EPIDEMIOLOGICAL STUDY

Enterobius gregorii — reality or fiction?

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Abstract

Authors present findings based on the actual epidemiologic situation of incidence of E. vermicularis (Linnaeus, 1758) which is till now the uniquely diagnosed parasite of Enterobius species in human population in Slovak Republic. They compared methods used in our laboratories for detection of propagation stages of E. vermicularis ova with methods used in other laboratories, which detect various propagation stages directly in stool. The authors found differences in detection and identification of adult female and male parasites. The relevance of sings for Enterobius gregorii (Hugot, 1983) diagnosis was assessed from the point of view of ontogenetic and morphologic development of E. vermicularis. Authors describe the isolation of a gravid female of Enterobius species from the patient, which showed some somato-morphological signs different from that found in E. vermicularis females. Possible explanations of such deviations are being discussed. The study is illustrated by pictures showing morphological differences in females. ***(Fig. 3, Ref. 28.)***

Key words: Enterobius vermicularis, Enterobius gregorii, enterobiosis.

Statement of (9) that *Enterobius vermicularis* (Linnaeus, 1758) has been invading the human population for more than 10 000 years seems to be beyond all doubts. This long-lasting existence of infection may by connected with its rigid and hardly affectable transformation in human organism, which is its only host. This phenomenon has been proved by results of prestigious parasitologic laboratories in 20th century, and WHO accepted the attributes of enterobiasis as sufficiently known. Parasitosis caused by *Enterobius vermicularis* was classified as the mostly spread infection from the global point of view.

Appearing of a new species always surprises and this fact is followed by many questions that can not be answered without certain practical and theoretical knowledge. The aim of our study is not to disprove or refuse the hitherto findings, but to turn attention to facts requiring a scientific explanation.

Material and methods

Our laboratory has been engaged in investigation of origin of enterobiasis diagnosed mainly in children, young people or in persons living in collectives, its spreading, elimination and prevention for a long-time. This fact enables us to analyze some aspects of this problem. The importance of this work has been confirmed in publications of prestigious institutes of parasitology.

In the 80s of the last century (19) diagnosed *E. vermicularis* in 17.97 % of children in Bratislava by worldwide used and proved methods. Ten years later (27) diagnosed the incidence of enterobiasis in 11.38 % of pre-school children and in 19.32 % of school children. She diagnosed enterobiasis in 5.96 % of employees of school institutions. The family members of infected children were invaded in 10.93 % cases and the family members of positive employees in 13.04 % cases (7) detected the incidence of *E. vermicularis* in 12.92 % of children in kindergartens, thus the findings of (27) from 1999 were stated.

Another parasitological laboratory dealing with enterobiasis systematically is situated in the Turiec region, it is (25) who stated the findings of *E. vermicularis* in 25.6 % of children. Koňáková (21) from the NRC for intestinal parasitoses in Slovak Republic stated 12.62 % prevalence in 31 554 examined persons in years 1997–1999.

Despite of the facts that findings of *E. vermicularis* are variable and that the decrease of pinworm incidence in children is

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not statistically significant, the observed changes in enterobiasis occurrence do not signalize the improvement of situation. This parasitosis will further have negative impact on the health of population.

The epidemiologic data on *E. vermicularis* are mentioned in order to make aware of the prevalence degree in our population, to help the hands-off to have knowledge of number of infected persons and of work done during investigation and evaluation of optimal methods considered for standardized tests by WHO, being however inappropriate for detection of taxonomic differences. These methods exclude the possibility to detect reliably the adult worms of both sexes, e.g. by recommended Graham–Brumpt method (5, 11).

To detect the male parasites is improbable, because they die after the fertilization of females and are expelled from the organism of the host during defecation. Male parasite can be detected by coprological concentration – decantation methods only.

In most cases the diagnosis of *E. vermicularis* is based on ovoscopic methods, i.e. finding of fertilized mature ova with developing larva is considered for positive. Females (after 1–2 hour lasting sleep) come out from anus and lay ova into the perianal region. The Graham–Brumpt method is the most appropriate to detect them. Adhesive transparent PVC tape is pressed on the skin of perianal region, and everything what occurs there, i.e. the ova of *E. vermicularis,* is confirmed microscopically. It is a cheap simple method leading to excellent results.

Fixation of a whole female on the adhesive tape can succeed with probability 1:100. The male parasite is found exceptionally. We did not find it, although we performed thousands of examinations. Ovoscopic methods are excellent for ova detection, but they do not enable to distinguish whether they belong to *E. vermicularis*, or to another parasite species.

