Prevalence of *Echinococcus granulosus* Cluj county, Romania, revealed by PCR

Prevalența cu *Echinococcus granulosus* în județul Cluj, România, relevată prin PCR

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**ABSTRACT**

The definitive hosts of *Echinococcus granulosus* are canids, especially dogs. In canids, *E. granulosus* causes a typical tapeworm infection and produces eggs that pass within the dogs' faeces to the environment and the intermediate hosts (herbivores, or humans) that ingest the eggs. In the intermediate host, eggs hatch into larvae that travel through the blood and form hydatid cysts in the hosts’ tissues. Human echinococcosis caused by the larval form of the parasite *Echinococcus granulosus*, is characterized by a slowly growing, fluid-filled cystic lesion (hydatid cyst) in the liver, lungs and other organs. **Methods:** The survey was conducted during October 2008 - February 2009. In order to confirm the presence of *Echinococcus granulosus* in dogs, 48 specimens were randomly selected from positive ones at ELISA test. The dogs belonged to rural areas of Cluj county, Romania. Faecal samples were collected and examined by nested-PCR. **Results:** Using the primers (P60.for) (P375.rev) and (Eg1.for.) (Eg1.rev.), we obtained an amplicon of 255 pb at 37/48 dogs. **Conclusion:** It has already been described the PCR diagnostic method in naturally infected dogs (Stefanic et al., 2004), but so far no such studies have been completed in Romania. The detection of dogs with *Echinococcus granulosus* infection is very important in our country, since the current control programs should be based mainly on definitive hosts' treatment, with the aim of interrupting the life cycle of the parasite. The high frequency of infection obtained in the studied dogs, should be an impulse for epidemiological surveys on a greater scale, and using diagnostic tools with high sensitivity and specificity, such as PCR, but the use of this technique is limited for the moment in Romania.

**Keywords:** *Echinococcus granulosus*, dog, prevalence, Cluj county, PCR

**Introduction**

In the last 15 years, molecular biology researches on parasites and parasitic diseases have evolved significantly. Many of the findings led to the development of new tools for the polymerase chain reaction (PCR). In the present, increasing of the diagnostic sensitivity, which can be achieved using PCR, allows to study changes even at cell level, which is more than necessary in the study of parasites. PCR had an important role in the discoveries made in the systematics and epidemiology of parasites, in immunology and host-parasite interaction, development of recombinant DNA vaccines and more recently whole genome analysis, by direct sequencing of DNA or by functional genomics. For *Echinococcus granulosus*, it has been previously described the existence of morphologically distinct strains, according to the species of intermediate hosts that are involved in the biological cycle. This diversity of strains was confirmed by genetic studies, leading today in recognition of at least 10 types of strains (G1-G10) (Thompson et al., 1995; Eckert and Thompson, 1997; Lavikainen et al., 2003).
The degree of susceptibility of the humans to different species/strains of *Echinococcus* seems significantly different, but epidemiological data are limited in this field. Most cyst isolates from humans were considered to be of *E. granulosus* G1 (sheep strain). Infection with *E. equinus* has not been described in humans, and only a few cases of infection with *E. ortleppi* and *E. granulosus* G6 (strain Camels) were raised. Nothing is known about the infection with *E. granulosus* G7 (pig strain), although it was described in humans a similar genotype, called G9 (Scott et al., 1997), but quantitative data on the importance of different genotypes and species evolution in humans with cystic echinococcosis is insufficient (Eckert and Thompson, 1997).

Taking into account the situation in echinococcosis/hydatidosis in Romania (Iacobiciu et al., 2005) it is a real need for epidemiological data on prevalence in dogs, as they are the main vector for this zoonosis, and in order to determine the human risk for infection with different genotypes/species. Using the PCR technique in the detection of *Echinococcus granulosus* infection in dogs, provides the real prevalence of this tapeworm. This is the first epidemiological study performed in Romania, using the copro-PCR technique to detect DNA of *Echinococcus granulosus* sheep strain in dogs, as definitive hosts. As for the other strains, further studies are needed.

However, there is a well set PCR method to examine a large number of animal definitive hosts (Dinkel et al., 2004). Because of the high cost and instability of the PCR primers, the technique is limited only to positive cases confirmed by other diagnostic methods (copro-ELISA, coproparasitology or necropsy).

**Materials and methods**

In order to confirm the presence of *Echinococcus granulosus* in dogs, 48 specimens were randomly selected from rural areas of Cluj County, Romania. Faecal samples were collected and examined by nested-PCR, using the *Echinococcus granulosus* sheep strain primers described by Abbasi et al. (2003) and Stefanic et al. (2004): (P60.for.) (P375.rev.) for the first PCR and (Eg1.for.) (Eg1.rev.) for the second PCR. The PCR steps were carried out after the protocol of Dinkel et al. (1998), but with reference to *Echinococcus granulosus*. The DNA extraction was performed as described by Mathis et al. (1996), based on the concentration of oncospheres from faeces.

**Results and discussion**

Following research for identification of *Echinococcus granulosus* DNA, sheep strain, by PCR technique from faecal samples, using the copro-PCR technique to detect DNA of *Echinococcus granulosus* sheep strain in dogs, as definitive hosts. As for the other strains, further studies are needed.

![Marker molecular](image)

**Fig. 1.** Identification of *E. granulosus* DNA, sheep strain, by PCR, 255 pb amplicon
Detecting infections with *E. granulosus* in dogs is very important because the current control programs are based primarily on the treatment of definitive hosts (Gemmel et al., 2001). It has already been described the usefulness of PCR in the diagnosis of naturally infected dogs during the patent period (Stefanic et al., 2004), but no studies have been made to detect infected dogs during prepatency. So far, the PCR diagnostic method in naturally infected dogs has not been completed in Romania.

