

Experimental Echinococcosis (Hydatidosis) in lambs: diagnostic imaging

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Abstract. The study was conducted during October 2004 – May 2006 on 10 lambs – crossbreed – aged 4-6 months which were assigned into 2 groups: group E – infected with *E. granulosus* (n = 5); group M – not infected, control group (n = 5). Three weeks before the infection, the lambs undergone a full parasitological examination (fecal examination), being dewormed with 7.5 mg albendazole/bw, followed by two examinations post-infection, respectively at 66 and 140 days p.i. For the experimental infection of the group E, 5600/animal oncospheres were orally administered. To confirm the infection with hydatid cysts, three ultrasound/X-ray examinations, at 62, 104 and 300 days post infection (p.i.) were performed. Laparoscopic examination was carried out on three lambs in group E and two lambs in group M in the day 316 of the experiment.

Keywords: *Echinococcus*; Ultrasound; X-ray; Laparoscopy; Lambs.

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Introduction

Echinococcosis or Hydatidosis is a major zoonosis, a parasitic disease with major repercussions, both in case of infestations in humans and animals. Although much is known about the hydatid infestation, the disease is still spreading on large both among humans and animals. It is basically prevalent worldwide, there are few and restricted areas as disease-free regions. The hydatid cyst, the larval stage of *Echinococcus granulosus* and the causative agent of human hydatid echinococcosis was already known in ancient

Greece. Perhaps one of the first references was that of Hippocrates (379 BC) (Kirby et al., 2006). He wrote in the 55th aphorism (even he was not aware of the causative agent of the disease) "when the liver is filled with water and break into the caul, the patient's belly fills with water and it dies". The recognition of the animal origin for the hydatid cyst can be attributed to Redi (1624-1694), followed by Pallas, who considered it as a parasite generating vesicles. Goeze recognized the scolex and assigned into visceral and intestinal teniosis, while naming the parasite *Taenia visceralis socialis granulosa*. The name

Echinococcus was introduced by Rudolph in 1801. It seems that Von Siebold was experimentally infecting dogs with *Echinococcus veterinorum* (= *Taenia echinococcus*). He was followed in 1836 by Naunyn in Berlin and Krabbe, in Iceland, who independently have managed to obtain adult tapeworms in dogs infected with protoscolexes contained in material obtained from humans. Haubner infested in 1855 for the first time sheep (Gemmell, 2000).

E. granulosus has a cosmopolitan range, being found in all continents, in tropical, subtropical, temperate and polar biomes. The highest prevalence of the parasite has been found in parts of Eurasia, Africa, Australia and South America. Only a few countries can be identified as free of *E. granulosus*.

Under current opinions the infections with this parasite are found in equilibrium in most endemic areas, except Cyprus, Falkland Islands, New Zealand and regions 11 and 12 in Chile, where it is considered eradicated.

Due to its ability to multiply in the intermediate host, we can say that *E. granulosus* is infectious in nature. Formation and development of hydatid cysts in various organs cause several types of actions: mechanics, obstructive and toxic.

The purpose of this article is to establish the relevance of diagnostics surveillance and periodical radiological screening, to confirm the presence of hydatid cyst infestation in small ruminants using laparoscopic technique. To determine and track the evolution of parameters of the body and especially the status of the immune response to hydatid disease, the experimental reproduction of the disease has been performed. While following the evolution of the disease, the aim of the present experiment was to compare the effectiveness of various methods of diagnostic imaging in cystic echinococcosis.

Materials and methods

The research was conducted during October 2004 – May 2006 in the Department of Parasitology and Parasitic Diseases, Medical

Pathology Clinic and the Emergency Hospital of the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

The experiment was made on 10 lambs – crossbreed – aged 4-6 months which were assigned into 2 groups: group E – the batch infected with *E. granulosus* (n = 5); group M – not infected, control group (n = 5). Housing and feeding conditions were identical for both groups during the entire experiment, the animals were clinically examined on daily basis. Three weeks before the infection, the lambs had undergone a full parasitological examination (fecal examination), being treated for worms with Unialben (7.5 mg albendazole/bw), according to the experimental scheme.

