the environment; in addition, moose are seldom found in habitats close to human settlements, thus their relevance is comparatively lower than other cervids (Vikøren et al., 2004). Reindeer are similarly considered to be of little relevance both in the wild and when farmed, since they inhabit mountainous areas at high altitudes with a cold climate and little human population, where cats are not found and the lynx is uncommon (Kapperud, 1978; Vikøren et al., 2004). Antibodies to *Toxoplasma* have been detected in Europe also in other cervids, such as in fallow deer (*Dama*

*dama*), and in wild ruminants (mouflon, ibex) as well, but data are limited compared to other species (Aubert et al, 2006; Gauss et al., 2006).

In Europe, also wild boars (*Sus scrofa*) have been found infected with *Toxoplasma* (Gauss et al., 2005; Ruiz-Fons et al., 2006; Bártoová et al., 2006; Antolová et al., 2007). Being omnivorous, wild boars are exposed to infection through ingestion of both oocysts shed by felids and tissue cysts which develop in intermediate hosts such as rodents and birds. In the last 30 years, the wild boar population has rapidly increased in many European countries, and there is evidence that population density factors are related to seropositivity to *Toxoplasma*. Higher seroprevalences have in fact been observed in wild boars living in smaller areas compared to more extended areas (Gauss et al., 2005). In addition, where hunters leave animal carcasses or leftovers of deer, boars or foxes out in the field, there is a chance that wild boars scavenge on them, leading to further chances of infection with *Toxoplasma*.

In Europe, severe epizootics of toxoplasmosis in the brown hare (*Lepus europaeus*) with high mortality have been frequently reported. The brown hare seems to be exceptionally susceptible to *Toxoplasma* and acute fatal infections are mostly observed. Subclinical infections in this species are uncommon at least in some European countries, and antibody titers to *Toxoplasma* range from low to null. However, a high seropositivity has been recently reported in populations of brown hares of Schleswig-Holstein (Frölich et al., 2003). Thus, monitoring of *Toxoplasma* infections in brown hares should rely both on the detection of *Toxoplasma* in tissues and organs of dead hares and on the detection of specific antibodies in sera.

In a review of toxoplasmosis in wild birds, Dubey (2002) summarized the results of a number of recent surveys. *Toxoplasma* has been isolated in many countries from wild avian species belonging to several families, and specific antibodies to *Toxoplasma* have been detected as well. Surveys about the natural occurrence of *Toxoplasma* in wild birds that can be farmed or hunted for human consumption in Europe have not been published. However, antibodies to *Toxoplasma* have been detected in experimentally infected pheasants, Japanese quails, bobwhite quails and red-legged partridges (Dubey et al., 1993; Dubey et al., 1994a; Dubey et al., 1994b; Martinez-Carrasco et al., 2005). Bioassays have been performed as well by inoculating brains, hearts and skeletal muscles of pheasants and quails in mice, and *Toxoplasma* has been detected in several such samples (Dubey et al., 1993; Dubey et al., 1994a; Dubey et al., 1994b). Thus, monitoring of *Toxoplasma* infections in wild birds should rely on both direct and indirect methods.

### 4.3. Detection methods in animals

Toxoplasmosis in food animals (e.g., sheep, goats, pigs) is generally diagnosed by direct and indirect laboratory methods in case of overt disease.

#### *Parasite detection*

##### *Histology and immunohistochemistry*

Tachyzoites and tissue cysts of *Toxoplasma* can be visualized in sections of tissues and organs of affected animals by histology (haematoxylin and eosin stain) and immunohistochemistry. Tissues/organs of choice for the analysis include the skeletal muscle, the heart and the brain. In abortion outbreaks, the placenta and fetal organs (brain, liver, lung and heart) are examined.

Tachyzoites can also be detected in impression smears from the same organs by Giemsa staining or immunofluorescence.

#### *In vitro culture*

The isolation and propagation of *Toxoplasma* in cell culture is feasible but too cumbersome, time consuming and expensive to be employed as a routine diagnosis. In addition, it is less sensitive than other methods, such as bioassays (see below).

#### *PCR*

Several polymerase chain reaction (PCR) assays, including real-time PCR, targeted to different genes of *Toxoplasma* have been described (Burg et al., 1989; Tenter et al., 1994; Lin et al., 2000; Hurtado et al., 2001). They can be highly specific, but the small size of the sample required for the tests may limit their sensitivity, since the distribution of the tissue cysts is random, and the density of the parasite in affected tissues can be low (Esteban-Redondo et al., 1999; Hill et al., 2006).

#### *Bioassay*

*Toxoplasma* can also be recovered and demonstrated in tissue of animals inoculated with tissue samples where the parasite is suspected. These tests (bioassays) are carried out in laboratory mice or in cats. Mice are either inoculated by the intraperitoneal or subcutaneous route or fed with a homogenate of tissues and maintained in observation for 3-4 weeks, when their brain is examined for the detection of tissue cysts and they are tested for antibodies to *Toxoplasma*. Cats are fed with muscle tissue and their faeces are examined for oocysts 3 days after inoculation. The sensitivity of bioassay is good, since it allows the detection of 1 cyst in 100 grams of tissue from naturally infected pigs (Dubey et al., 1995a). Cats are more susceptible than mice to the infection by *Toxoplasma*. Thus, the cat bioassay is considered the gold standard of detection methods.

#### *Serological methods*

Several indirect methods have been proposed for the detection of antibodies to *Toxoplasma*, generally in samples of serum and plasma. In addition, thoracic fluid of aborted fetuses, milk and samples of fluid obtained by freezing and thawing portions of muscular tissue (meat juice) can be tested as well for antibodies. There is a wide variety of serological assays available and in use. However, data from different studies may not always be directly comparable due to discrepancies in the procedures used for the detection of antibodies, to the modification of protocols, to the different strains of *Toxoplasma* used in the laboratories, and to the different cut-off points of tests. Not all assays are suitable for every animal species and cross reactions with antibodies to related parasites may result in false positives.

#### *The Modified agglutination test (MAT)*

In the last few years, serological surveys in animals (Dubey et al., 1995b) and game (Bártová et al., 2006; Antolová et al., 2007) have been mostly carried out with the MAT as it can be widely

employed without the need of species-specific reagents. The MAT is easy to perform, safe and reliable. It does not require species-specific reagents, but it employs a large amount of formalin-treated whole tachyzoites. Some drawbacks of the test are the length of time needed to perform it, and the subjectivity in the interpretation of the results, which may render the test less practical than others for widespread application.

#### *The Immunofluorescence antibody test (IFAT)*

The IFAT is a simple and widely used test. It employs formalin-treated tachyzoites and it requires species-specific conjugates, which may not always be available. Different commercial kits have been developed. Differences in the performance of the test can be related to the strain of *Toxoplasma* used, and variation of results are partly due to the subjective interpretation (reading) of the reaction via fluorescence microscopy.

