Toxoplasmosis is an infection of vertebrates caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii*. It is one of the most common parasitic diseases of humans, infecting approximately one third of the world’s population. It is a significant cause of congenital disease and an important opportunistic pathogen which has become an increasing problem worldwide due to the AIDS epidemic. There is limited historical information about the disease in South Africa. More knowledge is needed at a regional level to properly consider solutions aimed at reducing the risk for this disease. The seroprevalence of *T. gondii* in samples of selected populations at risk, namely HIV-positive individuals and a more general population sample biased towards pregnant women, was therefore investigated and found to be 9.8% (37/376) and 6.4% (32/497) in the respective samples. The Pastorex Toxo latex agglutination test was evaluated and found to be a cheap, reliable method to screen for *T. gondii* exposure. PCR-based diagnostics were developed for direct diagnosis on tissue samples. Rodent *T. gondii* infection prevalence was investigated, but did not yield any positive results. This study helped to answer questions relating to the seroprevalence and diagnosis of *T. gondii* in South Africa. Many questions still remain to be answered, however to fully understand the impact of this parasite in our country.