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Short review

On the determination of *Toxoplasma gondii* virulence in mice

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HIGHLIGHTS

- Life stages of *Toxoplasma gondii* affect parasite virulence in mice.
- Continuous passages in mice and cell culture affect *T. gondii* virulence.
- The routes of infection affect the outcome of *T. gondii* virulence in mice.
- Different lines of laboratory mice vary in their resistance to *T. gondii* infection.
- Proposed methodology in determining *T. gondii* virulence in mice.

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ABSTRACT

Toxoplasma gondii is one of the most successful pathogens on earth, capable of infecting an extremely broad range of mammals and birds and causing potentially fatal disease in humans. The house mouse (*Mus musculus*) has been used as the primary laboratory animal model for determining the virulence of *T. gondii* strains. Epidemiological evidence also suggests a potential association between virulence in mice and disease severity in human toxoplasmosis. However, many factors can affect virulence measurements, including route of infection, life stage of the parasite, number of passages of the parasite in mice or cell culture, and the mouse host line used. Variability among these factors makes it difficult to compare results between different studies in different laboratories. Here, we discuss important factors that should be considered when carrying out *T. gondii* murine virulence assays and propose a standardized methodology that should facilitate integration of *T. gondii* virulence data throughout the research community in future studies and thereby enable more efficient and effective analysis of genetic and virulence patterns for this important parasite.

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1. Introduction

Toxoplasma gondii is an obligate intracellular parasite capable of infecting almost all warm-blooded animals, including humans (Dubey, 2010). Felids are the only known hosts, in which sexual reproduction of the parasite occurs, resulting in the dissemination of oocysts into the environment via defecation, and thus play a critical role in the transmission of *T. gondii*. It is estimated that one third of the world's human population is chronically infected with this parasite (Dubey, 2010). Humans acquire infection by ingesting oocysts from contaminated food and water, consuming undercooked meat containing tissue cysts, or by vertical transmission from mother to fetus (Tenter et al., 2000; Dubey, 2010). The primary infection in immunocompetent individuals is mostly asymptomatic but in some cases it can lead to ocular toxoplasmosis (Holland, 2003). In immunocompromised individuals, such as AIDS patients, reactivation of chronic infection can cause life threatening encephalitis (Montoya and Liesenfeld, 2004; Dubey, 2010).

Susceptibility to *T. gondii* infection varies among different hosts: cattle, horses, rats, Old World monkeys and humans are resistant to infection, while Australian marsupials and New World monkeys are much more susceptible. The reasons for such differences are not clear, but one reason may be a consequence of host-parasite co-evolution (Cunningham et al., 1992; Dubey, 2010). Laboratory mice are generally sensitive to *T. gondii* infection and are often used as the preferred animal model to determine the virulence of the parasite.

Virulence of *T. gondii* strains in mice varies with the genetic background of the parasites. Some genotypes of *T. gondii* are lethal to all strains of mice regardless of the dose of parasites administered, whereas other genotypes are non-lethal with a low dose of inoculation and can readily establish chronic infection in mice (Darde et al., 1992; Sibley and Boothroyd, 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002; Khan et al., 2009a,b; Shwab et al., 2016).

Recent analysis of *T. gondii* genetic diversity has revealed geographical patterns of genotype distribution (Shwab et al., 2014). Archetypal type II and type III strains are dominant in Europe and north Africa, while types II, III, and 12 are dominant in North America, and Chinese type 1 is most prevalent in east Asia. In contrast, *T. gondii* strains in South America are highly diverse with no clear dominance of any particular genotypes. The genotypes prevalent in Europe, North America, north Africa and Asia are non-lethal to mice at low infection dose, whereas a large proportion of *T. gondii* strains identified in South America are highly virulent and lethal to mice (Shwab et al., 2016). Population genetics and epidemiological studies have indicated a correlation between the geographic variations of *T. gondii* genotype and disease manifestation in humans. For example, severe symptoms associated with ocular toxoplasmosis are more frequently reported in Brazil than in European countries (Holland, 2003; Grigg et al., 2015), and numerous incidences of severe systemic toxoplasmosis in immunocompetent adults from French Guiana have been reported, in some cases resulting in the deaths of the afflicted individuals (Ajzenberg et al., 2009; Darde et al., 1998; Demar et al., 2007). Taken together, virulence of different *T. gondii* strains in mice appears to be generally correlated with disease manifestations in human cases (Xiao and Yolken, 2015). Therefore, determination of *T. gondii* virulence in mice could be invaluable in predicting the potential outcome of human infections.

