

Original papers

Comparative analysis of morphometric features of the eggs of selected alimentary tract parasites and of the plant pollens[1]

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ABSTRACT. The aims of the study were: 1. to compare morphometric features of eggs of selected alimentary tract parasites and common garden plants pollen, which may accidentally contaminate a feces undergoing parasitological examination; 2. to make laboratory diagnosticians aware of a possibility of an error at coproscopic examination as well as in helminthologic evaluation of the environmental samples (soil, water). Eggs of 7 species of alimentary tract parasites and 52 common garden plants pollens were examined and compared; using of MultiScanBase v.8.08 computer program the perimeter, length and width of the given objects/images were measured and statistical calculations were made in Statistica 9.1 program. The following conclusions can be drawn: 1. The computer image comparative analysis of parasite eggs and plant pollen proved the existence of statistically significant differences between the particular morphometric features; 2. A routine direct microscopic observation of feces is performed with the naked eye which is not able to notice slight differences (up to a few µm in size), and therefore, despite lack of statistically significant differences, it is easy to make an error; 3. During a routine microscopic observation it should be remembered that the structure, shape or exine of many commonly occurring pollen grains are extremely similar to eggs of some parasites; 4. Both in the analysis of feces specimens and the evaluation of environmental samplings characteristic morphological features of eggs should be considered to facilitate differentiation between eggs and pollen grains; 5. Following the precautionary measures to avoid contamination of feces simples with plants pollens, quality control as well as reliability and awareness of risk eliminates danger of obtaining false positive results.

Key words: parasite eggs, plant pollens

Introduction

Parasite invasions often do not manifest typical symptoms of a disease and in the light of unsatisfactory clinical criteria it is usually difficult to identify them. Finding a parasite especially in the alimentary tract and blood is an evidence of invasion. Success and efficacy in finding and identifying a parasite depends on the selection of the appropriate material and the methods of its collection, its transport, storage time before diagnostics, selection of the appropriate method, and experience and qualifications of the person performing the examination [3,6].

In case of intestinal parasites coproscopic examination of stool still remains the basic and referential test. In spite of development of diagnostic methods, examination of such material is subject to great difficulties. Feces contains numerous components, such as undigested food waste, epithelial cells, mucus and a lot of various microorganisms (e.g. bacteria, fungi) as well as other elements, morphologically similar to the parasitic forms, which may be erroneously taken for parasites [2,5,8]. Inaccurate identification of these elements may lead to false positive results. For the patient's sake it is worthwhile to look for the casual factors of laboratory errors in greater detail [5].

The aims of the study were: 1. to compare morphometric features of eggs of selected alimentary tract parasites and common garden plants pollen, which may accidentally contaminate a

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feces undergoing parasitological examination; 2. to make laboratory diagnosticians aware of a possibility of an error at coproscopic examination as well as in helminthologic evaluation of the environmental samples (soil, water).

Materials and Methods

The material for examination consist of eggs of some the alimentary tract parasites and of plant pollens, collected in June, July and August, 2009 and in April and May, 2010 in the area of the Botanical Garden, 3rd May Park, Zdrowie Park (Lodz district) and in the gardens along 1st May Avenue in Lodz.

In the study direct and fixed preparations, not stained or stained with Lugol's solution of parasite eggs: *Ascaris lumbricoides*, *Diphyllobothrium latum*, *Enterobius vermicularis*, *Fasciola hepatica*, *Taenia* sp., *Toxocara* sp., *Trichuris trichiura* were used**.** The specimens were supplied by the Departments of Biology and Medical Parasitology of Medical Universities in Lodz, Warsaw and Poznan.

