Original Article

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IMPROVED DETERMINATION OF MACROSCOPIC PARASITE PREPARATIONS USING S10 MODIFIED PLASTINATION PROCEDURE

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ABSTRACT

Macroscopic preparations of parasites fixed in formaldehyde or alcohol don't fulfill in complete the requests for education, as well as their determination, mainly because of the toxic fumes and not enough visible structure of fixed parasite. Using the modified C10 plastination method, parasites from three different *phylum* were prepared: *Plathelminthes: Class Cestoda (Dipilidum caninum, Moniezia spp and larvae from T.Echinococcus granulosus - Echinococcus unilocularis, larvae from T. pisiformis - Cysticercus pisiformis, , larvae from T. hidatigena - Cysticercus tenuicollis), Phylum Nemathelminthes, Class Nematoda, (Ascaris suum, Macracanthorhynchus hirudinaceus, Dirofilaria immitis), Phylum Arthropoda, Class Arachnida (tick from the Ixodidae family) and Class Insecta (Gasterophilus intestinalis, Hypoderma bovis)*. The aim of this study was conserving the parasites in native condition with plastination method and improved determination according to their visible morphologic structure. Parasites were previously kept in 10% formaldehyde. Prepared parasites were dry, chemical free, not toxic and safe for the environment, flexible and with detained form and structure. There was a variation in the natural colors in some of the parasites, as a result from long-time formalin fixation. Preparations made with this method are permanent educative material which enables improved study of parasite's structure.

Key words: *Plastination; Parasites; Macroscopic preparation ; Determination*

INTRODUCTION

Parasitic diseases in animals are usually acquired through contaminated food and water, unhygienic conditions, crowded farms, inadequate dehelminthisation and food imbalance. (1, 2,) Most parasitic diseases in animals are caused by parasites from the genus of protozoa, nematoda, cestoda and artropoda (3) The parasites, isolated from invaded animals, in order to keep their natural color and form, are often kept in 10% formalin or 70% alcohol. Working with formalin fixed preparations are very unpleasant, due to the toxic steam which can harm the respiratory system, eyes, and they also require special room for storage. (5) From the other side, lately, plastination has been promoted as a method for permanent conservation of samples, which are not toxic. In the same time, this method allows keeping samples in original form, size and improved cellular level view.

In this study, using the standard silicone procedure - C10, a permanent conservation of some parasites previously fixated in formalin for 10 years, was made.

MATERIAL AND METHODS

A parasites from three different *Phylum* ***Plathelminthes****: Class* ***Cestoda*** *(Dipilidum caninum, Moniezia spp and larvae from T.Echinococcus granulosus -* ***Echinococcus unilocularis****, larvae from T. pisiformis -* ***Cysticercus pisiformis****, , larvae from T. hidatigena -* ***Cysticercus tenuicollis****),* ***P****hylum* ***Nemathelminthes****, Class)* ***Nematoda,*** *(Ascaris suum, Macracanthorhynchus hirudinaceus, Dirofilaria immitis), Phylum* ***Arthropoda****,* ***Class Arachnida*** *(tick from the Ixodidae family)* ***and C****lass* ***Insecta*** *(Gasterophilus intestinalis, Hypoderma bovis)* have been plastinated.

Echinococcosis cysts were fresh, while the other parasites were 10 years old, stored in 10% formalin. First, E. cysts were wash with running water in order to remove the blood The fluid from the cysts was removed and the cyst were filled with 3% formalin till maximum stretch of the walls was accomplished. Later, cysts were separated from the hepatic tissue and fixated in 3% formalin for the period of 5-7 days. (7). After the process of fixation, an openings were made on the surface of the cysts, in order to make the internal structures of the cysts visible. Before dehydration, to remove as much as possible formalin from the prepared samples, they were washed 24 hours in cooled water (+5 Cº). (6). For the process of dehydration, samples were placed in 100% acetone on - 25Cº in ratio 10:1. The parasites were dehydrated in 3 acetone baths, from which the first two lasted 10 days and the third 7 days. Acetone concentrations were measured by acetone-meter (8), to estimate the level of dehydration. (Table 1)

After the dehydration of the parasites, they were put in silicone mixture S10/S3/hylen in ratio 100:1:3. Vacuum was constantly controlled by digital reader. The impregnation was finished after 20 days on temperature - 25°C. Impregnation was finished when the production of bubbles stops and the vacuum stabilized on approximately 8mmHg. The whole proces lasted 20 days on - 25°C.