#### *The Enzyme-linked immunosorbent assay (ELISA)*

The ELISA is another test widely used for examination of animal sera. Several ELISA kits are commercially available for detection of *Toxoplasma* antibodies in certain animal species (e.g. small ruminants). ELISAs can be automated and thus are suited to the testing of large numbers of samples, and to routine screening on the farm or at slaughterhouse. Some ELISAs have been shown to be both highly sensitive and specific when compared to IFAT. Recently, a commercial ELISA for the detection of antibodies to *Toxoplasma* has also been validated in swine (Gamble et al., 2005), and it correlates well with MAT. Subsequently, Hill et al. (2006) compared six methods for the diagnosis of *T. gondii* infection in pigs, and they found that the validated ELISA is the most sensitive test for the analysis of sera. An IgG-avidity ELISA which may help in the identification of active or recent *T. gondii* infections, has been described (Sager et al., 2003).

#### *The Sabin-Feldman dye test (SFDT)*

The SFDT is based on the inability of *T. gondii* tachyzoites of taking up a dye when anti-*Toxoplasma* antibodies are present in test sera. Given its high specificity and sensitivity, the test has been widely used for the detection of antibodies in humans, and can also be employed for the analysis of animal sera, but not for testing bovine samples since a natural globulin in bovine sera may give false positive results (Dubey, 1988). Overall, as this method is technically difficult, expensive and hazardous since it employs live tachyzoites of *Toxoplasma*, it is rarely used in veterinary laboratories.

#### *Other serological tests*

The carbon immunoassay (CIA) is a simple test where *Toxoplasma* tachyzoites, after incubation with the test serum, are mixed with black India ink. If present, *Toxoplasma*-specific antibodies combined with *Toxoplasma* tachyzoites will bind carbon particles of the ink (Chinchilla et al., 1992; Arias et al., 1994) or carbon labelled anti-species rabbit IgG (Waller et al., 1983). The test has been shown to be cheap and specific and has been proposed for routine laboratory use. The direct agglutination test (DAT) is a simple and easy to perform test which is both sensitive and specific. It is commercially available. It is used mostly for the testing of wildlife sera (eg deer: Vikøren et al. 2004), since it does not require species-specific conjugate

sera. The latex agglutination test (LAT) and the indirect haemagglutination test (IHAT) have also been used, although at low titers cross reactions with other coccidia may occur. Another indirect test, the immunoblotting (IB) is not a method for the routine testing of sera, but is used to investigate in detail the reactivity of sera to separated *Toxoplasma* antigens.

#### 4.4. Future directions for surveillance and monitoring in animals

Due to the broad range of potential transmission of *Toxoplasma* from animals to humans, it is not feasible to monitor all animals used for human consumption for *Toxoplasma* infection. Considering the epidemiology of *Toxoplasma* infection in animals and the infection source to humans, the testing of animal species in the EU should be primarily targeted to sheep and goats. As reported above, high seropositivities in these species have been detected in the EU, and the association between consumption of undercooked meat from these species and the infection of humans has been documented. In this respect, specific consumer habits should be considered for the sampling procedure in certain regions.

As to other species, monitoring of *Toxoplasma* infection should also focus on pigs from organic farms and on free-ranging pigs, since they may be more exposed to *Toxoplasma* than animals conventionally reared indoors, under the assumption that biosecurity measures are implemented and correctly enforced in the latter situation. Testing of cattle would not be a priority in this scenario, since there is inconclusive information as to the infectivity of their meat due to the low viability of tissue cysts.

As to the detection methods that can be used for the monitoring and surveillance of *Toxoplasma* in animals, direct methods (i.e. examination of tissue samples by PCR after slaughter and detection of infective cysts by bioassay) are too expensive and cumbersome for routine application. However, their use can be advocated in targeted studies. Therefore, indirect serological tests (e.g. ELISA) for detection of *Toxoplasma* antibodies in the blood of animals (either sampled at farm or at slaughter) are considered more feasible. However, serological tests should undergo a standardisation and harmonisation by use of appropriate reference material before they are used for surveillance and monitoring of *Toxoplasma* in animals.

### 5. Surveillance and monitoring in food

There is a globalisation in the trade of animals and food within the European Union which is by far the biggest importer of food worldwide. For instance in 2005, 15,000 tons of pork (carcass weight) were imported into the European Union, and 1.309.000 tons of pork were subjected for export (<http://ec.europa.eu/agriculture/agrista/tradestats/eu15comments.htm>). Rules for trade of meat and meat products seek to guarantee that all imports fulfil the same high standards as products from EU Member States. This should also be addressed to the animal health status and high standard of meat and meat products in order to avoid human toxoplasmosis. Besides measures focussing on pre-harvest food safety (e.g. surveillance and monitoring in animals), post-harvest strategies at slaughter and during food processing have become more and more importance in recent years.

Under this consideration, surveillance and monitoring in food and food products could provide important information for a better risk assessment on *Toxoplasma* and toxoplasmosis from the consumer protection point of view. Due to the complex epidemiology of *Toxoplasma* (see chapter 2.2), meat and meat products as well as other food such as milk and water are reviewed

as possible diagnostic matrices in this chapter. Furthermore, recent studies are analysed in respect to feasible methods and testing schemes for isolation, detection and typing of *Toxoplasma* in food.

### 5.1. Meat and meat products

Sheep and goats are the main source of infected meat in Southern European countries (Berger et al., 2007). This is of particular importance, because tissue cysts have been found in many edible parts of sheep, and small ruminants are important in both milk and meat production throughout the world.

Other animals, e.g. chickens, camels and wild game may have infective *Toxoplasma* cysts in edible portions of meat and cysts remain alive in the body for the lifetime of the animal. However, there is no way to distinguish infected carcasses from uninfected carcasses during slaughter inspection (Dubey et al., 2002).

Of the meat sources, pork has also been considered to be a source of *Toxoplasma* infection, whereas beef has not been shown to contain infectious *Toxoplasma* parasites. In a field study, hearts and tongues from 55 six months old pigs from a farm in Massachusetts were bioassayed for *Toxoplasma* by feeding them to *Toxoplasma* free cats whereas 51 animals shed 25–810 million *Toxoplasma* oocysts in their faeces (Dubey et al., 2002). Examinations were conducted on the presence of *Toxoplasma* cysts in fresh pork sausages, produced in factories in Londrina, (Parana State), Brazil. After bioassay in mice, 13 (8.7%) sausage samples were positive, in one of them *Toxoplasma* was isolated and in the other 12 the mice seroconverted. In another study, 33 out of 70 fresh pork sausage samples obtained from commercial establishments in the municipality of Botucatu, SP were positive by PCR but not by bioassay (Dias et al., 2005).