The infection method

For reproducing experimentally the cystic echinococcosis in lambs, was chosen the natural way of infection (oral administration of *E. granulosus* oncospheres). This method was used because it is identical to the natural way of infection of the intermediate hosts.

Origin of pathogenic material

The infecting material came from dogs, which undergo necropsy in the University's Necropsic Pathology Department, corpses of four dogs (which came from the surroundings of slaughterhouses from Alba county) were investigated. For a more accurate verification, the small intestine was opened throughout its length and then examined with a magnifying glass, both its content and its mucosal surface. Scrapings of the mucosa were plated between two blades and then examined under a microscope with 10× objective. They emphasized tapeworms belonged to the family Taeniidae, *E. granulosus* species, both young and adult. Adult tapeworms were collected individually under microscope then preserved in mild saline solution in the laboratory. These were macerated, yielding a suspension of oncospheres which have been quantified (no. oncospheres/10 µl), forming the base of the infecting material. Feces were collected intrarectally from two dogs (with a higher degree of

infestation and higher percent of mature tapeworms) and using the method of successive decanting a concentrated solution of worms was prepared. To the material obtained from the last decantation, a saline suspension was added containing 1 mg/ml streptomycin and 2000 U.I. of penicillin. Also a quantification of the larvae was achieved, and the suspension was administered to 2 lambs from the infected group.

The experimental infection of the lambs from the group E was carried out by administering a suspension containing 5600 oncosphere / host – oral administration. For the lambs of group M a saline solution was administered in the same amount (to maintain identical conditions of stress). After infection, the animals were clinically examined (daily), also coprological examinations (66 and 140 days p.i.), blood sampling, laboratory and other diagnostic protocols were performed (according to the protocol established working).

To confirm infection with hydatid cysts in the sheeps experimentally infected, were performed three ultrasound/X-ray examinations, at 62, 104 and 300 days post infection (p.i.). At 316 days p.i., laparoscopic examination was conducted which intended to:

- highlight the formation of cysts in the abdominal cavity;
- collect biological samples for conducting histopathology sections;
- confirm the formal diagnosis based on ultrasound and X-ray examinations.

Laparoscopic examination

Besides the usual diagnostic imaging methods in cystic echinococcosis, a new method – the laparoscopy is emerging. The technique of endoscopic evaluation of serous membranes lining the large body cavities and the organs inside these cavities is known as laparoscopy. Laparoscopy is indicated when abdominal organs should be examined and biopsied, but laparotomy is not desired or required.

In our research an OLYMPUS laparoscopic line was used, with the specific handheld laparoscopy instruments. Laparoscopic

examination was carried out on three lambs in group E and two lambs in group M in the day 316 of the experiment. Lambs were undergoing a diet for 18 hours before the examination. For preparing the area a local trimming was performed on the dorsal part by trimming and shaving perigastric area.

Neuroleptanalgesia was used, premedication was performed with acepromazine (0.03-0.1 mg/kg), followed by administration of ketamine, within 10 min at a dose of 10-20 mg/kg. During the intervention the anesthesia was maintained by administering subdoses of ketamine (Mateş, 1997). In addition a local disinfection was conducted using ethyl alcohol and Betadine applied to the operating field. It was followed by the localization of the Veress's needle insertion place and one 0.5 cm incision was made.

By blowing gas, a moderate pneumoperitoneum was produced, and then introduced the trocar. Using the endoscope a general examination of the organs was made, insisting on the liver. Under endoscopic control were introduced the other probes.

After examination of the liver and highlighting pathologic formations, a biopsy was resorted from such areas. Biopsy technique used was to capture a portion of the organs with forceps and an electrocautery probe was inserted through the other probe, to detach the desired portions of the liver, achieving concomitant hemostasis. Finally, after extracting the instruments, we sutured the incisions using surgical silk. A preventive postoperative antibiotic was administered by parenteral route.

Results

Results of the laparoscopic examination

The examination was conducted in day 316 p.i., using laparoscopic technique in the 3 infested lambs belonging to group E and in two lambs from group M, highlighting in the liver the parenchyma formations of cystic type, measuring up to 2.5 cm in lambs from group E.