Research in PCR for echinococcosis, made by Stefanic et al. (2004), used the primers (Eg1f) CATTAATGTATTTGTAAAGTTG and (Eg1r) CACATCATCTACAATAACACC, extracted from mitochondrial 12S RNA and specific for *E. granulosus* sheep strain. The primers were used for the investigation of 131 faecal samples from dogs in Kazakhstan, harvested by purgation with arecoline. Of the total number of samples, 18 had adult *Echinococcus* forms and of 13 samples in which were found oncospheres, 8 were positive by PCR technique for *E. granulosus*, 4 for *E. multilocularis* and a single sample had mixed infection.

The main primers used in our PCR study for diagnosis of *E. granulosus* infection in dogs from Romania, were those described by Abbasi et al. (2003) and Stefanic et al. (2004). But these are only for the *E. granulosus* sheep strain, so this test is not relevant for all strains. Dinkel et al., 2004, elaborated the specific primers for 3 strains of *E. granulosus* (sheep strain - G1, bovine strain - G5 and a combined strain camel/pig - G6/7) and used them for identifying genotypes of the larval form. The diagnostic value of these primers for copro-DNA remains to be demonstrated. To date, there is a copro-PCR test able to identify all strains of *E. granulosus*, but the research is to conduct a pan-specific primer to be used in single or multiple PCR with primers specific for *E. multilocularis* and *Taenia* spp., in order to identify all strains (Mathis and Deplazes, 2006).

In Romania, studies on the genetic characterization of strains of *E. granulosus* were made in the western part of the country, on cysts from intermediate hosts, and two genotypes were detected: the sheep G1 type and the pig strain G7 genotype (Morarui, 2004).

For conclusive epidemiological results, we need to perform studies on a greater number of dogs, but the high frequency (37/48) of infection in the dogs from Cluj county, must be of concern about the real situation of this zoonosis.

As in Romania, the Xinjiang plateau of western China has been shown to have a high prevalence for human cystic echinococcosis (CE) caused by *Echinococcus granulosus*. The endemic dog is suspected to be the primary definitive host for the transmission of both *E. granulosus* to humans. Seventeen of 30 stray dogs from Hejing County of Xinjiang were found positive for *E. granulosus post mortem*. Furthermore, gene target DNA fragments were amplified for formal identification of the two parasite species, based on 12s rRNA mitochondrial gene. The PCR products were purified and sequenced. 100% identity being *E. granulosus* (sheep strain, G1 genotype) (Zhang et al., 2006).

Our results are the first molecular studies in intestinal echinococcosis of dogs in Romania. The PCR technique would be the best diagnostic tool, as we can identify the species and strains of *Echinococcus granulosus*, but the technique is limited for the moment to the financial resources of our country.

**Conclusions**

Following research for identification of *Echinococcus granulosus* DNA, sheep strain, by PCR, we obtained a high frequency of infection, from the 48 dogs examined 37 being positive.

**REZUMAT**

Gazdele definitive pentru *Echinococcus granulosus* sunt canidele, în special câinii. La canide, *E. granulosus* provoacă o infecție tipică teniozelor și se produc ouă (oncosfere) care trec prin fecale, în mediul exterior și sunt preluate de gazdele intermediare (erbivore, sau om). În gazda intermediară, ouăle eclozionatează, embrionul hexacant eliberat ajunge prin sânge în țesuturile gazdei și formează chistul hidatic. *Echinococcoza chistică* la om produsă de forma larvară a teniei *Echinococcus granulosus*, este caracterizată printr-o creștere lentă și formarea lezii chistice pline cu lichid (chist hidatic), localizeate în ficat, placăni și alte organe. **Material și metode:** Studiul a fost efectuat în perioada octombrie 2008 - februarie 2009. În scopul confirmării prezenței speciei *Echinococcus granulosus* la câinii, au fost selectate aleator 48 de exemplare din cele pozitive pentru testul ELISA. Câinii au provenit din zonele rurale din județul Cluj, România. Probele de fecale au fost colectate și examineate de prin copro-PCR. **Rezultate:** La testul PCR, utilizând primerii (P60.for) (P375.rev) și (Eg1.for.) (Eg1.rev.), am obținut un amplicon
de 255 pb, la 37/48 câini. **Concluzie:** Tehnica PCR a fost deja descrisă ca metodă de identificare a câinilor în infestații natural (Stefanic et al., 2004), dar până acum nici un astfel de studiu nu a fost realizat în România. Detectarea câinilor infestați cu *Echinococcus granulosus* este foarte importantă în țara noastră, în măsura în care programele de control trebuie să se bazeze în principal pe tratamentul gazdelor definitive, cu scopul întreruperi ciclului biologic al parazitului. Frecvența ridicată a infestației la câini studiați, ar trebui să fie un impuls pentru realizarea de anchete epidemiologice la o scară mai mare, și prin utilizarea de metode de diagnostic, cu sensibilitate și specificitate ridicate, cum ar fi tehnica PCR, dar utilizarea acestei tehnici este limitată pentru moment în România.

**References**


12. ZHANG Y., BART J.M., GIRAUDOUX P., CRAIG P., VUITTON D., WEN H., 2006. Morphological and molecular characteristics of *Echinococcus multilocularis* and *Echinococcus granulosus* mixed infection in a dog from Xinjiang, China, Veterinary Parasitology, 139, 1-3, 244-248.