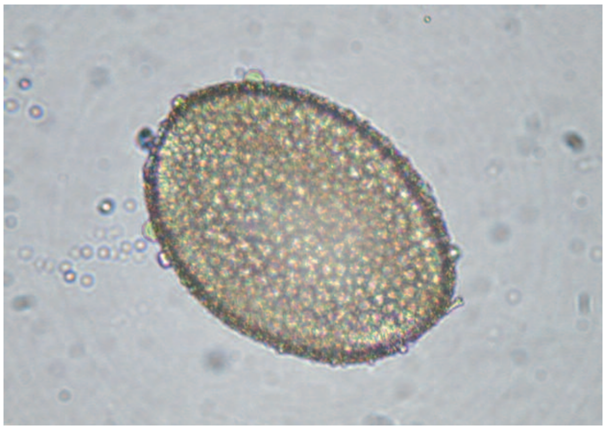
The pollen grains of the following plants were collected: *Acer platanoides* L. (Norway maple), *Aesculus hippocastanum* L. (common horsechestnut), *Alcear osea* L. (hollyhock), *Alopecurus pratensis* L. (meadow foxtail), *Alstroemeria ligtu* and *Alstroemeria aurea* (alstremeria), *Anemone sylvestris* L. (windflower), *Anethum graveolens* L. (garden dill), *Asclepias syriaca* L. (common milkweed), *Astilbe chinensis* (Chinese astilbe), *Bellis perennis* (English daisy), *Betula pendula Roth* (common white birch), *Carpinus betulus* L. (hornbeam), *Centaurea cyanus* L. 1753 (bluebottle), *Cerasus vulgaris* Mill. (cherry), *Chaenomeles Lindl.* (quince), *Chelidonium majus* L. (celandine), *Corylus avellana* L. (European hazel), *Crataegus laevigata* (Poir.) DC. (hawthorn), *Crocus sativus* L. 1753 (saffron), *Cydonia oblonga* Mill. (common quince), *Fagus sylvatica* L. (common beech), *Forsythia* Vahl (forsythia), *Fragaria vesca* L.(woodland strawberry), *Fraxinus excelsior L.* (European ash), *Fuchsia×hybrida, Hemerocallis fulva* (hemerocallis), *Inula britannica* L. (British yellowhead), *Iris sibirica* L. (Siberian iris), *Larix decidua* Mill. (European larch), *Lilium candidum* L. (Madonna lilly), *Lilium* sp. variety 'Wiener Blut' (red lilly), *Lonicera xylosteum* L. (dwarf honeysuckle), *Malus sylvestris* (common apple), *Narcissus jonquilla L.* (daffodil), *Papaver argemone L.* (long prickly head), *Peperomia caperata* (emerald ripple peperomia), *Philadelphus coronarius* L. (mock orange), *Pinus sylvestris* L. (wild pine), *Populus alba* L. (white poplar), *Quercus petraea* (Mattuschka) Liebl. (durmast oak), *Rhododendron catawbiense* Michx. (rosebay), *Rosacanina* L. (briar rose), *Rubus fruticosus* L. (black raspberry), *Salix fragilis (*crack willow), *Sorbus aucuparia* L. (European mountain ash), *Spiraeabetulifolia Pallas* (birch-leaved spirea), *Taraxacum officinale* F.H. Wigg. (common dandelion), *Tilia platyphyllos* L. (largeleaf linden), *Tolpis barbata*, *Tulipa* L. 1753 (tulip), *Ulmus laevis* (white elm). To identify species, professional literature sources were used [1,4,7]

Direct specimens were made in 0.98% NaCl to create conditions similar to those in which such specimens are prepared in parasitological feces examination. The pollen which resembled helminth eggs was photographed. Both the parasite eggs as well as plant pollen were photographed with the use of Nikon ECLIPSE E 200 microscope with Nikon E950 camera, always zoomed in 400x and with a fixed zoom in the camera.

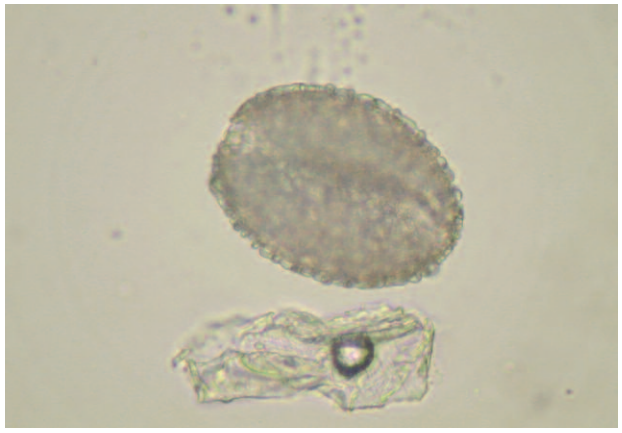
Afterwards, with the use of MultiScanBase v.8.08 computer program the perimeter, length and width of the given objects/images were measured. The program was then scaled with a photo/image with a measuring scale. Statistical calculations were made in Statistica 9.1 program using the Kruskal-Wallisand Mann-Whitney U tests. The Kruskal-Wallis test is a rank, non-parametric statistical test which does not assume normality of distribution. It was used to compare the distribution of a variable in $k>3$ populations. The Mann-Whitney U test is a non-parametric variability test and it was used to check whether the values of the specimens from two independent populations are the same.