No cystic formations were observed in lambs in group M comparable in size or form to those observed in lambs in group E. In the latter group we observed hemorrhagic lesions, suggesting a possible outbreak of recent parasitic re-infestation. In the histological preparations (color H.E and Tricom-Masson) prepared from the material collected by biopsy, granulomatous inflammations were present in the center showing a necrotic area bounded by epithelioid cells and giant cells that had more nuclei concentrating at one of the poles of the cells. Around them we observed infiltrated lymphocytes and eosinophils. It is known that such proliferative inflammation are caused mainly by infectious agents that act locally (*Mycobacterium*, *Brucella*, fungi, parasites) and *E. granulosus* is one of them. Larval form of the parasite that develop in internal organs of intermediate hosts, are covered in early ontogeny by a granuloma, with epithelioid and giant cells present. But these may be present even in cysts that have formed in other cavities.

Discussion

This was the first case of using laparoscopic method for diagnosing hydatid cysts in sheep in our country. However, this revolutionary new method has already used in the surgical treatment of hydatid cyst in humans. Thus, for the first time echinococectomy was performed using video-guided intervention (laparoscopy) on liver in humans, mentioned by Khoury et al. (1996, 1998) from Beirut.

Diagnostic thoracoscopy was performed in 8 human patients with complicated hydatid cyst (CHP) erupted in the pleural cavity. It is to be noted that in the case of complicated hydatid cyst with overflow in the pleural cavity thoracoscopy was used both for diagnostic and curative purposes. Thoracoscopic diagnosis could confirm the diagnosis of CHP evoked by clinical-radiologic test. Especially through this guidance the processes and pleural cavity drainage technique is specified as thoracoscopy or curative surgery. Laboratory investigations following classic methods are routinely mentioned as important for patients ready for surgery.

Celioscopic surgical technology development has broadened the spectrum of surgical methods in a minimally invasive manner. Thus, thoracoscopic treatment of lung hydatid cysts using video-assisted methods has become an alternative to traditional surgery treatment. Although the techniques used in this method correspond to classic technical processes, the postoperative evolution makes the last to be elective surgery, especially for uncomplicated hydatid cyst, located solitary and in peripheral locations. Surgery offers the classic lung hydatid cyst treatment, but with important costs of visceral and parietal tissues sacrificed. It is thought that this is the minimally invasive approach of lung hydatid cyst treatment and is a feasible and easy solution. Provided the use of modern equipment, continuous professional training, the current information synthesis constitutes basic elements in promoting the method, especially for countries where echinococcosis has a great impact both at medical and socio-economic levels.

Video assisted thoracoscopy interventions were performed on 64 (38.32%) humans, and video-assisted thoracoscopy associated with the mini-toracotomy was used in 92 (55.1%) human surgical operations. Surgery is the mainstay of treatment in hepatic hydatid disease (Sangani et al., 2014). Thus for 78 (35.6%) of CHP, with size up to 4-5 cm the favorite performance was followed by perichistomy and partial toracoectomy with the hydatid cavity left open to drain into the pleural cavity, forming the symphysis in parietal pleura. For 81 cysts (46.1%) post-hydatid dissolution cavity was performed by headlining (Balaban, 2006).

This method was used successfully in our country by Furcea et al. (2007), who has treated 33 patients with hepatic hydatid cyst between 1996-2005. The mean period of hospitalization after laparoscopic surgery being only 5.6 days. The available treatment options for *E. granulosus* infection include open surgery, percutaneous interventions, and pharmacotherapy (Mihmanli et al., 2016).

Studies targeting the current imaging methods evaluating the diagnosis of the evolution of hydatid cysts in lambs suggest that only a

radiological examination fails to detect early infestations with hydatid cyst, but is recommended to be conducted as always orients, and sometimes even further certify the diagnosis. Ultrasound examination is a sensitive method, very useful in the diagnosis of hydatid cysts, especially in the diagnosis of localized liver cysts, but should not be neglected neither in pulmonary locations.

Laparoscopic technique offers some of the most reliable data in the diagnosis of cystic formations identified by the methods mentioned above, but its use important not only in the diagnostic phase, although it is more commonly and widely used in the therapy of human hydatid echinococcosis.

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