References confirm (18, 28, 8, 4), that there are still laboratories in some countries where the prevalence of *E. vermicularis* is investigated directly from the stool of patients. The detection of *Enterobius vermicularis* or *Enterobius gregorii* males was successful only by these direct methods, although it was rarely the case.

If we used one of the concentration methods, our aim would not be the detection of ova exclusively, but also male and female worms. Investigation of ontogenetic development and morphogenetic signs of parasite is possible when only sufficient number of adult parasites of both sexes is available.

These coprologic methods should be associated with anthelminthics with strong expelling effect after application. Irrigation of large intestine can be taken into consideration.

Results

2451 persons were examined by Graham–Brumpt method (using adhesive transparent PVC tape) in Bratislava from 1995 to 1999. (27) 345 of them (14.08 %) were positive for pinworm ova. There has not been detected any living males and females in there cases only.

Discussion

In some laboratories where *E. vermicularis* is investigated in stool exclusively, the researches were able to find both adult worms of male and female. However, positive findings are infrequent – to about 0.1 %, (18, 1), from 0.2 % to 1.0 % (28) – 0.3 %; (23) – 0.5 %; (3) – 0.8 %; (20) – 0.9 %; (8) – 1.0 % and more than 1.0 % (4) 1.2 %; (1) – 1.4 % and (26) 2.84 %. In one case (22) it was possible to observe both types of males *E. vermicularis* and *E. gregorii.*

Searching for worm ova directly in stool specimen is not appropriate for objective estimation of pinworm incidence because the findings are rare, only one tenth of infections diagnosed by Graham–Brumpt method is detected. The detection of adult males and females is also not sufficient, this can be substantially improved by expelling the adult worms by anthelminthics or by irrigation of distal portion of large intestine followed by examination of the expelled stool by decantation – flotation method. This is the only way how to collect higher number of males as described by (12), they succeeded to collect 849 males. Another group of authors isolated 194 males in 17 school children positive for *E. vermicularis*. 76.86 % of males were of *E. vermicularis* and 23.2 % of *E. gregorii* species (2).

First observation of *Enterobius gregorii* males were made by (13). He described its morphology as a younger stage or less developed species with shorter spicule 70 to 80 μm than the spicules of *E. vermicularis* genus, being 100 to 122 μm (13). This statement is in contradiction with earlier parasitological literature and great parasitological compendia e.g. “Parazitologie pro lékaře” (16), where the spicule longitude of 70 to 80 μm is considered to be characteristic of *E. vermicularis*. It seems that the spicule of male *E. vermicularis* grows with age from 70–80 μm gradually to 100–122 μm.(14) described the spicule longitude of *E. vermicularis* males being from 100 to 140 μm and as a further differential sign the circular drawing around the anal opening. Hasegawa et al (12) detected in an 64 years old Japanese man 87 individuals of *E. vermicularis* species and 754 individuals of *E. gregorii* species*,* including six larvae and two worms without spicules. They stated that *E. vermicularis* was significantly longer and more voluminous than *E. gregorii*. The shape and longitude of spicule distal tubular portion was identical in both worms species, nevertheless the basal portion was different. Prematurely adult exemplars of both species immediately after the sloughing or still during the forth cuticular stage had the distal tubular portion only what confirms that the basal portion is definitively formed during their further development. Various transitional forms of *E. gregorii* spicule and its body shape were observed including the transformation into the type of *E. vermicularis.* The basal portion of *E. vermicularis* spicule continues in formation even at the stage of definitive development of basal part of *E. gregorii* body type. These facts lead to assumption that *E. gregorii* could represent only a part of ontogenetic development or an early stage of *E. vermicularis.*

During the investigation of greater number of worm males, if it is possible to detect their various developmenal stages, the

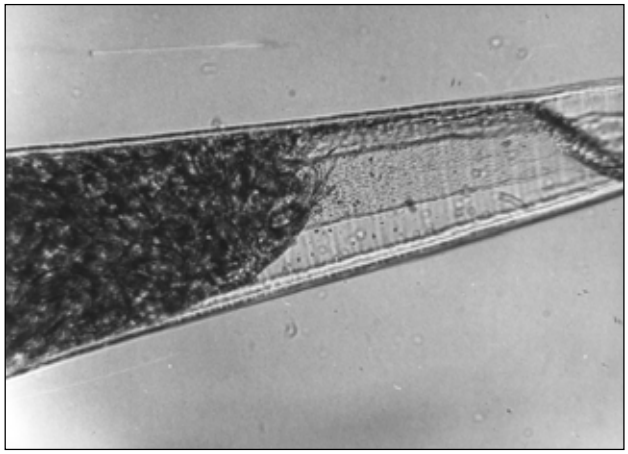

Fig. 1. Enterobius vermicularis.