Results

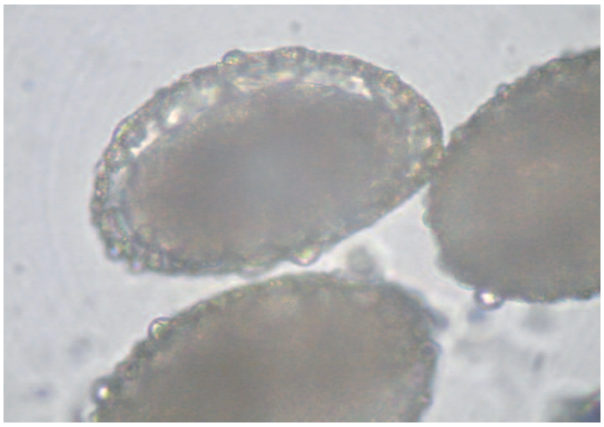
During subjective eye examination of pollen grains (according to their size, shape, colour and the structure of their exterior and inside) a resemblance to the eggs of the selected intestinal parasites was observed in 37 out of 52 cases. Subsequently, the length, width, length/width coefficient and circumference of the selected parasite eggs were compared with the parameters of the resembling pollen. During the microscopic observation it was noticed that out of pollen grains collected from 52 species, grains of three species: *Lilium* variety „Wiener Blut" (phot.1), *Iris sibirica* (phot.2) and *Lilium candidum* (phot.3) corresponded with



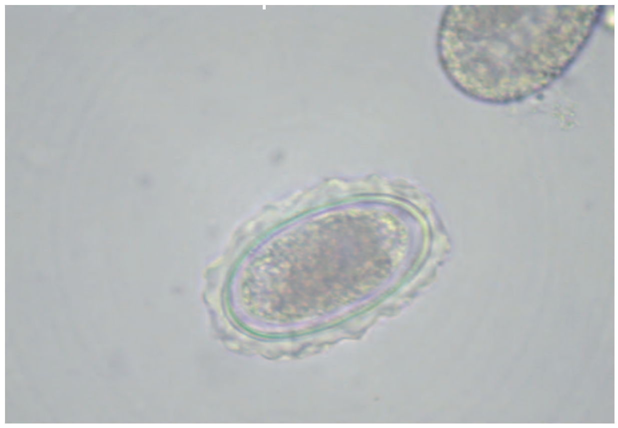
phot. 1 *Lilium* variety „Wiener Blut" pollen



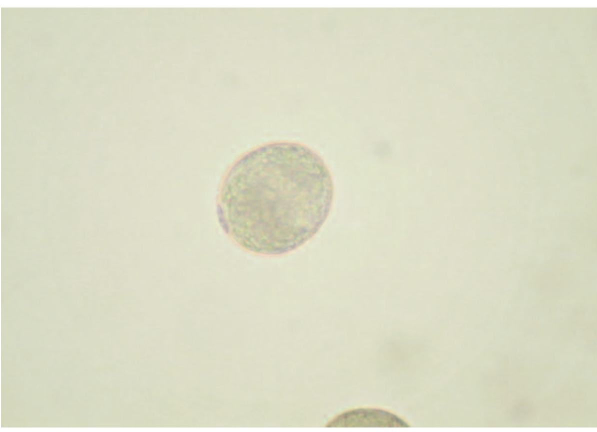
phot. 2 *Iris sibirica* pollen



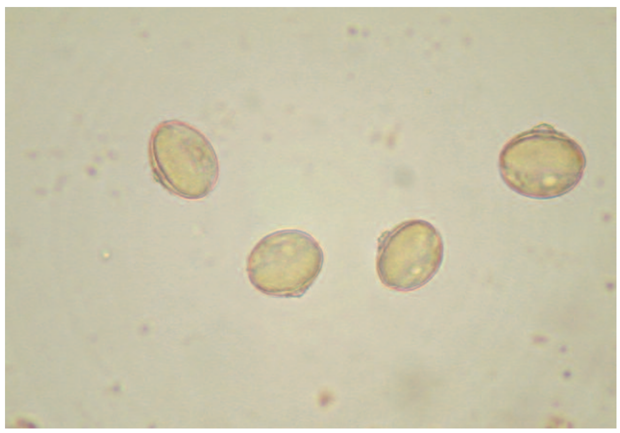
phot. 3 *Lilium candidum* pollen



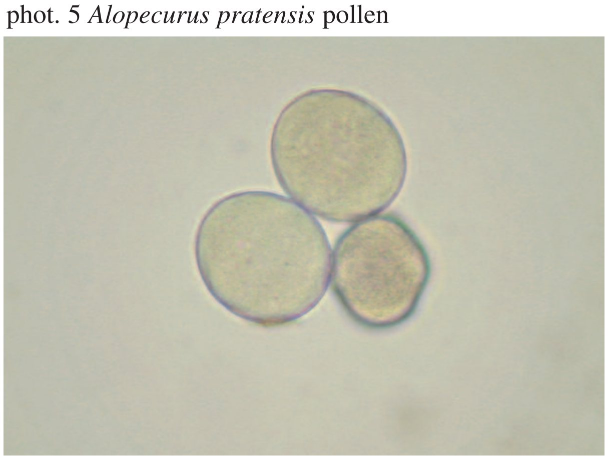
phot. 4 *Ascaris lumbricoides* fertilized egg