In a comprehensive study, the prevalence of *Toxoplasma* was determined in 2,094 meat samples each of pork, beef and chicken, obtained from 698 retail meat stores from 28 geographic areas of the United States. A pool of 6 samples each weighting 100g were fed to *Toxoplasma* free cats and faeces were examined for oocyst shedding. Overall, the prevalence of viable *Toxoplasma* in retail pork was very low with a total of 10 isolates whereas none of cats fed chicken or beef samples became positive (Dubey et al., 2005b). In contrast to absence of detected tissue cysts in beef the seroprevalence in cattle may be high reflecting the problem that positive serology may not correlate with presence of parasites in the meat.

It is estimated that the meat of one pig is eaten by approximately 300 to 400 hundred individuals, and all edible parts of a pig tested seropositive for *Toxoplasma* should be considered infectious (Dubey, 1986). The human dose response for *Toxoplasma* is not established, but extrapolation from animal studies could be exploited in the future (AFSSA, 2005; Remington et al, 1995). Dubey et al. (1996) have estimated that less than 1 cyst per 50g of tissue is likely to be found in *Toxoplasma*-infected pigs. Low oocyst counts in the bioassay may be related to the fact that the meat was inadvertently frozen during transport from the slaughterhouse to the store. Furthermore, low level infections which cannot be detected by laboratory methods may be attributed to post-harvest processing such as hard chilling and pumping that inactivate tissue cysts. This should be considered for future surveys for *Toxoplasma* infection in livestock (Dubey et al., 2005b).



## 5.2. Milk and milk products

The excretion of *Toxoplasma* in milk was studied by inoculation of mice with milk samples from 7 experimentally infected and 7 naturally infected goats. *Toxoplasma* was demonstrated in the milk of a goat infected with 10,000 infective oocysts but not in goats infected with 100 or 1,000 oocysts or in naturally infected goats. It was concluded that the risk of human toxoplasmosis after ingestion of milk containing tachyzoites of naturally infected goats is small since tachyzoites are most likely be destroyed by gastric juice (Dubey, 1980). However, human infection by ingestion of raw goat milk has been documented in humans (Skinner et al., 1990) and unpasteurized goat milk is considered a source of *Toxoplasma* infection in children of rural areas (Riemann et al., 1975). Transmission of *Toxoplasma* is attributed both to tachyzoites in the milk and suckling trauma but also to tissue cyst excretion due to specific exocytic lipid secretion in the mammary cell (Pettersen, 1984).

## 5.3. Shellfish

Recent studies demonstrated that *Toxoplasma* oocysts could sporulate in seawater and that they remained viable for at least 6 months in seawater suggesting that oocysts can survive for extended periods of time and be available to a variety of potential transport hosts (Lindsay et al., 2003). Experimental studies revealed that sporulated *Toxoplasma* oocysts were filtered out of seawater by mussels (Arkush et al., 2003) and Eastern oysters (Phelps et al., 2001) and that they were orally infectious in mice bioassay. In a longitudinal study, Eastern oysters readily removed *Toxoplasma* oocysts and remained viable for up to 85 days in mice bioassay (Lindsay et al., 2004).

## 5.4. Water

The Directive 98/83/EC on the quality of water intended for human consumption does not contain specific regulatory measures for water in regard to contamination with *Toxoplasma* oocysts. If the water originates from or is influenced by surface water and in the event of non-compliance with prescribed parametric value, the water supply must be investigated to ensure that there is no potential danger to human health arising from the presence of pathogenic micro-organisms. Detection of *Toxoplasma* in water is difficult and no standardized methods are available. The methodology is based on the experience gained from other coccidians such as *Cryptosporidium* and involves concentration of oocysts using centrifugation, filtration, immunomagnetic separation and fluorescence-activated cell sorting (Dubey, 2004). Direct identification by microscopy is not reliable due to a high resemblance with oocysts from other coccidian species.

The general safety of public drinking water in Europe concerning *Toxoplasma* is not known. Villena et al (2004) showed that 1 out of 98 tested samples did contain *Toxoplasma* DNA, but were not able to show that the samples contained infective material by mouse bioassay.

A recent survey from France showed that 7 % of raw surface water samples tested were positive for *Toxoplasma* parasites (Villena et al. 2004). Well water is less likely to become contaminated with oocysts although Villena et al. (2004) have shown that 9% of tested samples were positive. The degree of contamination of well water may depend upon the degree and type of filtering and the depth of the well.

In a study performed in Lublin province (Poland), a total of 114 drinking water samples (80 from shallow household wells with a windlass, 16 from deep wells with a pump, and 18 from the water supply system) were analysed by microscopic examination and PCR with *Toxoplasma* being detected in 15 (13.2%) and 31 (27.2%) of samples, respectively. *Toxoplasma* DNA was significantly more frequent in water samples from the shallow windlass operated wells than in those from deep wells ( $p < 0.05$ ) and water supply system ( $p < 0.01$ ). A positive correlation was observed between the consumption of unboiled well water and the presence of *Toxoplasma* antibodies ( $p < 0.05$ ), this correlation was remarkable for farms with poor hygiene conditions enclosing shallow wells ( $p < 0.001$ ) (Sroka et al., 2006).

## 5.5. Detection methods in food

### *Bioassay*

The established reference method for the isolation of *Toxoplasma* from foodstuffs is gavage or inoculation into animals. However, mouse and cat bioassays require use of live animals for 6 to 8 weeks, they are time-consuming and not suitable for slaughterhouse testing or monitoring of commercial meat products (Warnekulasuriya et al., 1998). Bioassay in cats is more likely to detect *Toxoplasma* in meat than in mice because more tissue can be fed (Dubey et al., 2005b). Tissue culture is less sensitive than animal inoculation but produces results in a shorter period of time at a lower cost (Holliman, 1990). Since there may be less than one cyst per 50 g of tissue, it is generally not practical to demonstrate the presence of the parasite by histological examination (Dubey et al., 1996). One of the major difficulties in detection of *Toxoplasma* in tissue from large animal species, such as sheep and cattle, is the limitation of sample size which can be examined. Thus, the parasite may be undetectable in certain parts of tissue (Esteban-Redondo et al., 1999).

To demonstrate the presence of *Toxoplasma* in milk, pregnant specific pathogen free cats were inoculated orally with *Toxoplasma* (300 to 1000 oocysts per animal) at various times prior to parturition. Faecal samples were examined for oocyst shedding and milk was collected for detection of the B1 gene by PCR and bioassay in mice. In preliminary experiments, the sensitivity of PCR was determined with 10 tachyzoites/ml in peptide buffered saline (PBS) and 100 tachyzoites/ml in spiked cow milk. By means of PCR *Toxoplasma* was detected intermittently in milk after parturition over a span of 4 to 23 days. Oocysts were last detected in the faeces between 1 and 26 days before *Toxoplasma* was detected in milk (Powell et al., 2001).

