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A NEW METHOD FOR SELECTIVE DIAGNOSTIC STAINING OF HOOKS OF ECHINOCOCCI, CYSTICERCI AND TAPEWORMS IN HISTOLOGICAL SECTIONS

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Abstract. This paper describes a new method for selective staining of hooks of echinococci, cysticerci and tapeworms in histological sections. The method is based on pre-staining in hematoxylin, then the preparations are stained overnight in dilute polychrome blue and differentiated with tartrazin in Cellosolve (Ethylene glycol monoethyl ether or Ethylene glycol monomethyl ether). The hooks stain blue, nuclei brown to brown-green, and cell plasma is yellow. The hooks stain very brightly, which enables the identification of parasite remnants in granulomas and scars. The intensity of staining depends on the grade of maturity and sclerotization of hooks and these are readily observed, even in lower magnifications. The staining was successfully used in a variety of parasites.

Having studied histopathology of echinococcosis in humans and animals (Štěrba and Šlais 1972, 1974, Prokopič and Štěrba 1976, Prokopič et al. 1983, Štěrba 1986), tissue reactions on some species of cysticerci (Šlais 1960, Štěrba and Šlais 1972, 1974, Štěrba 1978, 1986) and the pathology of some other parasitoses (Štěrba and Baruš 1976, Štěrba et al. 1976, Prokopič and Štěrba 1976, Štěrba 1978, 1986) we faced the problem of histological verification of hooks both in larval and adult stages. The verification of hooks in histological sections as well as the determination of their shape, number and size are decisive for final diagnosis, particularly when the parasites are dead or regressively changed.

Because the verification of hooks with the hitherto used staining methods was difficult, we have tried to prepare a staining method which eliminates these difficulties. This paper contains results of the new method for selective staining of hooks. The advantages of this method and its usefulness both in human and veterinary pathology and parasitology are also discussed.

MATERIALS AND METHODS

The staining and verification of hooks were tested in two cases of generalized cysticercosis of man, caused by *Cysticercus cellulosae* (a total of 46 cysticerci). Sixteen cases of dystrophic and partly calcified human cysts of echinococci and material from four human specimens containing fertile cysts of echinococci were also studied. In addition, another group of live or regressively changed cysticerci with hooks, both from sporadic findings in humans and predominantly veterinary material, was examined. The new method has been compared with conventional methods (Štěrba 1978, Štěrba and Šlais 1972, 1974), used for study of hooks of *Strobilocercus fasciolaris* (*Taenia/Hydatigera taeniaeformis* (Botsch, 1786) — larvae) from common veterinary necropsied and unique material (Štěrba and Baruš 1976 and Štěrba et al. 1977). This method was also used for the study of unique strobilocercosis of pheasants. The material was provided by Academician Ryšavý.

The staining was tried out on adult tapeworms of the following species:

Davainea proglotina (Davaine, 1860) — the poultry tapeworm — hooks on suckers of scolex and rostellum. *Raillietina tetragona* (Molin, 1858) — the chicken tapeworm — hooks on suckers and rostellum. *Raillietina echinobothrida* (Megnin, 1881) Fruhman, 1924 — hooks on rostellum and suckers. *Raillietina cesticillus* (Molin, 1858) Joveux, 1923 — hooks only on rostellum. *Dicranotaenia collaris* (Batch, 1786) — scolex with 10 hooks. *Drepanidotaenia lanceolate* Bloch, 1782 — scolex with 8 hooks.

Hymenolepis anatina (Krabbel, 1869) — rostellum armed with a single circle of 10 hooks. *Hymenolepis setigera* (Froelich, 1789) — thin rostellum with 10 hooks. *Hymenolepis tenuirostris* (Rudolphi, 1819) — thin rostellum bearing 10 hooks. *Choanotaenia infundibulum* Bloch, 1779 — rostellum with a single row of hooks.