Fig. 2. Enterobius vermicularis.

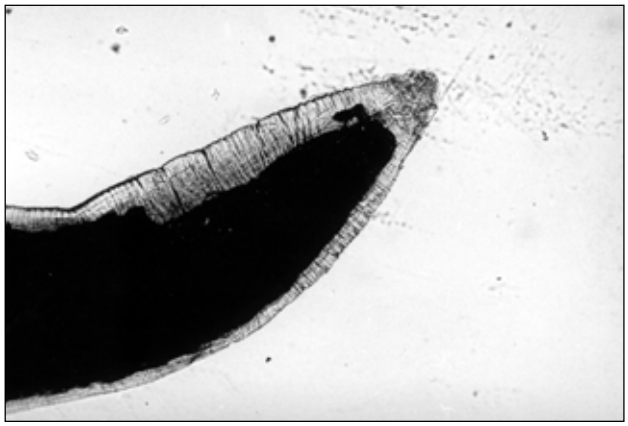

Fig. 3. Enterobius sp.

best guideline is the changing shape and longitude of spicule. The authors agree with (12) with addition that in the continuity of development of basal portion of spicule, the development of *E. gregorii* basal portion is completely included, thus *E. gregorii* represent an early stage of *E. vermicularis*. In details, *E. vermicularis* changes morphological signs and size during its development, what has been considered by Hugot for sign of a "sister species". The term "sister species" is most frequently used in experiments of taxonomic classification of slightly differing new species. The only acceptable evidence of *E. gregorii* existence as an independent genus could be its distinguishing on the basis of polymerase chain reaction (PCR).

The finding of (2) of *E. gregorii* in 23.2 % of persons and *E. vermicularis* in 76.8 %, and the finding of (12) who collected 849 male worm from one person – 10.2 % of them were *E. vermicularis* and 88.8 % of *E. gregorii* – can be considered for nothing else than for various ontogenetic development stages of parasite present in organism of the host. Answers to question why the difference between the species is manifested only in males could bring more light at the problem of new species.

It has been already mentioned that we had not been able to detect any male parasites during examination of enterobiasis in 2451 persons (27) using Graham-Brumpt method, but we had found 3 gravid females with reproductive organs filled with ova.

The microscopic examination illustrated with photographic documentation revealed a female different from others by its caudal portion of body.

The female of *E. vermicularis* has been described by several authors (16, 24, 17, 10, 6, 7, 15) its measures are 10 to 12x0.4 to 0.6 mm and it is bigger than the male. The opening of vulva is located ventrally in first third of its body. Digestive organs are in the middle of the body, oral opening is composed of three lips, oesophagus is separated from the further part of digestive system which ends by anal opening.

The proximal part of *E. vermicularis* female body becomes progressively narrower until it ends in pointed tail (Figs 1 and 2). The morphology of described *E. vermicularis* female and that by us detected female is identical concerning the head, the anterior part of body with vulva opening. Surprising are the anatomical – morphological differences in the bigger female at the caudal end of the body. Its body did not become narrower to the pointed tail, it was more or less cylindrical up to minimally pointed, more rounded tail. The anal opening was located at the end of the body, respectively of the tail (Fig. 3). Further differential sign in the described female in comparison to a typical female of *E. vermicularis* is the cuticula covering the whole body with fine ring – shaped drawing. The circular drawing of *E. vermicularis* female ends at the site where the body is becoming narrower. The rest of its body is covered with smooth cuticula.

More questions arise from these facts – are these morphologic and anatomic deviations genetically coded or can we talk about changes due to accidental mutations?

Our observations of the signs characteristic for a new *E. gregorii* species or the morphological deviations in gravid female isolated from the patient positive for enterobiasis could belong to the group of biological events induced by quantitative signs, or they can be the result of misinterpretations. In the case of the detected female it is not clear whether the mutagenic alterations only are involved into the changes of original signs specific to *E. vermicularis* female. The mutagenic deviation are per-

haps not so rare and we were able to detect them by chance. Concentrated investigation of *Enterobius* females (not only the males) could give the correct answer soon.

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