The fixation was made by standard gas conservation procedure. Before the procedure, the parasites were removed from the silicone and left on room temperature for 12 hours, so that the excess of silicone gets out of the surface. Every parasite was placed in appropriate position, with filter paper between. To gain the wanted form, Echinococcus cysts were filled with piece of material. The excess of silicone was removed in certain periods. The parasites were completely fixated after 24 h.

Picture 1. *Preparation of the samples for gas conservation*

Picture 2. *Gas conservation chamber*

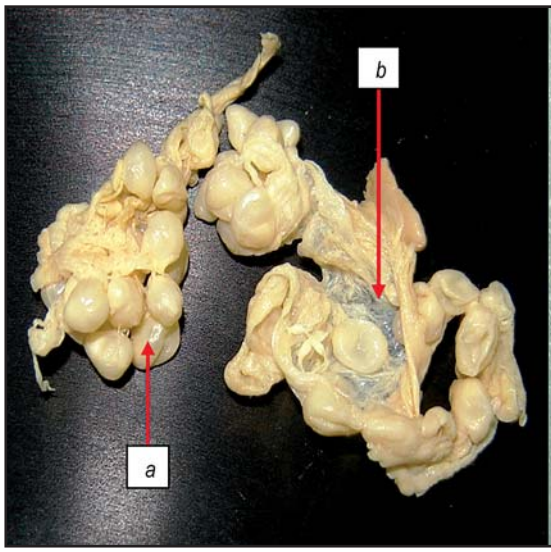
RESULTS

After plastination the parasites were dry, chemical free, completely harmless and not toxic for the environment with clearly detectable structure. The parasites were not flexible, but they kept their natural form.

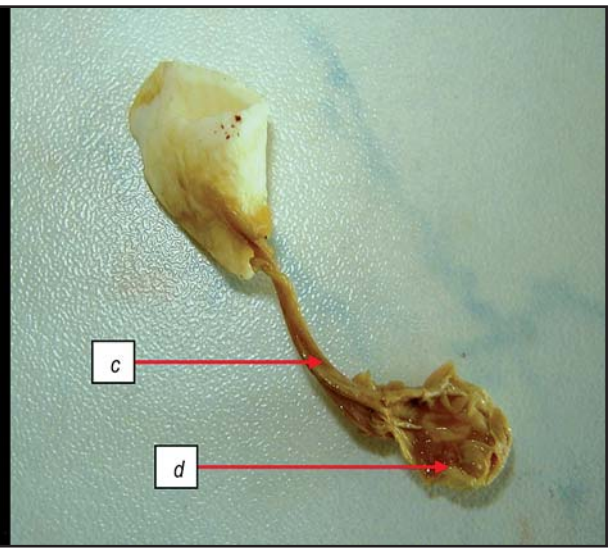
Table 1: *Level of dehydration*

Bath	percent of dehydration
I acetone bath	98%
II acetone bath	98.7%
II acetone bath	99.3%