Examined parasites having hooks both in cysticercus and tapeworm are the following:

Cysticercus tenuicollis (*Taenia hydatigena* Pallas, 1766 — larvae), *Cysticercus pisiformis*/*Taenia pisiformis* (Bloch, 1780) — larvae.

Tissue excisions with cysticerci and control material of adult tapeworms with hooks were fixed in 10% formol and processed with standard paraffin method.

New staining method — Hematoxylin — Polychrome Blue — Tartrazin (HPBT):

Fixation and sections: Formalin and other fixatives, paraffin sections.

Technique:

1. Remove wax with xylene and take sections to water.
2. Stain nuclei with Weigert's or other iron hematoxylin, differentiate in acid alcohol and blue in tap water.
3. Rinse in distilled water.
4. Stain in dilute solution of Unna's polychrome methylene blue (subst. Merck) add 0.5—1 ml 1.5% stock solution to 100 ml distilled water/overnight.
5. Rinse in distilled water.
6. Rinse briefly in 95% alcohol.
7. Rinse in Cellosolve for a few seconds, until no more clouds of dye leave section.
8. Differentiate and counterstain with saturated solution of tartrazin (Lachema) in Cellosolve until the section is yellowish-green or yellowish-brown.
9. Rinse in Cellosolve.
10. Clear 2 changes xylene and mount in synthetic resin medium or Canada balsam.

RESULTS AND DISCUSSION

With the new staining method, the hooks stain blue, nuclei brown to grown-green, and cell plasma is yellow. The hooks stain wery brightly, the intensity of staining depending on grade of their maturity and sclerotization. They stain blue on yellow background and are readily observed even at lower magnifications. They are very well differentiated from surrounding tissues, and stained with great contrast that enables the easy identification of parasite remnants in granulomas and scars.

Figs. 1—3 show a comparison of routine staining of histological sections stained with hematoxylin-eosin (H—E), wet method after Giemsa and the new Hematoxylin-Polychrome Blue Tartrazin (HPBT) stain.

Pl. I, Figs. 1—3 show parallel sections of granuloma of regressively changed *Echinococcus granulosus* in the liver of man. Pl. I, Fig. 1 shows a great number of hooks quite readily visible even by H—E staining, especially when the microscope illumination is reduced. Pl. I, Fig. 2 shows Giemsa stained hooks. When strongly stained calcareous corpuscles are differentiated away, the hook staining is less visible. Pl. I, Fig. 3 demonstrates strongly counterstained hooks, stained with the HPBT method.

Pl. I, Figs. 4—6 shows parallel sections of hooks in *Cysticercus cellulosae* from human brain. It is characteristic that the verification of hooks of cysticerci is very difficult (Šlais 1970, Štěrba 1978) and it is possible to pick them up only sporadically in complete series of histological sections. In the examples in Pl. I, Figs. 4—6: in only 3 sections, from several hundreds of sections examined, was one hook picked up in a connective tissue sheath of necrotic and calcified cysticercus. By H—E staining, the verification of such a hook (Pl. I, Fig. 4) would take tens of hours of careful microscopy. With the new staining method, it is possible to identify the intensely stained hook at lower magnification during routine examination of preparations.

The same difference in stainability of hooks is emphasized in parallel sections of *Cysticercus crassiceps* (Pl. II, Fig. 1 — H—E, Fig. 2 — HPBT). Another hook is

shown in Pl. II, Fig. 3; the example is of *Cysticercus crassiceps*. It shows that HPBT staining method enables the verification of more detailed morphological structures of hooks of cysticerci even in adult tapeworms. This is also shown in the section of the poultry tapeworm Davainea proglotina (Pl. II, Fig. 4). The new HPBT staining does not enable only detection of hooks. Because the method permits visualization of undistorted parasite structures, the same preparation can be used for both morphometric and differential diagnostic studies.

