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Recent trends in molecular diagnostics for *Toxoplasma gondii* infections

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ABSTRACT

Toxoplasmosis is an important parasitic infection of man and animals. It is well-known that the progression and severity of disease depend on the immunological status of the host, but recent studies suggest that the genetics of the parasite can also play a role. Diagnosis based on clinical appearance and serology is not always easy. However, molecular methods do not depend on an immune response, and allow direct detection of the parasite in biological samples. Thus they can be used to establish a diagnosis when serological tests are not definitive. Multicopy sequences specific for *Toxoplasma gondii*, e.g., the B1 gene or the 529-bp sequence, are especially useful in molecular tests. Real-time PCR is very sensitive and is a promising technique that is capable of providing a quantitative result. Molecular methods are also used for genotypic characterisation of *T. gondii* isolates. Analysis of polymorphic sequences determines the precise strain. The choice of sequence is critical when undertaking studies on the correlation between clinical signs and symptoms of disease and the *T. gondii* genotype. Further studies involving direct genotyping of *T. gondii* from clinical samples are needed.

Keywords Diagnostics, genotyping, molecular, PCR, review, *Toxoplasma gondii*

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects warm-blooded vertebrates, including man. The parasite is distributed widely in the human population, and is estimated to affect more than a billion individuals worldwide. Farm animals in the food chain are significant reservoirs of *T. gondii*, which is important because of possible transmission to man, and toxoplasmosis also causes significant veterinary losses.

Toxoplasmosis has a variable outcome, depending on the interaction of many factors, including the functions of the immune system. Most infections are asymptomatic or take the form of a mild, self-limiting illness. However, the patient is left with life-long latent infection caused by the presence of tissue cysts. The disease is dangerous when contracted *in utero*, and also for patients with immunodeficiency, especially if adaptive immunity is involved (e.g., AIDS patients, leukaemia patients, transplant patients). Acute toxoplasmosis acquired during pregnancy may result in fetal death or in serious complications such as blindness, deafness or central nervous system disorders. These complications may manifest in neonates, or later in life for children infected congenitally.

Serological diagnosis can be difficult in prenatal cases or in patients with immunodeficiency. The use of molecular diagnostic techniques is particularly appropriate for such patients, as these techniques do not depend on the immunological status of the host. However, although the immune response plays a very important role in the development of toxoplasmosis, several studies have advanced the hypothesis that the outcome and clinical presentation are also related to the virulence of specific genotypes of *T. gondii* [1–3].

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LIFE-CYCLE AND VIRULENCE OF *T. GONDII*

The life-cycle of *T. gondii* is well-established. Sexual and asexual reproduction of the parasite take place in the definitive hosts, which are all members of the family Felidae (e.g., domestic cats). In the asexual phase of the cycle (schizogony), maternal cells divide to form daughter cells termed merozoites. The sexual phase occurs in the small intestine of a cat. The zygotes are formed in epithelial cells and, after formation of a solid wall, are discharged into the intestinal lumen as oocysts. Sporulation occurs a few days after a cat sheds oocysts. Sporulated oocysts are resistant to adverse environmental conditions. Each oocyst contains four sporozoites which are the invasive form of *T. gondii* and remain infectious for *c.* 1 year. Soil and plants contaminated with oocysts are the source of infection for intermediate and definitive hosts. There is also evidence of transmission as a consequence of drinking contaminated water [4,5].

In an intermediate host (e.g., man), *T. gondii* undergoes an asexual phase in its life-cycle that includes two forms, namely rapidly multiplying tachyzoites and slowly multiplying bradyzoites. Tachyzoites invade the host cells and are responsible for the acute phase of the disease. Bradyzoites, characterised by slow metabolic processes, remain in the tissues as cysts. Tissue cysts can be formed in many organs and tissues, e.g., brain, heart and muscles, and these are the sources of infection for carnivorous and scavenger intermediate and definitive hosts.

There are few opportunities in such a life-cycle for genetic recombination through sexual reproduction (which requires infection in a cat with two different toxoplasmas simultaneously), so the population structure of *T. gondii* is of a clonal type. Many pathogenic bacteria and protozoans have such a population structure, with certain clones being responsible for particular disease patterns. In the case of *T. gondii*, such an association is evident in animal models. For example, there are *T. gondii* strains of high (type I) and low virulence in mice. A single parasite of a virulent strain forms an LD_{100} (a dose lethal for 100% of animals tested), whereas an LD_{100} for a strain of low virulence requires several thousand parasites [5]. Interestingly, there have been no reports of intermediate values, so the association between strain and disease pattern in mice seems to be clearly defined. Similarly, in human foreskin fibroblast cultures, type I strains multiply three times faster than parasites belonging to type II or III. There have also been reports of different immune responses in animal models against different strains of *T. gondii* [6]. In addition, virulent strains isolated from different hosts comprise a single genetic lineage [1]. Thus, the question arises as to whether there is a correlation between the virulence factors of *T. gondii* and the signs and symptoms of disease in man. If so, could these virulence factors be used in the development of tools to improve diagnosis, treatment and prognosis? For example, could the determination of *T. gondii* virulence factors contribute to the development of new drugs or vaccines?

Current studies on toxoplasmosis are focused mainly on the use of molecular methods. Rapid developments in molecular biology during recent years have opened up new research possibilities, with the advent of new technology often driving the direction of research. The remainder of this review describes the use of molecular techniques in the diagnosis of toxoplasmosis.

IDENTIFICATION OF *T. GONDII* WITH MOLECULAR TECHNIQUES

The presence of *T. gondii* in a biological sample can be diagnosed by molecular techniques aimed at detecting its genetic material. A specific fragment of the genome can be amplified by PCR so that it can be visualised on an agarose or a polyacrylamide gel following staining, on an automated sequencer by laser detection, or directly as an amplification product by means of real-time PCR techniques. The sensitivity and specificity of PCR-based methods depend on an appropriate technique for isolation of genetic material from samples, the characteristics of the DNA sequence chosen for amplification, and the parameters of the amplification reaction itself.

The first stage in amplification involves the hybridisation of specific primers to a chosen genome sequence. The sensitivity of the PCR is enhanced if the target sequence exists in multicopies that are species-specific. For detection of *T. gondii*, the sequence used most frequently is the B1 gene, first identified in 1998 by Burg *et al.* [7], of which there are 35 copies in the genome. The