

A nested PCR for *Toxoplasma gondii* detection from different animal samples

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A nested PCR to detect *Toxoplasma gondii* DNA from different samples such as tissues, milk and faeces has been used to start a molecular screening for toxoplasmosis in Sicily, Italy.

Toxoplasma gondii is a coccidian protozoa that can infect all warm blooded animals and all different cells. Seroprevalence ranging from 20 to 80% is observed worldwide in both humans and animals depending on age, life style and hygienic conditions. The protozoa perform a sexual life cycle in the intestine of felids, while an asexual life cycle is present in all warm blooded vertebrates resulting in tissues cysts formations. The infectious forms are represented by tachyzoites, (rapidly dividing in the acute phase of the infection), bradyzoites (slowing growing inside tissue cysts) and sporulated oocysts eliminated in the faeces of infected felines. Toxoplasmosis can be spread by ingestion of contaminated food and by trans-placental transmission. Infections are quite often asymptomatic but can be particularly dangerous for the foetus when the primary infection occurs during pregnancy (congenital toxoplasmosis) and in general in the case of immunodepression. In Sicily, a general serological screening on ovine populations, revealed positive serology up to more then 60% in several ovine flocks depending on the age of the animals, the type of farm management and the presence of cats We started a molecular and serological screening focusing on specific farms and different animal species. The molecular screening is based on a nested PCR targeting the rRNA locus. The first primer set amplifies the entire intergenic region between 18S and 25S rRNA gene amplifying a fragment of almost 1000 bp. The amplified product of this PCR can be utilized to distinguish by restriction enzymes, DNA patterns from *Toxoplasma* and *Neospora* isolates. The nested primers amplify the internal transcribed spacer 1 (ITS1) region. DNA was extracted from the different samples by the Gene Elute mammalian kit (Sigma cat N° G1N- 350) following manufacture's instructions. Analyses were performed by a first direct PCR using NC 18S RNA sense primer (5'TGCGGAAGGATCATTCACACG 3', Invitrogen) and NC25S RNA antisense primer (5'CCGTTACTAAGGGAATCATAGTT3', Invitrogen). The amplified fragment is approx. 1000bp. This PCR was performed for 7 min at 95°C followed by 40 cycles of 40 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final extension of 7 min at 72°C. A nested PCR using Toxo ITS1sense primer (5'GATTTGCATTCAAGAAGC TGATAGTAT3', Invitrogen) and Toxo ITS1 antisense primer (5'AGTTAGGAAGCA ATCTGAAAGCACATC, Invitrogen) was performed for 7 min at 95°C followed by 40 cycles of 40 s at 94°C, 30 s at 60°C, 1 min at 72°C and a final extension of 7 min at 72°C. The fragment amplified is approx. 333bp. Amplified products were loaded on 2% agarose gels and visualised on chemi doc (BIORAD).

By this method a general survey has started in different animal samples in Sicily. An overall 2–3% of *Toxoplasma* PCR positive samples have been detected so far from different animal species. All samples resulted negative after the first PCR showing the higher efficacy and sensitivity of a nested PCR for "field samples" analysis.