### *Filtration, centrifugation and flocculation*

When drinking water emerged as a possible source for toxoplasmosis, a laboratory method was needed to attempt detection of this waterborne parasite. Based on the method for detection of *Cryptosporidium* oocysts, a technique was developed for detection of *Toxoplasma* oocysts in large-volume drinking water samples passing a filter cartridge (1  $\mu\text{m}$  nominal porosity) at a flow rate of 4 to 10 litres per minute. After washing, centrifugation and keeping oocysts for sporulation, the filter retentate was tested for *Toxoplasma* in a bioassay. The evaluation of bioassay revealed that at least 250 inoculated oocysts from a human strain induced a clinical infection and seroconversion in rodents (Isaac-Renton et al., 1998).

In another study, centrifugation and flocculation using coagulants were evaluated for recovering *Toxoplasma* from water samples inoculated with sporulated or unsporulated oocysts ( $1 \times 10^4$  and  $1 \times 10^5$ ). The mean recovery rate for oocysts was high for both methods, whereas flocculation was generally more successful than centrifugation. The infectivity of recovered sporulated oocysts was tested in mice. Seroconversion of all inoculated mice suggests that oocysts retain their infectivity after mechanical treatment and purification. Authors conclude that both methods are appropriate for detection of *Toxoplasma* oocysts in water (Kourenti et al., 2003).

### PCR

A number of assays based on PCR amplification of different DNA sequences of *Toxoplasma* have been developed (Holliman, 1994). In a study on detection of *Toxoplasma* in ready-to-eat cured meat samples by amplification of the parasite's P30 gene, PCR was able to detect parasite contamination down to a level of  $5 \times 10^3$  trophozoites/g while viable *Toxoplasma* could be detected in tissue culture at a level of  $10^3$  trophozoites/g cured meat. The high salt content of some cured meats limited sensitivity of the PCR assay by inhibition of the polymerase enzyme and reduced the sensitivity of tissue culture due to osmotic pressure causing cytopathic effect (Warnekulasuriya et al., 1998). Investigations of various meat products showed that many samples containing ingredients of porcine origin harboured detectable *Toxoplasma* DNA. With respect to parasite distribution, tissue cysts are likely to be disrupted during processing giving samples that are more homogeneous than might be expected of traditional cuts of meat, where PCR detection is likely to be a more stochastic process. The presence of DNA shows that the meat originates from a *Toxoplasma*-infected animal but does not necessarily mean that the product contains infectious organisms (Aspinall et al., 2002).

An 18S-rRNA nested PCR was developed for natural and drinking water samples of different quality and origin tested for *Toxoplasma* DNA after flocculation and purification by discontinuous sucrose gradients. Up to 100 oocysts per 50 ml could be detected in seeded tap water replicates, whereas for environmental water samples collected by flocculation, the highest detection sensitivity was 100 and 10 oocysts for river water and well or sea water, respectively. The protocol was finally applied for detection of *Toxoplasma* DNA in water samples obtained from different countries and four out of 60 samples (7%) were found positive by PCR examination. The method offers an alternative approach for *Toxoplasma* monitoring in environmental drinking water sources, and particularly during outbreak events, where high contamination levels are anticipated (Kourenti and Karanis, 2006).

In an experimental seeding assay, filtered and purified water samples were examined by PCR and mouse inoculation test to determine the presence and infectivity of recovered *Toxoplasma* oocysts. A parasite density of 1 oocyst per litre was detected by PCR in deionized water in 60% of cases whereas 10 oocysts per liter were detected in all samples. The sensitivity varied from less than 10 to more than 1000 oocysts per litre, depending on the sample source. However, PCR was always more sensitive than the mouse inoculation test. This detection strategy was then applied to examination of environmental water samples collected over a 20-month period and 10 out of 125 samples (8%) were tested positive for *Toxoplasma* by PCR. In contrast, none of samples revealed positive in mouse inoculation test. To distinguish between DNA from living and from dead oocysts, reverse transcription-PCR could be used as an important indirect technique in order to check oocyst viability (Villena et al., 2004).

### **Real-time PCR**

A highly sensitive and specific real-time PCR has been developed to detect and quantify *Toxoplasma* burden in animal tissue samples using *Toxoplasma* ITS1-derived primers. For this purpose, tissues from experimentally *Toxoplasma*-infected mice and pigs as well as bradyzoite-spiked pig muscle samples were used to test and standardize this technique. Assay specificity was confirmed against a panel of DNA samples from *Toxoplasma* and other common protozoa (*Neospora caninum*, *Hammondia hammondi*, and *Hammondia heydorni*) as well as host animal tissue. When applied to bradyzoite-spiked muscle samples with a weight of 50 g, the TaqMan test successfully detected as few as about four bradyzoites per g of pig tissue. Findings by real-time PCR corresponded with both serological response of all infected animals and presence of tissue cysts in the brain of infected mice. Results indicate that this assay is applicable to examination of swine carcasses and commercial pig products and can diagnose and quantify *Toxoplasma* in animal tissues (Jauregui et al., 2001).

A real-time TaqMan PCR targeting B1 and *ssrRNA* was developed for detection of *Toxoplasma* in experimentally exposed mussels under laboratory conditions. *Toxoplasma*-specific *ssrRNA* was detected in mussels as long as 21 days post exposure ( $1.5 \times 10^4$  and  $1.6 \times 10^5$  oocysts per 25 and 10 litre of filtered saltwater, respectively). Parasite *ssrRNA* was most often detected in digestive gland homogenate (31 of 35, i.e. 89%) compared with haemolymph or gill homogenates. Infectivity was tested in mice inoculated with any one of the mussel sample preparations (haemolymph, gill, or digestive gland), but only digestive gland samples remained positive in bioassay for at least 3 days post exposure. The total proportion of mice inoculated with each of the different tissues from *Toxoplasma*-exposed mussels well correlated with the proportion of exposed mussels from the same treatment groups that were positive via TaqMan PCR. Parallel analysis performed on extracted DNA showed a 10- to 100-fold increased sensitivity of the TaqMan PCR when compared to the conventional gel-electrophoresis PCR protocol. The TaqMan PCR assay may prove to be a valuable method for the identification of other marine hosts and routes of *Toxoplasma* transmission (Arkush et al., 2003).