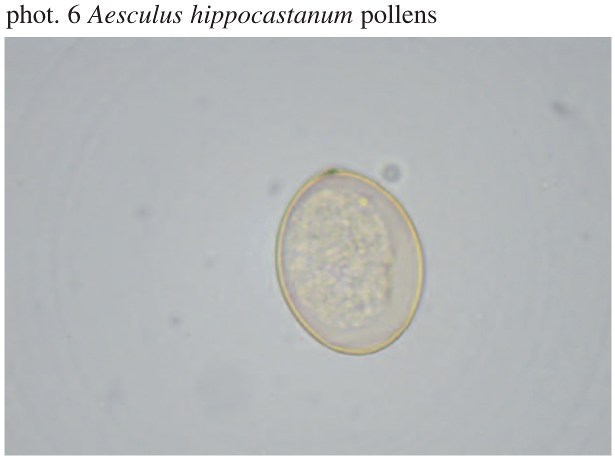
phot. 5 *Alopecurus pratensis* pollen



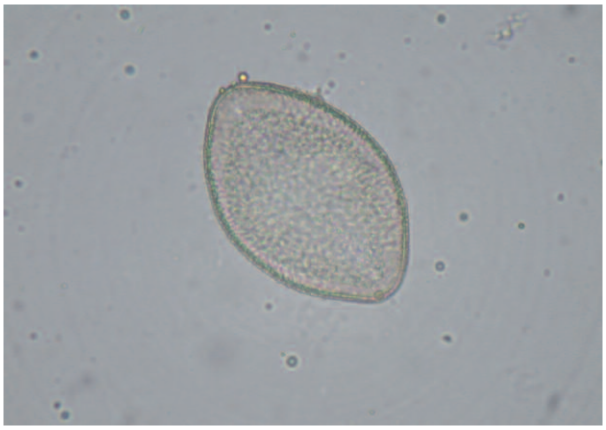
phot. 6 *Aesculus hippocastanum* pollens