The verification and staining of hooks, particularly in cestodes, were studied especially by Šlais (1966); later the range of methods of examination was extended to other staining. It was found that the histological and histochemical verifications of hooks of echinococci and adult tapeworms are in fact more or less the same as that of cestodes. Examining the hooks by phase contrast microscopy and with various histological methods, its medulla resembles a cavity, appearing sometimes as if it also contained a fibrous structure stainable with eosin. Its delimination from the cortex layer is uneven and seems to strongly articulate with the inner surface of the cortex layer. With PAS and Best's carmine, either its delamination line or the entire medullar part become pale coloured. The cortex layer of the blade and a major portion of the base are refractory to staining with a number of histological methods and have their own greenish colour (Šlais 1966). However, the cortex layer and the base of hooks stain yellow with picric acid by van Gieson's method, yellowish-green with Hale's method and feebly red by Goldner. Of importance is also the fact that the hooks always stain various shades of brown with methods for the reduction of silver nitrate (e.g., methods after Kossa, Masson, Gomori). Through results of these stainings are explicit, they do not have any significance for the practical and prompt verification of hooks. The same results are obtained in histochemical reactions carried out on hooks, known from Šlais' descriptions (1966).

The hooks of echinococci, cysticerci and tapeworms belong to those sclerotized formations that are conserved for the longest time after the death of parasites. Mostly they are not resorbed by host reaction. Their finding, and the possibility of subsequent morphometry, contribute to the identification of the parasite. In addition to other histological methods (Štěrba and Šlais 1972, 1974, Prokopič et al. 1983), the result of which is not very decisive or rather informative, the regressive staining after Giemsa is used (Šlais 1960, Štěrba and Šlais 1972) to verify the hooks in histological sections. The result of this staining depends on experience. Not only does differentiation depend upon adequate staining, but also the search for stained hooks in intensively stained surrounding tissue is difficult. Verification by methods available until now is difficult, laborious and not entirely reliable. In addition a great deal of differential diagnostic experience and patience are required. For the above reasons we have tried to develop a staining method in which the hooks have a clearly different colour from the surrounding tissue. The result of our newly developed method is presented in this paper.

НОВЫЙ МЕТОД ИЗБИРАТЕЛЬНОГО ДИАГНОСТИЧЕСКОГО ОКРАШИВАНИЯ КРЮЧКОВ ЭХИНОКОККОВ, ЦИСТИЦЕРКОВ И ЦЕСТОДОВ В ГИСТОЛОГИЧЕСКИХ СРЕЗАХ

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Резюме. В работе описан новый метод избирательного окрашивания крючков эхинококков, цистицерков и цестодов в гистологических срезах. Принцип метода состоит в предварительном окрашивании гематоксилином, затем препараты оставляют на ночь в разведенной полихромной синьи и дифференцируют тартразином в целлосольве (этиленгликол-моноэтилэтер или этиленгликол-монометилэтер). Крючки окрашиваются в синий цвет, ядра

в коричневый-коричневозеленный цвет, цитоплазма клеток желтая. Крючки окрашиваются очень интенсивно, что дает возможность диагностицировать и остатки паразитов в гранулемах и рубцах. Интенсивность окраски зависит от степени созревания и склеротизации крючков и последние хорошо заметны и при небольших увеличениях. Метод окрашивания проверен на ряде паразитов.