Picture 3. *Plastinated parasites samples*

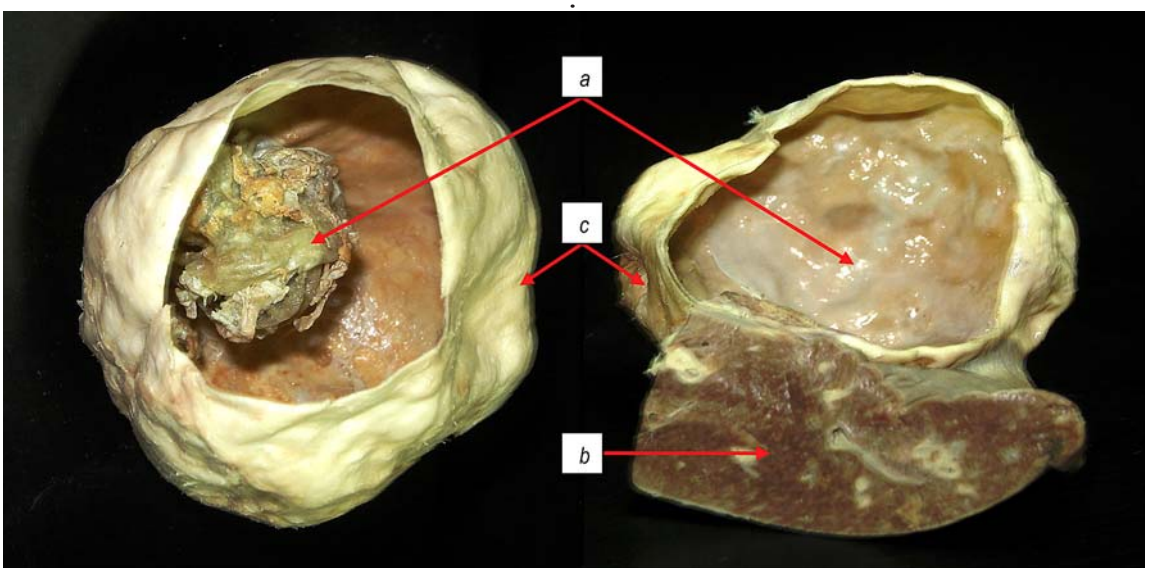


Picture 4. *Cysticercus pisiformis*



Picture 5. *Cysticercus tenuicollis*

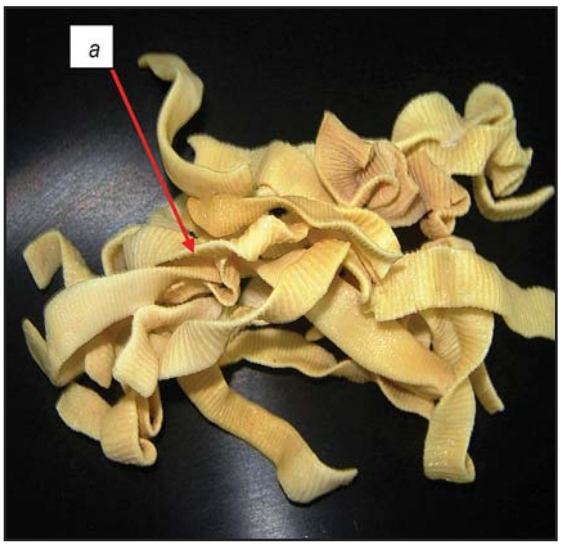
Cysticercus pisiformis – larval stage from T. pisiformis, together with the mesentery of rabbit (b), with completely conserved grape form ***(a)*** where the cysts of a pea size, filled with fluid, can be noticed (cistricercus) ***(a).*** On the picture 5, Cysticercus tenuicollis originated from mesentery of sheep is presented, where the handle (colum) can be seen ***(c)*** and the single hanging cyst enclosed in a membrane (cisticercus) ***(d).***



Picture 6. *Echinococcus unilocularis- with calcified*

Picture 7. *Echinococcus unilocularis- with calcified*

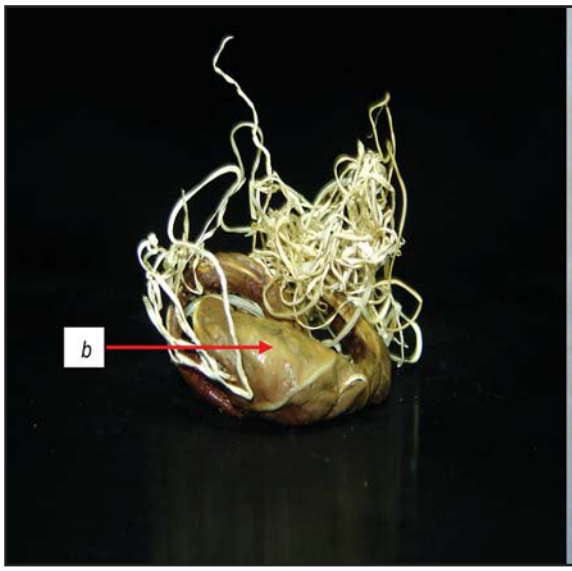
Two larva samples from T. Echinococcus granulosus - Echinococcus unilocularis with the main cyst body, that have kept the round form and white to pail yellow color can be seen on the pictures 6 and 7. The host-derived fibrovascular capsule (adventitial layer) (c) and the inner, nucleated germinal layer (a) of Echinococcus unilocularis is evident on the pictures 6 and 7. On the picture 6, there is a calcification of the germinal layer, because the cyst was old, under which acellular laminated layer can be distinguished. Cyst on the picture 7 has been plastinated together with the hepatic tissue from sheep. (b).