phot. 7 *Chaenomeles* pollens

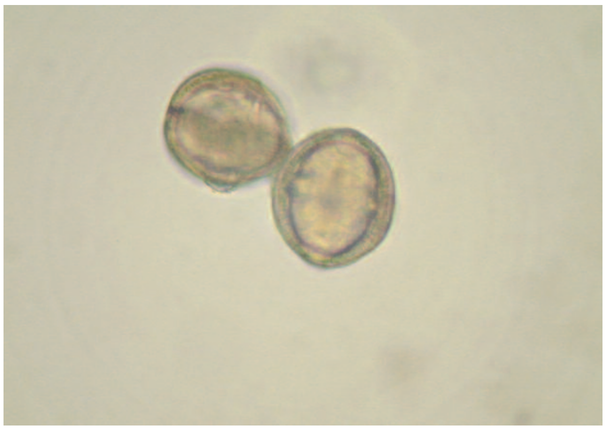


phot. 8 *Diphyllobothrium latum* egg



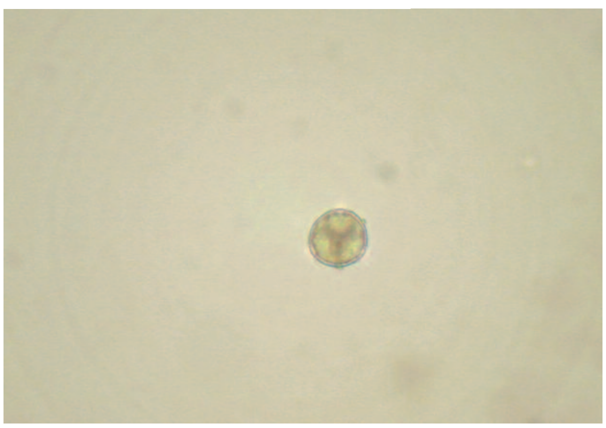
phot. 9 *Alstroemeria ligtu* pollen

phot. 10 *Fasciola hepatica* egg

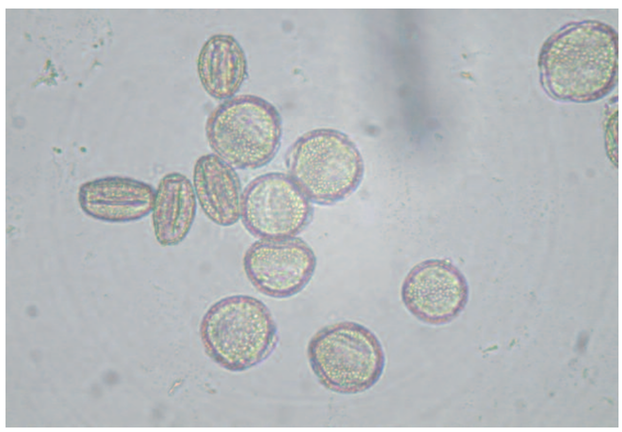


phot. 11 *Centaurea cyanus* pollens

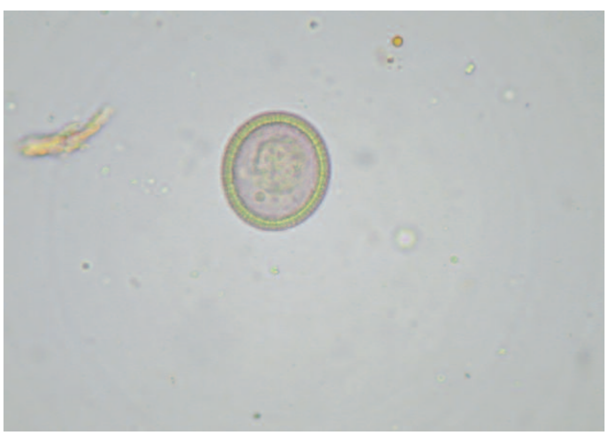
phot. 12 *Papaver argemone* pollen



phot. 13 *Anemone sylvestris* pollen

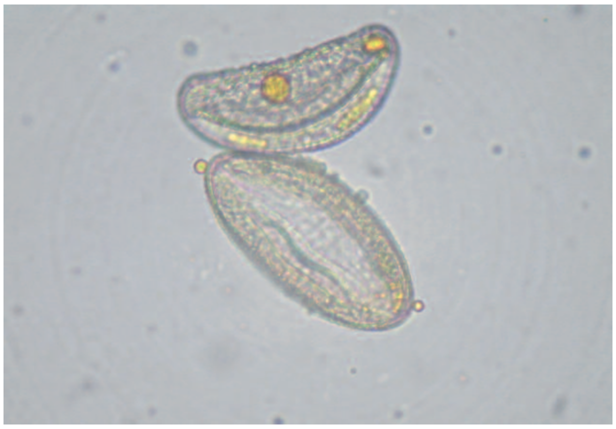


phot. 14 *Salix fragilis* pollens

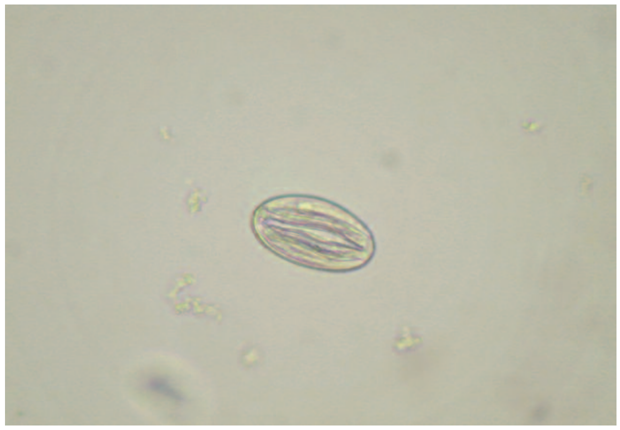


phot. 15 *Taenia* sp. egg

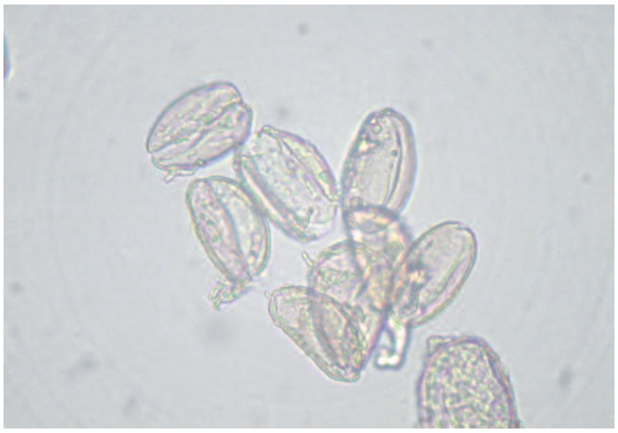
fertilized eggs of *A. lumbricoides* (phot.4). These pollens were enclosed by undulating exine which could bring to mind egg shell of giant roundworm. Pollen grains of three species: *Alopecurus pratensis* (phot.5), *Aesculus hippocastanum* (phot.6) and *Chaenomeles* (phot.7) resembled *D. latum* eggs (phot.8), while *Alstroemeri aligtu* (phot.9) grains were similar to *F. hepatica* eggs (phot.10). Grains of four species: *Centaurea cyanus* (phot.11), *Papave rargemone* (phot.12), *Anemone sylvestris* (phot.13), *Salix fragilis* (phot.14) most resembled



