MOLECULAR STUDIES ON THE ETIOLOGY AND EPIDEMIOLOGY OF ECHINOCOCCOSIS/ HYDATIDOSIS IN ANIMALS FROM SOUTHERN ROMANIA; PARASITOLOGICAL CONTROL PROGRAM

SUMMARY

Key words: *Echinococcus granulosus*; echinococcosis/hydatidosis; etiology; molecular biology; cattle; sheep; coproparasitological examination; coproELISA; parasitological control.

Echinococcosis is a major public health issue, especially in the regions with limited economic resources (Otero - Abad and Torgerson, 2013). In these areas there are signs of an increase in the number of cases of echinococcosis / hydatidosis (E/H), so this parasitosis is considered in progress of expansion (Grosso et al., 2012; Schweiger et al., 2007; Torgerson et al., 2006). E/H is usually maintained through the domestic life cycle (dog / domestic ungulates) and is a persistent zoonosis especially in the rural areas of farming, where people co-live with dogs that they feed with the viscera of parasitized animals (Torgerson and Budke, 2003).

The economic burden of E/H arising from affecting the livestock industry, globally has been estimated at over 2 billion $ per year (Budke et al., 2006). A similar value is also recorded consecutive cystic echinococosis (CE) in humans (hospitalization, medical rest, treatments carried out, etc.). Atkinson et al. (2013) states that worldwide 2 - 3 million people are affected by this zoonosis and about 200.000 new cases of E / H are diagnosed annually. Despite the substantial socioeconomic impact, the CE is still present in some areas with a high incidence (Craig et al., 2007). Recently, the World Health Organization included human echinococcosis in the group neglected zoonoses (WHO, 2010).

The studies included in this thesis were conducted in the 2009 - 2015 period and had as objectives the study of etiology and molecular epidemiology of echinococcosis / hydatidosis in animals in Southern Romania and integrating the results into a program of parasitological control of this important zoonoses.

The thesis is structured in two parts. The first part is entitled "Bibliographic study" and contains six chapters and the second part of "Personal research" consists of five

chapters. This thesis contains 50 figures (photo images, schematic representations and graphics) and 16 tables (Annex I). The bibliography that underlies this thesis includes a total of 314 titles.

The first part of the thesis is a summary of the literature consulted to deepen knowledge about echinococcosis / hydatidosis.

Chapter 1 provides an overview of E/H, including history, taxonomy, systematic classification and life cycle with the description of adult tapeworms, but also the larval stage of the parasite *Echinococcus granulosus*.

Chapter 2 illustrates the spread of echinococosis / hydatidosis and implicitly the genotypes of *Echinococcus granulosus* at global level and in our country.

Chapter 3 treats the aspects of pathogenesis, anatomopathological changes and clinical manifestations encountered in animals (GI and GD) with echinococcosis / hydatidosis.

Chapter 4 is a review of various methods of diagnosis of echinococcosis / hydatidosis in living animals and post- mortem, including differential diagnosis of E/H.

Chapter 5 presents data on the economic and social impact of this serious parasitosis. Are showed some data about the costs resulted from the losses determined by animal echinococcosis from various countries.

The sixth chapter consist of the description of certain aspects related to the combating of E /H, that were presented in the two subchapters: the treatment and prophylaxis of this parasitosis.

The second part of the thesis is meant to convey the own research results, followed by general conclusions and the bibliography.

The 7-th chapter of the thesis presents the epidemiology of hydatidosis in animals from the South of Romania.

In subchapter 7.1., is presented the evaluation of the incidence of hydatidosis in animals from the Southern of Romania evaluated by the parasitological and necropsy exams and also the recording of the hydatid cyst. The four species of animals examined

after slaughter were: pigs (n = 3780), cattle (n = 1878), adult sheep (n = 642), lambs (n = 589), and equine (n = 24). Those animals originated from ten counties in Romania and were slaughtered in four official slaughterhouses (Buzău, Dâmbovița, Teleorman and Vâlcea).

The prevalence of hydatidosis in studied animals varied depending on the species, being 65.6% in adult sheep, 40.1% in cattle, 25% in horses and pigs and lambs 0%. In ruminants, the predominant localization of hydatid cyst was pulmonary (65.1% of cattle and 63.8% for sheep), followed by liver (31.2% for cattle and 36% sheep), while in horses cysts prevailed in the liver (58.3%).