### **Genotyping**

Several genetic markers have been developed and utilized to identify the *Toxoplasma* isolates for genetic analysis of the parasite population. Three major *Toxoplasma* genotypes have been initially characterized: type I, consisting of mice-virulent isolates, type II, containing mouse avirulent isolates, and type III, comprising both avirulent and intermediately virulent isolates (Howe and Sibley 1995). Due to the clonal population structure of *Toxoplasma* in Europe and USA, a monocus approach based on *SAG2* gene polymorphism analyzed by PCR-RFLP using two restriction sites has been widely used to differentiate with a single PCR the three main genotypes of *Toxoplasma* (Parmley et al., 1994; Sibley and Boothroyd 1992). However, this monocus approach relying entirely on the *SAG2* locus leads to the misidentification of atypical or recombinant genotypes which are found notably outside Europe (Fazaeli and Ebrahimzadeh, 2007, Ajzenberg et al; 2004). Multiplex strategies for genotyping based on 11 loci analyzed by PCR-RFLP (Su *et al.*, 2006) or on 5 microsatellite markers (Ajzenberg et al. 2005) were recently described and are more suitable to identify atypical genotypes. Fingerprinting methods based on association of highly polymorphic microsatellites have been developed and used for tracing outbreak (Demar et al. 2007).

In a study on prevalence of *Toxoplasma* in commercial meat products obtained from retail outlets in United Kingdom, samples consisting of 57 pork, nine lamb, four beef and one pork and beef mix from a variety of commercial sources were examined by PCR targeting SAG2 locus. 27 out of the 71 meat samples (38%) were found to be contaminated with *Toxoplasma*, including six of the nine lamb samples, and 20 of the 58 samples containing pork (Aspinall et al., 2002). Restriction analysis and DNA sequencing showed that 21 of the contaminated meats contained parasites genotyped as type I at the SAG2 locus, whilst six of the samples contained parasites of both genotypes I and II. A further analysis of DNA products for sulfonamide resistance in *Toxoplasma* revealed that none of them carried the drug-resistant dihydropteroate synthase gene. In another study on occurrence of *Toxoplasma* in pork, molecular characterization of oocysts obtained from bioassay using the SAG2 locus and 5 hypervariable microsatellite loci demonstrated genotypes I, II and III (Dubey et al., 2005b).

In an outbreak study in Santa Isabel do Ivaí (Brazil), samples from the suspected water supply were positive for *Toxoplasma* in bioassay. Viable cysts obtained from subpassage in mice were subjected for molecular characterisation using SAG2 locus and restriction fragment length polymorphism (RFLP) identified *Toxoplasma* genotype I which is of high virulence (de Moura et al., 2006).

Other studies based on SAG2 PCR-RFLP (Owen et al., 1999), as well as on a multilocus approach (Dubey et al. 2006, Dumetre et al. 2005, Sousa et al., 2006) revealed the predominance of type II strain in meat (sheep, chicken) in several countries of Europe, but type III have also been detected.

## 5.6. Future directions for surveillance and monitoring in food

Results of data analysis reveal that *Toxoplasma* monitoring should focus on meat and meat products from sheep, goat and pigs. Priority should be given to raw meat and meat-derived products that are processed at less than 67°C, as these are most likely to contain viable *Toxoplasma* tissue cysts.

Also water seems to be a suitable matrix for detection of *Toxoplasma*. However, more facilitated techniques are necessary for the isolation of oocysts under field conditions and further field and experimental studies are needed to better clarify the role of water and its role for contamination of vegetable and fruits with oocysts in the transmission to humans.

A low concentration of tissue cysts in meat or oocysts in water may lead to false-negative results. Therefore, an optimal sensitivity is attributed to several factors such as the choice of sample matrix, sampling site, sample weight and respective method used for the detection of tissue cysts in meat or oocysts in water.

Predominantly, bioassays in cats and mice were used for detection of *Toxoplasma* for example in pork, beef and chicken but these methods are not feasible for routine monitoring considering high costs, expenditure of time and ethical aspects (animal welfare). There are new approaches for detection and typing of *Toxoplasma* in meat and water by molecular techniques (e.g. PCR, RFLP). However, various questions have to be clarified such as inhibitory effects or ingredients which may limit sensitivity and specificity of the test system. Furthermore, these methods are applied as in-house tests which are neither standardised nor harmonised. Recent detection methods cannot be considered as feasible for routine surveillance and monitoring of *Toxoplasma* in food unless they are validated in term of sensitivity and specificity on the basis of suitable reference material.

## CONCLUSIONS

1. Recent studies from a few Member States indicate that the disease burden of toxoplasmosis may have been underestimated and is uncertain. Therefore the collection of representative data on the disease burden and the role of food including water in the EU is crucial for any future decision regarding food safety intervention.
2. There is increasing evidence that acquired *Toxoplasma* infection is the most important cause of infectious posterior uveitis. Only a better monitoring of prenatal and postnatal *Toxoplasma* infections and follow-up of the infected patients will allow conclusions on the overall incidence of postnatally acquired ocular toxoplasmosis in the different Member States.
3. Commercial serological tests are available for monitoring in humans and are standardised and subject to quality control. However, there is no biological test to distinguish between infections from oocyst ingestion transmitted by cats or from tissue cysts ingestion from infected meat.
4. The relative role of *Toxoplasma* oocysts in the environment as a source of direct exposure and contamination for water and fresh produce, versus tissues cysts in meat and meat products as a source of infection for humans is not known.
5. Data on the prevalence of *Toxoplasma* infection in food producing animals are not comparable due to differences in sampling and methodology.
6. Eating raw or undercooked sheep and goat meat remains an important risk. The role of beef as a source of infection is still unclear.
7. The extent of *Toxoplasma* infection in other food animals (pigs, poultry) depends on the farming system where outdoor access leads to a higher prevalence.
8. All game including farmed game has to be considered as a further source of foodborne *Toxoplasma* infection if consumed raw or undercooked.
9. Data on the prevalence of *Toxoplasma* oocysts in feed, fresh food and water are scarce or not available.
10. There appear to be more virulent strains of *Toxoplasma* outside of Europe.
11. Several diagnostic methods are being used for serological testing in animals but have not been standardised because there are no species specific reference sera.
12. With respect to *Toxoplasma* in food such as meat, milk or water, currently available detection methods are still experimental (molecular techniques) or not appropriate for routine monitoring (bioassay).

## Answering the Terms of Reference

### *Second ToR*

The analytical methods to be used to detect and identify *Toxoplasma* in food and animals need to be characterised in terms of sensitivity, specificity and other performance parameters associated with the reliability and consistency of such methodologies. In order for such characteristics to be attained, there is an absolute requirement for reference materials and reagents. As a first step, identification and long term availability of such materials and reagents would be a matter for CRL to undertake, after which field trials could be initiated to establish the suitability of current and future tests/assays for the above purpose.

### *First ToR*

Once such standardised methods are available, the Panel recommends that *Toxoplasma* monitoring should start on pre-harvest sector in sheep, goats, pigs and game.