To obtain a better understanding of large-scale patterns of *T. gondii* virulence, it is essential to have a standardized methodology for parasite virulence determination which allows for direct comparison of the results obtained from different studies. Currently, a variety of mouse strains, different life cycle stages of the parasite, and different routes of inoculation are used in *T. gondii* virulence assays. This variation hinders meaningful comparisons and complicates integration of data. In order to address this issue, in this review we summarize the common methodologies used to determine *T. gondii* virulence in laboratory mice, and put forth a simple standardized methodology that will facilitate more productive comparisons for future studies. Establishment of a cohesive database for studying the relationship between *T. gondii* genotype and virulence in mice should greatly enhance our understanding of parasite virulence patterns and aid in predicting the outcome of *T. gondii* infection in humans.

2. Current methods for virulence assessment of *T. gondii* in mice

2.1. Virulence of tachyzoites, bradyzoites and oocysts of T. gondii

Toxoplasma gondii has a complex life cycle, and the specific stages of the parasite used for inoculation may lead to marked differences in the outcomes of virulence in mice. The three infectious forms of the parasite include: the rapidly dividing tachyzoite, responsible for systemic invasion during primary infection; the slowly growing bradyzoite, associated with chronic infection; and the sporozoite, sexually produced in mature oocysts. All three forms may be used to infect mice, but infection with different forms may have varied results in terms of virulence. For example, mice inoculated orally with a single oocyst of the strain M-7741 were found to exhibit 100% mortality after approximately two weeks, whereas 10^3 bradyzoite-containing tissue cysts were required to produce this same mortality rate, and mice infected orally with 10^4 tachyzoites failed even to establish infection (Dubey and Frenkel, 1973). Higher pathogenicity of oocysts is also evident from a previous study in which mice infected orally with 10 oocysts died within two weeks but mice orally infected with 10 tissue cysts failed to cause any infection (Dubey et al., 1981). Among the three infectious stages of the parasite, oocysts in general are more virulent (Dubey and Frenkel, 1973; Dubey et al., 1981; Dubey, 2006). In addition, oocysts are environmentally resistant, highly infectious, and thus hazardous to work with, whereas tachyzoites and bradyzoites are readily killed even in water.

2.2. Change of T. gondii strain phenotypes after multiple passages in mice and cell culture

Changes in biological characteristics occur in *T. gondii* strains after passage in mice or cell culture (Jacobs and Melton, 1954; Frenkel et al., 1976; Dubey, 1977; Lindsay et al., 1991; Villard et al., 1997; Dubey et al., 1999; Khan et al., 2009a). *Toxoplasma gondii* strain M-7741, initially isolated from a sheep in 1950, was found to have lost the capacity to produce oocysts in cats after 30–35 continuous passages in mice (Frenkel et al., 1976). Similarly, after maintained in cell culture for 40 passages, the type I strain GT1 lost the ability to produce oocysts in cats (Lindsay et al., 1991). The most commonly used *T. gondii* RH strain which was isolated from a six-year old child in 1939, has been found to no longer produce oocysts in cats following prolonged maintenance through passage in laboratory mice or cell culture, presumably due to unknown biological changes in parasites over time (Dubey et al., 1999). A variety of phenotypic changes among several RH-derived clonal lineages were also observed (Khan et al., 2009a). Differences among these lineages included larger plaque formation, enhanced survival outside the cells, faster growth, and decreased differentiation. Enhanced virulence in mice for *T. gondii* strains maintained in cell culture for several passages has been reported previously (Frenkel and Ambroise-Thomas, 1997). In that study, 28 out of 31 original