In subchapter 7.2. are summarized the results of morphological characterization of 2869 hydatid cysts collected and analyzed in the laboratory, with respect to the morphometry and the determination of viability.

Most of the analyzed hydatid cysts had middle sizes between 2 and 5 cm (966/1716 - 56.3% in cattle, 794/1141 - 69.6% for sheep and 9/12 - 75% in equine). The collected cysts were classified according to their appearance: normal (fertile or sterile) caseating or calcified.

The viability of hydatid cysts was evidenced by microscopic examination of the various components and observation of protoscoleces. The proportion of the viable cysts in sheep was very high (73%), while in cattle was very low (0.17%); in horses were not registered viable hydatid cyst. It can be concluded that sheep is the specie with the highest cysts fertility and thus confirms the essential role of this host in the transmission of echinococcosis.

The objective of the study described in the **8-th chapter** was to characterize and identify, the species / strains of *Echinococcus,*with the aid of the molecular biology methods (PCR) using genetic material obtained from hydatid cysts from ruminants slaughtered in a slaughterhouse. These ruminants were from households and specialized farms located in seven different counties of Romania.

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The research carried out to identify *Echinococcus* strains were made during two internships performed in the Laboratory of Molecular Biology from the Faculty of Veterinary Medicine in Naples, Italy.

A total of 128 hydatid cysts (39 sheep and 89 cattle) were collected for analysis. After performing the polymerase chain reaction (PCR), the primer pair used (JB3 and JB 4.5) amplified a region of about 450 bp of mitochondrial gene subunit 1 (CO1) for 114 of samples.

Of the 67 samples from cattle, 53 (79.1%) were 100% identical with the genotypes of *E. granulosus complex* s.s. namely: G1 (n = 29), G2 (n = 12) and G3 (n = 12) (GenBank: U50464, AY462129, M84662, M84663). Also different variants have been detected: three samples (4.5%) were identical to the G1c (GenBank CO1: AY686565.1), one (1.5%) with G2b (CO1 GenBank: AY686560 0.1) and two (3%) of G2c version (GenBank CO1: AY686563.1). Eight (11.9%) of the samples were identical to the bovine variant G2 which have a transition mutation of cytosine (C) to thymine (T) at position 257 (T257C variant G2).

Of the 31 samples from sheeps, 23 (74.2%) had 100% identity with the complex genotypes of *E. granulosus* s.s. G1 (n = 16) and G3 (n = 7) (GenBank: U50464, AY462129, AY686559, M84663). There have been identified also variants of G1: one sample (3.2%) was identical to the G1a (GenBank: AY686564.1) and six samples with G1c variant (GenBank: AY686565.1). A sample (3.2%) was identical to the G2a (GenBank: AY686561.1).

Chapter 9 comprises research conducted to assess the prevalence of intestinal parasitic infections in dogs from southern Romania, focusing on *Taenia* spp. / *Echinococcus* spp .. The methods used to obtain the results were the fecal examination and ELISA immunoassay technique.

For the coproparasitological examination, 787 dogs feces samples were collected from urban (n = 544) and rural (n = 109) dogs, but also from guard dogs in ruminants (n = 134). The overall prevalence of endoparasitosis infestations in dogs was 71.5%. Oncosfere of *Taenia* spp. were identified in the proportion of 1.1% (9/787) from the analyzed fecal samples. There were significant statistically differences (P = 0.005) between the three categories of dogs examined they were found eggs of *Taenia* spp. (dogs from rural areas - 3.7%; in urban areas 0.4% and the guard ruminant 2.2%).

A total of 132 fecal samples were processed and examined by an enzyme immunoassay for the detection of *E. granulosus* coproantigens. CoproAg ELISA Chek - Echinotest was positive for eleven fecal samples of the total analyzed, showing an overall prevalence of 8.33%.

Chapter 10 refers to a development of a parasitological control program of E / H in animals from Romania, correlated with the epidemiology and pathogenesis of identified genotypes.

The last chapter, **Chapter 11** summarizes the general conclusions drawn from the personal research.