## Recommendations

1. The collection of representative data on the disease burden and the role of food including water in the EU is crucial for any future decision regarding food safety intervention.
2. Serological methods should be standardised and harmonized for each animal species which is relevant (primarily sheep, goats, pigs and game) and feasible methods for the routine detection of *Toxoplasma* in meat, meat products and water should be developed and validated. This requires the production and distribution of suitable reference materials, including reference material for related organisms that may cause cross-reactivity in diagnostic tests for *Toxoplasma*.
3. The correlation between serology and number of infective tissue cysts in edible parts of animals should be established.
4. When appropriate serological methods are available, *Toxoplasma* monitoring should start on pre-harvest sector in sheep, goats, pigs and game.
5. Raw and minimally processed meat products from the above animal species could be monitored according to the results of serological studies.
6. The relative importance of water in the transmission to humans should be clarified before inclusion in monitoring programs.
7. Data are needed concerning the *Toxoplasma* risk from imported meat and meat products from third countries with special consideration given to genotypes which are more virulent to humans.
8. Genotyping should be further developed to allow fingerprinting which can be used for tracing circulation of strains and source attribution in outbreaks.

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APPENDIX

Table 1. Annual economic costs of congenital toxoplasmosis in the USA for the year 1992 (Roberts, et al., 1994)

	US\$ Million per case	US\$ Million all cases
Medical costs	0.01	45
Annual productivity losses	0.67	2835.2
Special education and residential care	0.57	2376
Total	1.26	5256.2

Estimates are based on 1 case per 1000 live births, or 4179 US cases annually.

Table 2. Seropositivities of *Toxoplasma* infection in farm animals in Europe in 2005 (EFSA, 2007)

Country	Farm animals	N	Pos	% Pos
Finland	Cattle	396	0	0
	Pigs	852	0	0
	Sheep	76	0	0
Germany	Cattle	562	6	1.1
	Pigs	1257	0	0
	Sheep	595	146	24.5
	Goats	48	5	10.4
	Solipeds, domestic	71	0	0
Italy	Cattle, monitoring	47	17	36.2
	Pigs, monitoring	31	0	0
	Sheep, monitoring	2016	859	42.6
	Goats, monitoring	531	102	19.2
Norway	Sheep	44	18	40.9
Poland	Cattle	31	0	0
Portugal	Sheep	40	21	52.5
	Goats	33	15	45.5
	Pigs	333	53	16
The Netherlands	Pigs <sup>1</sup>	845	22	2.6
Slovakia	Goats	32	10	31.3

N= number of animals tested; Pos = number of animals positive to *Toxoplasma*; % Pos = percentage of animals positive to *Toxoplasma*.

<sup>1</sup> data from The Netherlands are from a survey carried out in 2004 and pigs originate from different farming systems.



Table 3. Seroprevalences of *Toxoplasma* infection in sheep (1990-2005)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n) <sup>b</sup>	Method <sup>c</sup>	Reference
<i>Slaughter sheep</i>					
Norway	1993	18	2070	ELISA	[Skjerve et al. 1996]
	1993	16	1940	ELISA	[Skjerve et al. 1998]
<i>Farmed sheep</i>					
Austria	<1991	72	531	CFT	[Schweighardt 1991]
	<1996	66	4079	IFAT	[Edelhofer & Aspöck 1996]
Croatia	<1994	4	95	DAT	[Rajkovic et al. 1994]
Czech Republic	1982-89	55	886	SFDT	[Hejlíček & Literak 1994b]
	1982-89	40	484	CFT	[Hejlíček & Literak 1994b]
	1986-90	#46-74	*661	SFDT	[Hejlíček & Literak 1994b]
	1986-90	#13-23	*650	CFT	[Hejlíček & Literak 1994b]
France	<1997	92	642	IFAT	[Cabannes et al. 1997]
	2006	##38	257	MAT	[Dumètre et al. 2006]
Germany	1993-95	33	1122	ELISA	[Seineke et al. 1996]
	<1997	21	151	IFAT	[Sting et al. 1997]
Greece (Crete)	<1995	23	8700	ELISA	[Stefanakis et al. 1995]
Ireland	<1990	56	837	IHAT	[O'Brien & Geraghty 1990]
Italy	1998-01	78	1056	LAT	[Gaffuri et al. 2006]
	1999-02	28	7194	IFAT	[Masala et al. 2003]
	2004-05	50	1876	ELISA	[Vesco et al. 2007]
Serbia	2002-03	85	511	MAT	[Klun et al. 2006]
Slovakia	1988-91	10	1939	CFT	[Kovacova 1993]
Spain	<1991	40	550	DAT	[Moreno et al. 1991]
	<1991	35	550	IFAT	[Moreno et al. 1991b]
	1992-93	12	541	MAT	[Mainar et al. 1996]
	<1996	38	3212	DAT	[Marca et al. 1996a]
	<1996	35	2306	MAT	[Marca et al. 1996b]
Sweden	<1996	34	2306	IFAT	[Marca et al. 1996b]
	<1992	19	704	ELISA	[Lunden et al. 1992]
United Kingdom	1990	29	202	LAT	[Samad & Clarkson 1994]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by "<". Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b Figures marked with "\*" were calculated from the published data. Seroprevalences marked with "#" varied with the herd examined. ##65.6% in ewes, 22% in lambs

c CFT, complement fixation test; DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFAT, indirect immunofluorescent antibody test; IHAT, indirect haemagglutination test; LAT, latex agglutination test; MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.

d Seineke P. Seroprävalenz von Antikörpern gegen *Toxoplasma gondii* bei Schafen, Ziegen und Schweinen in Niedersachsen. Thesis, Dr vet med, Tierärztliche Hochschule Hannover, 1996.

Table 4. Seroprevalences of *Toxoplasma* infection in goats (1990-2002)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n) <sup>b</sup>	Method <sup>c</sup>	Reference
<i>Farmed goats</i>					
Austria	<1996	69	687	IFAT	[Edelhofer & Aspöck 1996]
Croatia	1992	#4-14	*179	MAT	[Rajkovic et al. 1993]
Czech Republic	1981-90	61	54	SFDT	[Hejlíček & Literak 1994b]
	1981-90	21	54	CFT	[Hejlíček & Literak 1994b]
Germany	1993-95	42	69	ELISA	[Seineke 1996]
	<1997	19	829	IFAT	[Sting et al. 1997]
Greece (Crete)	<1995	14	2320	ELISA	[Stefanakis et al. 1995]
Italy	1999-02	12	2445	IFAT	[Masala et al. 2003]
Netherlands	<1998	47	189	DAT	[Antonis et al. 1998]
Reunion	1987	75	395	ELISA	[Roger et al. 1991]
Spain (Grand Canary Island)	<1995	63	1052	ELISA	[Rodríguez-Ponce et al. 1995]
<i>Unclassified</i>					
Czech Republic	1994	20	247	CFT	[Literak et al. 1995]
	1994-96	*30	202	CFT	[Slosarkova et al. 1999]
	1996	#60-66	*159	IFAT	[Slosarkova et al. 1999]
	1994-97	*45	203	CFT	[Slosarkova et al. 1999]
France	<1997	#0-77	765	ELISA	[Chartier et al. 1997]

- a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by "<". Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.
- b Figures marked with "\*" were calculated from the published data. Seroprevalences marked with "#" varied with the herd examined.
- c CFT, complement fixation test; DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFAT, indirect immunofluorescent antibody test; IHAT, indirect haemagglutination test; MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.
- d Seineke P. Seroprävalenz von Antikörpern gegen *Toxoplasma gondii* bei Schafen, Ziegen und Schweinen in Niedersachsen. Thesis, Dr vet med, Tierärztliche Hochschule Hannover, 1996.