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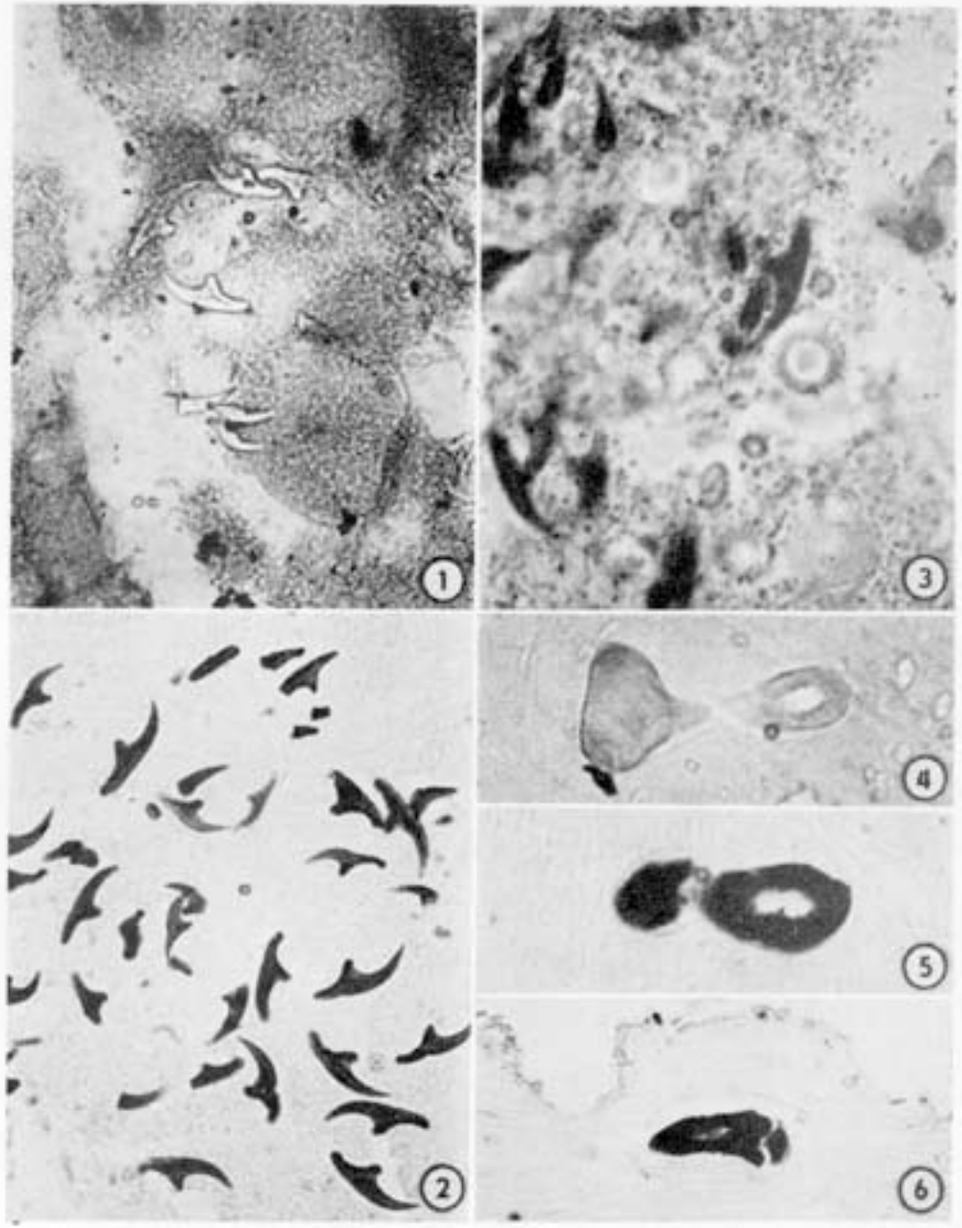
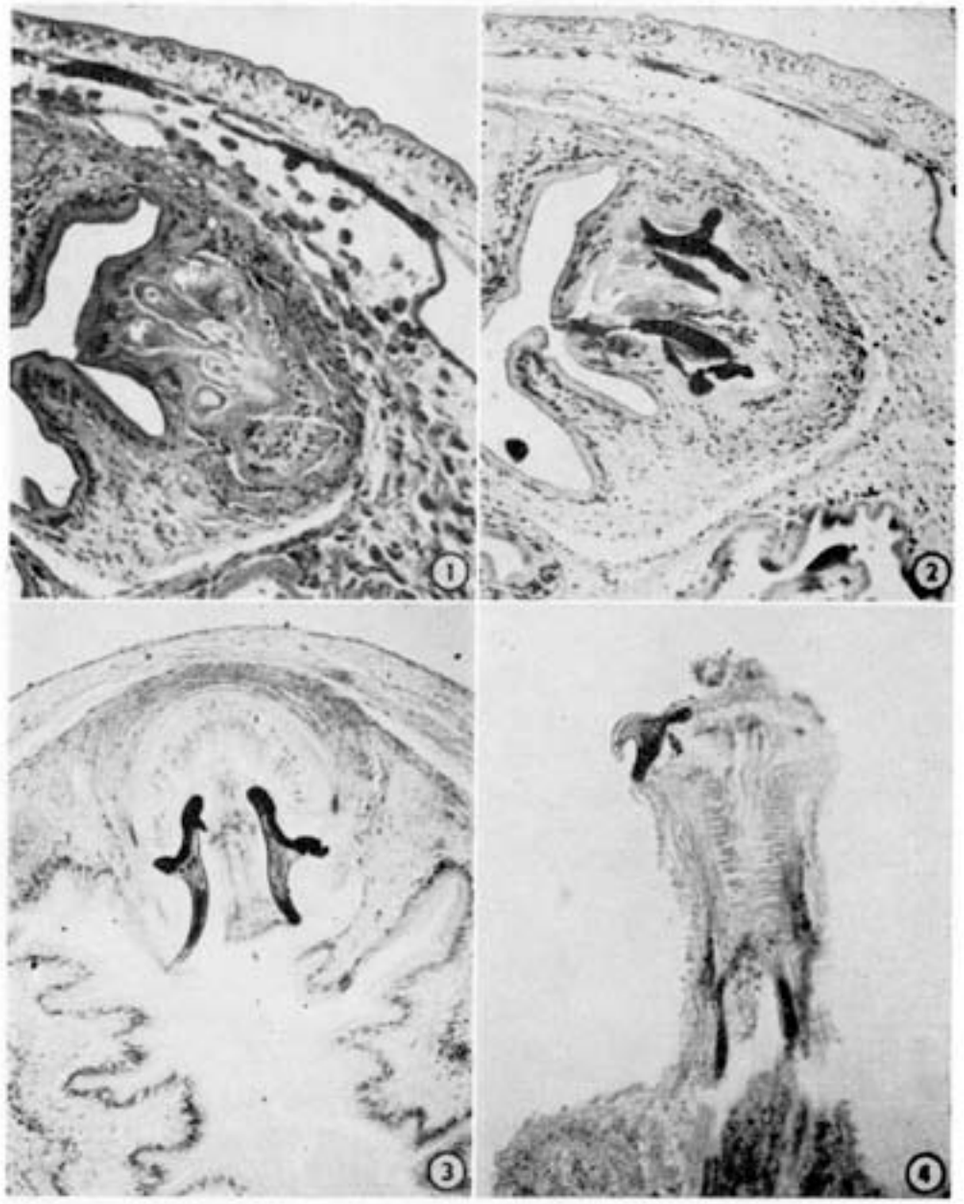


Fig. 1—3. Parallel sections of the parasite *Echinococcus granulosus* in the liver of man stained: **Fig. 1.** H—E, × 100. **Fig. 2.** Giemsa, × 120. **Fig. 3.** HPBT. **Figs. 4—6.** Section of hooks in *Cysticercus cellulosae* in the connective tissue sheath of dead cysticercus from human brain. **Fig. 4.** H—E × 400; **Fig. 5.** HPBT, × 400; **Fig. 6.** HPBT, × 350.



Figs. 1, 2. Parallel sections of parenchymal part of *Cysticercus crassiceps* with hooks. **Fig. 1.** H—E, × 175; **Fig. 2.** HPBT × 175. **Fig. 3.** *Cysticercus crassiceps*, HPBT, × 175. **Fig. 4.** Adult tapeworm *Dovinea proglotina*, HPBT, × 250.