phot. 16 *Alstroemeria aurea* pollens

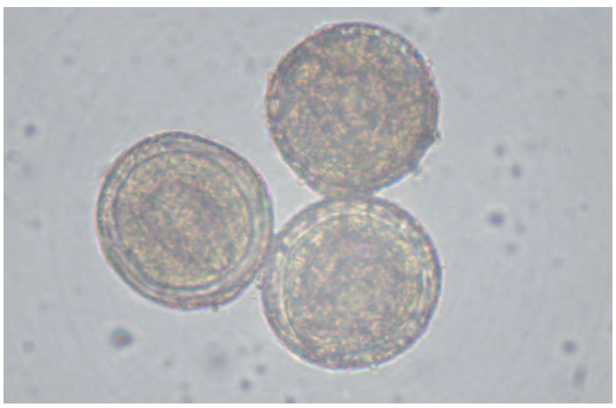


phot. 17 *Cydonia oblonga* pollen

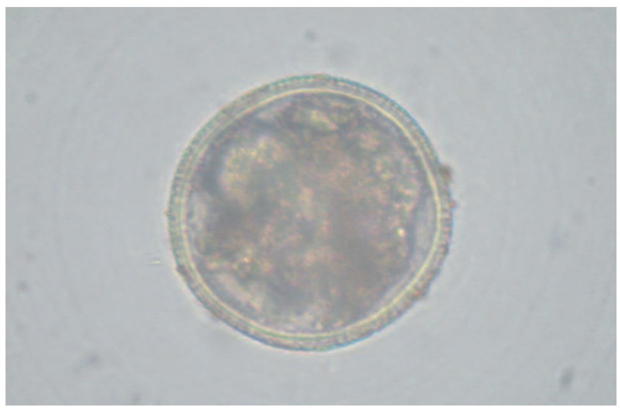


phot. 18 *Narcissus jonquilla* pollens

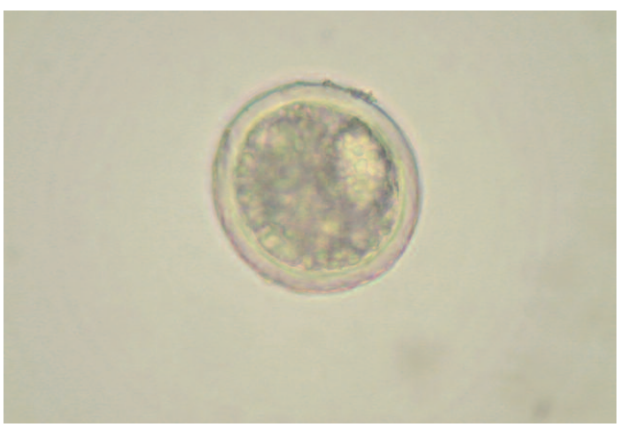
phot. 19 *Enterobius vermicularis* eggs



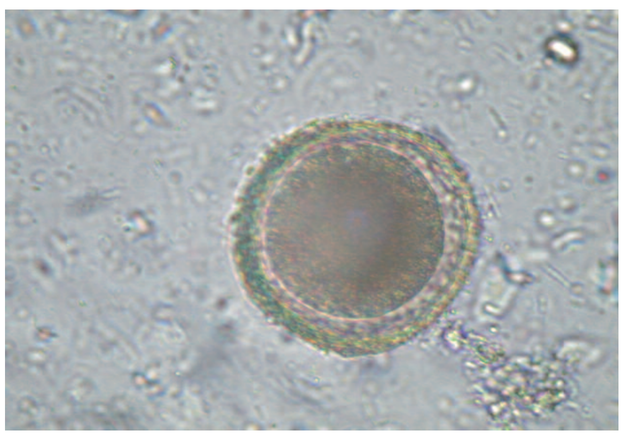
phot. 20 *Tulipa* pollens



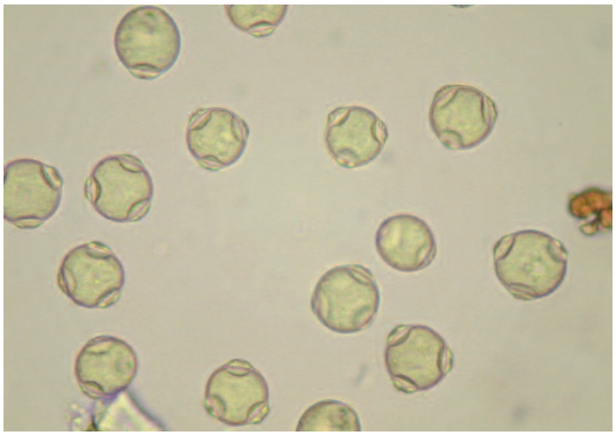
phot. 21 *Crocus sativus* pollen



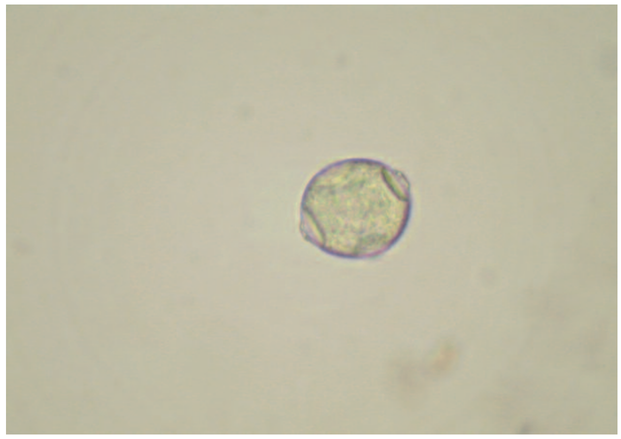
phot. 22 *Larix decidua* pollen



phot. 23 *Toxocara* sp. egg

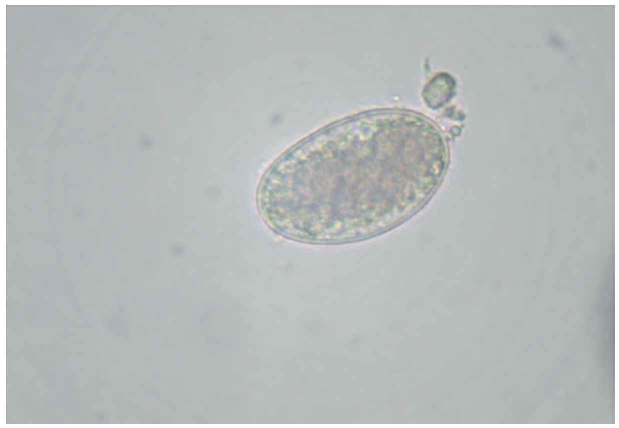


phot. 24 *Betula pendula* pollens

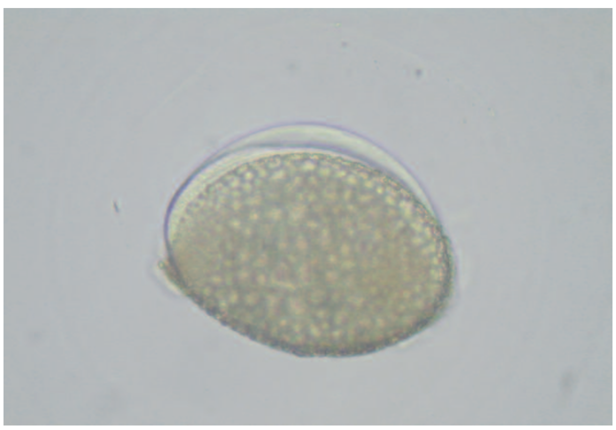


phot. 25 *Carpinus betulus* pollen

phot. 26 *Trichuris trichiura* egg



phot. 27 *Ascaris lumbricoides* not fertilized egg



phot. 28 *Hemerocallis fulva* pollen

Taenia sp. eggs (phot.15). The exine of most of these pollens was striated almost like the vitelline layer surround the oncosphere. Grains of three species: *Alstroemeria aurea* (phot.16), *Cydonia oblonga* (phot.17) and especially *Narcissus jonquilla* (phot.18) resembled *E. vermicularis* eggs (phot.19). In turn, above mentioned grains were transparent with opalescent exine, often with adhesitive matter/substance. It also important to note that pollen grains of three species: *Tulipa* (phot.20), *Crocus sativus* (phot.21) and *Larix deciduas* (phot.22) were exceptionally similar to *Toxocara* sp. eggs (phot.23). Some of the pollen

Table 1. Comparison of width for pairs of parasites eggs and pollen grains. No significant differences were found.

Pairs of parasites eggs and pollens	Width of eggs [μm]±σ (median)	Width of pollen grains [μm]±σ (median)
Taenia sp. and *Papaver argemone*	36.1±2.52 (36.9)	36.9±1.35* (37.1)
Enterobius vermicularis and *Alstroemeria aurea*	35.1±2.61 (35.0)	37.8±5.20** (37.7)
Trichuris trichiura and *Carpinus betulus*	34.0±2.93 (34.9)	35.0±1.38*** (34.8)
Toxocara sp. and *Larix decidua*	71.2±2.38 (71.2)	70.3±4.61**** (71.1)

*p=0.3746 **p=0.0505 ***p=0.4386 ****p=0.6641

Table 2. Comparison of the length/width coefficient for pairs of parasites eggs and pollen grains. No significant differences were found.