Table 5. Seroprevalences of *Toxoplasma* infection in pigs (1990-2006)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n) <sup>b</sup>	Method <sup>c</sup>	Reference
<i>Fattening / slaughter pigs</i>					
Austria	<1990	4	2755	CFT	[Quehenberger et al. 1990]
	1992	<1	2300	IFAT	[Edelhofer 1994]
Czech Republic	1979-90	6	2616	SFDT	[Hejlíček & Literak 1993]
	1979-90	<1	1179	CFT	[Hejlíček & Literak 1993]
	1981-90	32	287	SFDT	[Hejlíček & Literak 1994a]
	1981-90	11	215	CFT	[Hejlíček & Literak 1994a]
	1988-90	35	57	SFDT	[Hejlíček & Literak 1993]
	1988-90	14	57	CFT	[Hejlíček & Literak 1993]
Finland	1984	3	1847	ELISA	[Hirvela-Koski 1992]
Germany	1993-95	0	60	ELISA	[Seineke 1996]
Italy	<1991	64	90	IFAT	[Genchi et al. 1991]
Netherlands	<1991	2	23348	ELISA	[Berends et al. 1991]
	<1995	2	994	ELISA	[Van Knapen et al. 1995]
Norway	1993-94	3	1605	ELISA	[Skjerve et al. 1996]
Poland	<1991	36	925	ELISA	[Bartoszcze et al. 1991]
Portugal	1988-90	5	300	DAT	[Fortier et al. 1990]
	2004-05	16	333	MAT	[de Sousa et al. 2006]
Sweden	1999	3	695	ELISA	[Lundén et al. 2002]
<i>Sows</i>					
Austria	<1990	3	1162	CFT	[Quehenberger et al. 1990]
	1992	4	46	IFAT	[Edelhofer 1994]
Germany	1993-95	8	90	ELISA	[Seineke 1996]
	1997-99	18	>2000	ELISA	[Damriyasa et al. 1999]
	<2005	9	1500	ELISA	[Damriyasa & Bauer 2005]
Netherlands	<1995	31	1009	ELISA	[Van Knapen et al. 1995]
Sweden	1999	17	110	ELISA	[Lundén et al. 2002]
<i>Not classified</i>					
Czech Republic	1981-90	31	230	SFDT	[Hejlíček & Literak 1993]
	1981-90	10	158	CFT	[Hejlíček & Literak 1993]
Poland	<1991	36	925	ELISA	[Bartoszcze et al. 1991]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by "<". Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b Figures marked with "\*" were calculated from the published data. Seroprevalences marked with "#" varied with the herd examined.

c CFT, complement fixation test; DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFAT, indirect immunofluorescent antibody test; IHAT, indirect haemagglutination test; MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.

d Seineke P. Seroprävalenz von Antikörpern gegen *Toxoplasma gondii* bei Schafen, Ziegen und Schweinen in Niedersachsen. Thesis, Dr vet med, Tierärztliche Hochschule Hannover, 1996.

e Damriyasa IM, Bauer C, Volmer R, Failing K, Tenter AM, Lind P, Zahner H. Survey on endo- and ectoparasite infections in sow farms in Southern Hessen, Germany. In: Proceedings of the 17th International Conference of the World Association for the Advancement of Veterinary Parasitology, 15.-19.8.1999, Copenhagen, Denmark, ISBN 87-987513-0-1.

Table 6. Seroprevalences of *Toxoplasma* infection in cattle (1990-2003)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n) <sup>b</sup>	Method <sup>c</sup>	Reference
<i>Cattle</i>					
Czech Republic	1979-90	4	1926	SFDT	[Hejlíček & Literák 1992]
	1979-90	2	1238	CFT	[Hejlíček & Literák 1992]
	1981-90	22	218	SFDT	[Hejlíček & Literák 1992]
	1981-90	3	176	CFT	[Hejlíček & Literák 1992]
France	<1997	69	364	IFAT	[Cabannes et al. 1997]
Greece	<1992	40	1890	CFT	[Kritsepi-Konstantinou 1992]
Italy	<1993	92	255	DAT	[Avezza et al. 1993]
Netherlands	<1995	#13-43	*6976	ELISA	[Van Knapen et al. 1995]
Norway	1989	5	1053	ELISA	[Skjerve et al. 1996]
Portugal	1988-90	43	60	DAT	[Fortier et al. 1990]
Reunion	1987	54	780	ELISA	[Roger et al. 1991]
Serbia	2002-03	76	611	MAT	[Klun et al. 2006]
Spain	<1991	41	304	MAT	[Moreno et al. 1991a]
	<1991	40	304	IFAT	[Moreno et al. 1991a]
Switzerland	1994	14	148	ELISA	[Busato et al. 1997]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”. Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b Figures marked with “\*” was calculated from the published data. Seroprevalences marked with “#” varied with the herd examined.

c CFT, complement fixation test; DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFAT, indirect immunofluorescent antibody test; IHAT, indirect haemagglutination test; MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.

Table 7. Seroprevalences of *Toxoplasma* infection in domestic fowl (1990-2000)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n) <sup>b</sup>	Method <sup>c</sup>	Reference
<i>Chickens</i>					
Austria	2004-05	36	830	MAT	[Dubey et al. 2005a]
Czech Republic	1981-90	#1-5	*4458	SFDT	[Literák & Hejlíček 1993]
		24	225	MAT	[Dubey et al. 2006]
<i>Ducks</i>					
Czech Republic	1981-90	2	297	SFDT	[Literák & Hejlíček 1993]
<i>Geese</i>					
Czech Republic	1981-90	16	32	SFDT	[Literák & Hejlíček 1993]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”. Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b Figures marked with “\*” were calculated from the published data. Seroprevalences marked with “#” varied with the herd examined.

c MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.