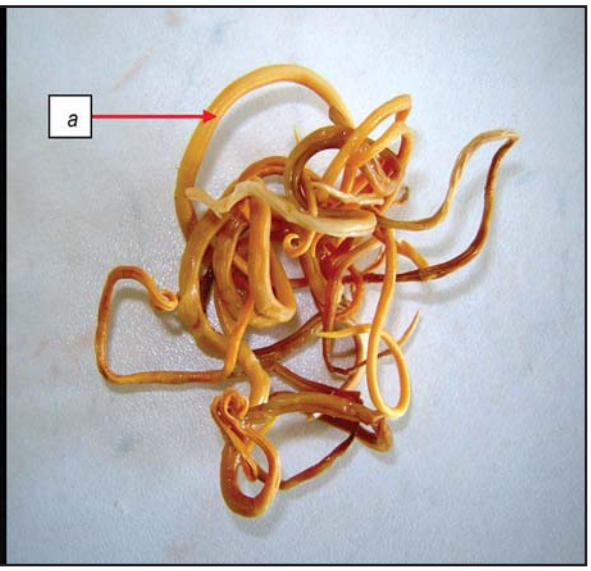
Picture 8. *Moniezia spp.*

Picture 9. *Dipylidium caninum*

Parasites from the class Cestoda (Moniezia spp. and Dipilidum caninum), have kept the natural white to yellow color, structure and segmentation of the strobila. Mature proglotides of Monieza spp., that are much wider than long, are easuly distingushable on the preparation ***(a).*** On the picture 9, proglottids from D. caninum of all degrees of maturity can be noticed. The gravid proglottids show a cucumber seed shape. Both parasites, due to their length, became not flexible and easily breakable.

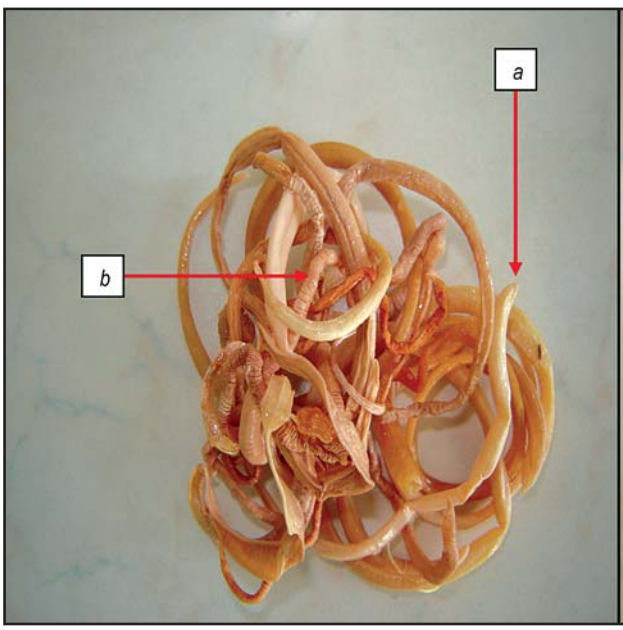


Picture 10. *Dirofilaria immitis*

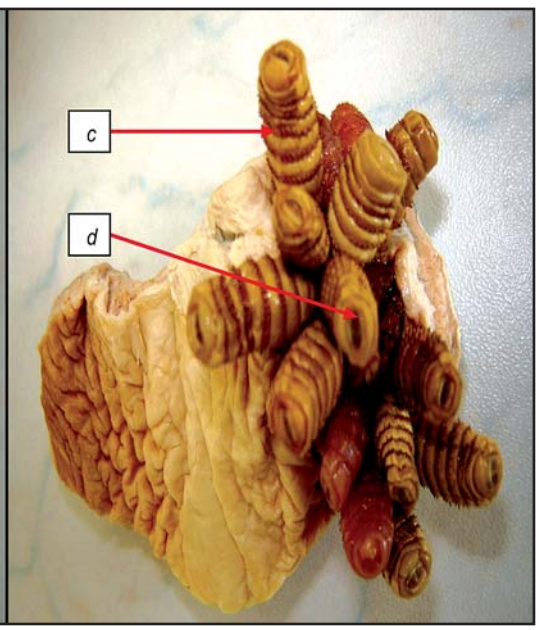


Picture 11. *Ascaris suum*

The nematodes **Ascaris suum and Dirofilaria immitis** have kept the original morphology. Their oval form is distinguishable ***(a). Ascaris suum*** thick cuticle**,** mouth in the anterior end and posterior end, which is curved ventrally in males, are clearly distinguishable. **Dirofilaria immitis** was plastinated with part of the hart muscle and arteria pulmonalis ***(b)*** where they make the obstruction. It's white, thread-like, with spirally coiled tail in mails. The preparations gained lighter color, as a result of long-time fixation with formalin.