Table 8. Seroprevalences of *Toxoplasma* infection in horses (1990-2006)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested (n)	Method <sup>c</sup>	Reference
Czech Republic	1987	8	2886	SFDT	[Hejlíček & Literák 1994c]
Sweden	1986-87	<1	219	ELISA	[Uggla et al. 1990]
	1992-93	1	414	DAT	[Jakubek et al. 2006]
	1992-93	<1	414	IB	[Jakubek et al. 2006]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”. Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b Seroprevalences marked with “\*” were calculated from the published data.

c ELISA, enzyme-linked immunosorbent assay; SFDT, Sabin-Feldman dye test; DAT, direct agglutination test; IB, immunoblotting.

Table 9. Seroprevalences of *Toxoplasma* infection in rabbits (1990-2003)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%)	Number of samples tested (n)	Method <sup>b</sup>	Reference
Czech Republic	1981-86	53	366	SFDT	[Hejlíček & Literák 1994d]
France	<1990	6	187	IFAT	[Chalupsky et al. 1990]
Poland	<2003	22	9	DAT	[Sroka et al. 2003]

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”. Data from the 1980s are included if the study was published in the 1990s and if no recent data were available for the area.

b IFAT, indirect immunofluorescent antibody test; SFDT, Sabin-Feldman dye test; DAT, direct agglutination test.

Table 10. Seroprevalences of *Toxoplasma* infection in game and wild animals (1990-2007)

Country	Year of sampling <sup>a</sup>	Seroprevalence (%) <sup>b</sup>	Number of samples tested ( <i>n</i> ) <sup>b</sup>	Method <sup>c</sup>	Reference		
<i>Wild boars</i>							
Austria	1990-93	19	269	IFAT	[Edelhofer et al. 1996]		
Czech Republic	1981-90	15	124	SFDT	[Hejlíček et al. 1997]		
	1999-05	26	565	IFAT	[Bártová et al. 2006]		
Germany	1993-94	25	130	IFAT	[Lutz 1997]		
	1997	21	81	IFAT	[Tackmann 1999]		
	1997	19	81	ELISA	[Tackmann 1999]		
	1997	15	81	SFDT	[Tackmann 1999]		
Slovak Republic	2003	8	320	ELISA	[Antolová et al. 2007]		
Spain	1993-04	38	507	MAT	[Gauss et al. 2005]		
	2000-03	36	91	MAT	[Ruiz Fons et al. 2006]		
France	2005-06	44	79	MAT	[Aubert et al. 2006]		
<i>Deer</i>							
Czech Republic	1981-90	#14-100	401	SFDT	[Hejlíček et al. 1997]		
Italy	1998-01	roe 13	207	LAT	[Gaffuri et al. 2006]		
		cha 5	108	LAT	[Gaffuri et al. 2006]		
		rd 8	571	DAT	[Vikøren et al. 2004]		
Norway	1993-99	roe 34	760	DAT	[Vikøren et al. 2004]		
	1994, 99-00	roe 100	3	MAT	[Sroka 2001]		
Poland	2001	rd 16	441	MAT	[Gauss et al. 2006]		
Spain	1993-05	fd 24	79	MAT	[Gauss et al. 2006]		
		roe 21	33	MAT	[Gauss et al. 2006]		
		mou 15	27	MAT	[Gauss et al. 2006]		
		cha 20	10	MAT	[Gauss et al. 2006]		
		bs 10	10	MAT	[Gauss et al. 2006]		
		Si 33	3	MAT	[Gauss et al. 2006]		
		France	2005-06	Roe 72	25	MAT	[Aubert et al. 2006]
		Red 6.6		16	MAT		
		Fd 25		4	MAT		
		<i>Moose</i>					
Norway	1992, 94-00	13	2142	DAT	[Vikøren et al. 2004]		
<i>Reindeer</i>							
Finland	1993-96	#0-25	*900	DAT	[Oksanen et al. 1997]		
Norway	1993-94	<1	*1677	DAT	[Oksanen et al. 1997]		
	1999-00	1	866	DAT	[Vikøren et al. 2004]		

a Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”.

b Figures marked with “\*” were calculated from the published data. Seroprevalences marked with “#” varied with the geographical area or the species examined (rd = red deer; roe = roe deer; fd = fallow deer; mou = mouflon; cha = chamois; bs = barberry sheep; Si = Spanish ibex).

c DAT, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFAT, indirect immunofluorescent antibody test; IHAT, indirect haemagglutination test; LAT, latex agglutination test; MAT, modified agglutination test; SFDT, Sabin-Feldman dye test.

**GLOSSARY / ABBREVIATIONS**

<b>AF</b>	Amniotic fluid
<b>ARC</b>	AIDS-related complex
<b>BMT</b>	Bone marrow transplant
<b>BSN</b>	Basic Surveillance Network
<b>CIA</b>	Carbon immunoassay
<b>CSF</b>	Cerebrospinal fluid
<b>CT</b>	Congenital toxoplasmosis
<b>DALY</b>	Disability Adjusted Life Year
<b>DAT</b>	Direct agglutination test
<b>DSN</b>	Dedicated Surveillance Network
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay.
<b>EUROTOXO</b>	EC financed project (Contract No. QL4-CT-2002-30262) “Prevention of congenital toxoplasmosis. A European initiative on the state-of-science”.
<b>HAART</b>	Highly active antiretroviral therapy
<b>IB</b>	Immunoblotting
<b>IFAT</b>	Indirect immunofluorescence antibody test
<b>IHAT</b>	Indirect haemagglutination test
<b>InVS</b>	Institut National de Veille Sanitaire, France
<b>ISAGA</b>	Immunosorbent agglutination assay
<b>LAT</b>	Latex agglutination test
<b>MAT</b>	Modified agglutination test
<b>Monitoring</b>	Monitoring is a system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto. As opposed to surveillance, no active control measures are taken when positive cases are detected. According to OIE definitions (Terrestrial Animal health code, 2006) monitoring is the continuous investigation of a given population or subpopulation, and its environment, to detect changes in the prevalence of a disease or characteristics of a pathogenic agent. In this respect, prevalence is defined as the total number of cases or outbreaks of a disease that are present in a population at risk, in a particular geographical area, at one specified time or during a given period.
<b>NAT</b>	Nucleic acid technology
<b>PCR</b>	Polymerase Chain Reaction.
<b>RFLP</b>	Restriction fragment length polymorphism

<b>RKI</b>	Robert Koch-Institut
<b>SFDT</b>	Sabin Feldman dye test
<b>Surveillance</b>	Surveillance means the investigation of a given population or subpopulation to detect the presence of a pathogenic agent or disease; the frequency and type of surveillance will be determined by the epidemiology of the pathogenic agent or disease, and the desired outputs.
<b>SYROCOT</b>	A systematic review (meta-analysis) from individual data of observational cohorts to assess the effect of prenatal treatment on mother-to-child transmission and occurrence of Congenital Toxoplasmosis clinical manifestations. Part of the EC financed EUROTOXO project
<b>YLD</b>	Years lived with a disability
<b>YLL</b>	Years of life lost due to mortality