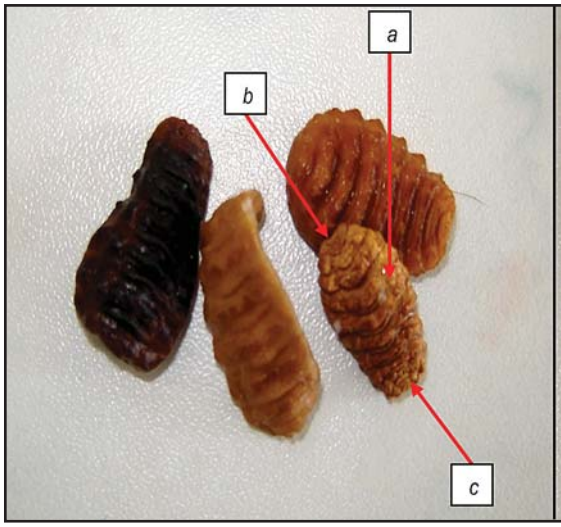
Picture 12. *Macracanthorhynchus hirudinaceus*



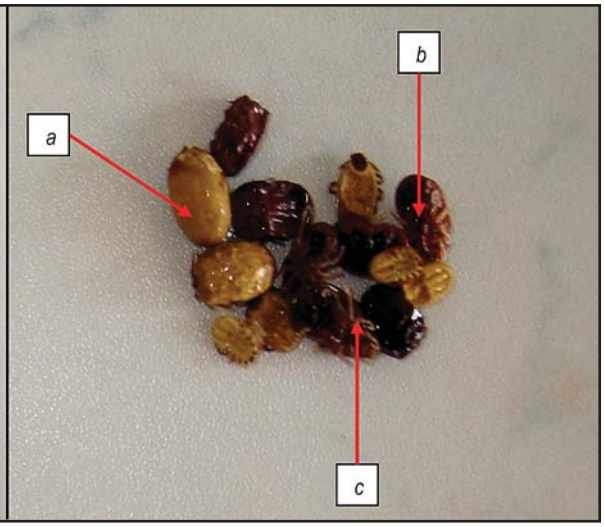
Picture 13. *Gasterophilus intestinalis larvae*

The picture 12 reveals the **Macracanthorhynchus hirudinaceus** preparation, where the anterior retractile armed proboscis with hooks can be seen ***(a).*** Also, the oval body form with typical folded and creased cuticle is showing some pseudosegmentation ***(b.*** This sample suffered shrinking due to its oldness and dehydration.

From the class of **Insects - Gasterophilus intestinalis,** larvae were plastinated together with non glandular part of a gastric tissue. During the procedures of fixation, dehydration and silicone impregnation, the parasites were unhooked from the mucosa. By special glue, during the gas conservation, Gasterophilus larvas were attached to the stomach mucose on the same place where the lesions were. Gasterophilus intestinalis larvae are stout, with bends of large spines on all segments, except the last one, which are important for identification of species. There are a mouth-hooks dorsally not uniformally curved and two rows of coarse spines per segment that are blunted at their tips. ***(c).***Clearly visible are also the spiracle of the last segment (d). These parasites have kept their natural color, the older are brown, while the younger ones are reddish.



Picture 14. *Hypoderma bovis - larvae*



Picture 15. *Ticks from ixodes spp.*

The plastinated sample from **Hypoderma bovis,** clearly shows laterally segmented body with 12 segments ***(a)*** two frontal hooks ***(b)*** and stigma on the last segment ***(c).*** This parasite has kept the natural yellow-orange color.

Class **Acarinae** (*tiks from Ixodidae family*) have kept the form, color and structure. The dorsal structure of the body can be seen (idiosoma) with the chitin layer (scutum) ***(a).*** On the ventral side, the mouth (gnathosoma), anus, anal and genital grooves can be seen ***(b.)*** Four pair of legs, with their six segments are also visible.***(c)***

DISCUSSION

Plastinated parasitological samples have huge advantage over those persevered in alcohol or formaldehyde, because they are characterized as being less permanent, having regularly needs of changing the immersion, the unpleasant smell and having hardly recognizable parts of the parasites. Students can't manipulate with the fixated samples. Plastinated educative samples are: always available, palpable, with clearly visible structure, can be observed from every perspective, have grand permanence, and can be storage on room-temperature. M.H.Asadi ет al. with C10 procedure have plastinated human Ascaris Lumbricoides (6). In our study we plastinated Ascaris suum in pigs that are from the same gender. Beside this nematode we plastinated parasites from three Phila, that were 10 years old. The new approach of S10 techique was modifying this method adding hylen in the process of fixation. It results in better flexibility, especially important in long parasites .

Plastinated parasitological samples are excellent educative material, which should be studied in future.

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