Pairs of parasites eggs and pollens	Length/width coefficient of eggs [μm]±σ (median)	Length/width coefficient of pollen grains[μm]±σ (median)
Taenia sp. and *Centura cyanus*	1.12±0.05 (1.11)	1.10±0.05* (1.09)
Ascaris lumbricoides and *Hemerocallis fulva*	1.37±0.19 (1.35)	1.40±0.28** (1.42)
Enterobius vermicularis and *Narcisus jonquilla*	1.78±0.11 (1.77)	1.72±0.21• (1.67)
Enterobius vermicularis and *Cydonia oblonga*	1.78±0.11 (1.77)	1.88±0.25•• (1.87)
Toxocara sp. and *Larix decidua*	1.06±0.02 (1.05)	1.06±0.04♦ (1.05)
Toxocara sp. and *Tulipa*	1.06±0.02 (1.05)	1.06±0.04♦♦ (1.05)

*p=0.0971 **p=0.0796 •p=0.1815 ••p=0.4386 ♦p=0.6472 ♦♦p=0.3323

grains (*Betula pendula* (phot.24), *Carpinus betulus* (phot.25) have lateral air bags or bladders resembling characteristic bipolar plugs of eggs of whipworm (phot.26). The statistically significant differences of the morphometric parameters were revealed in case of 31 comparisons of taxa parasite eggs.

No significant discrepancies were revealed in:

a. the width of the following pairs parasites eggs and pollens: *Taenia* sp. and *Papaver argemone*; *Enterobius vermicularis* and *Alstroemeria aurea*; *Trichuris trichiura* and *Carpinus betulus*; *Toxocara* sp. and *Larix decidua* (Table 1, Fig.1)

b. the length/width coefficient of the following pairs: *Taenia* sp. and *Centura cyanus*; not fertilized eggs *Ascaris lumbricoides* (phot.27) and *Hemerocallis fulva* (phot.28); *Enterobius vermicularis* and *Narcisus jonquilla*, *Cydonia oblonga*; *Toxocara* sp. and *Larix decidua*, *Tulipa* (Table 2, Fig.2-4)

c. and the length of *Toxocara* sp. and *Larix decidua* (Table 3).

Taking into consideration the above mentioned results (no statistically significant differences) the risk of error during identification is very high.

Discussion

In the direct analysis of feces we can encounter elements which resemble parasite eggs, parasites themselves or their parts. These fragments might be epithelium cells of human intestines, animal or plant cells, fungal spores, Acari eggs, as well as small

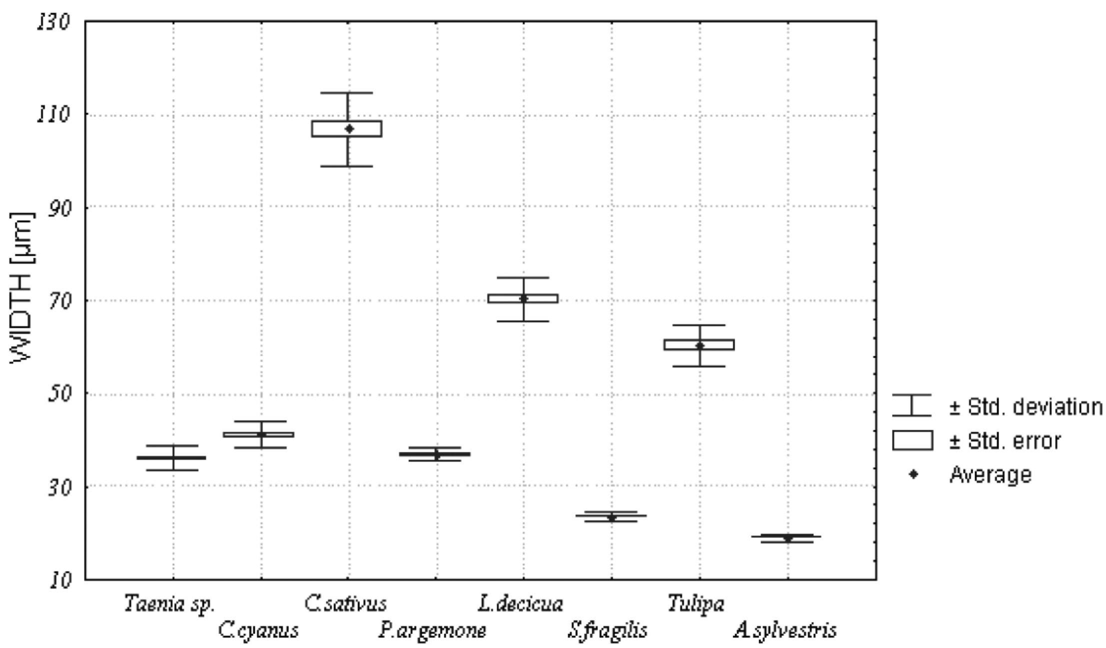


Fig. 1. The mean values [μm] of width of eggs of *Taenia* sp. and selected pollen grains

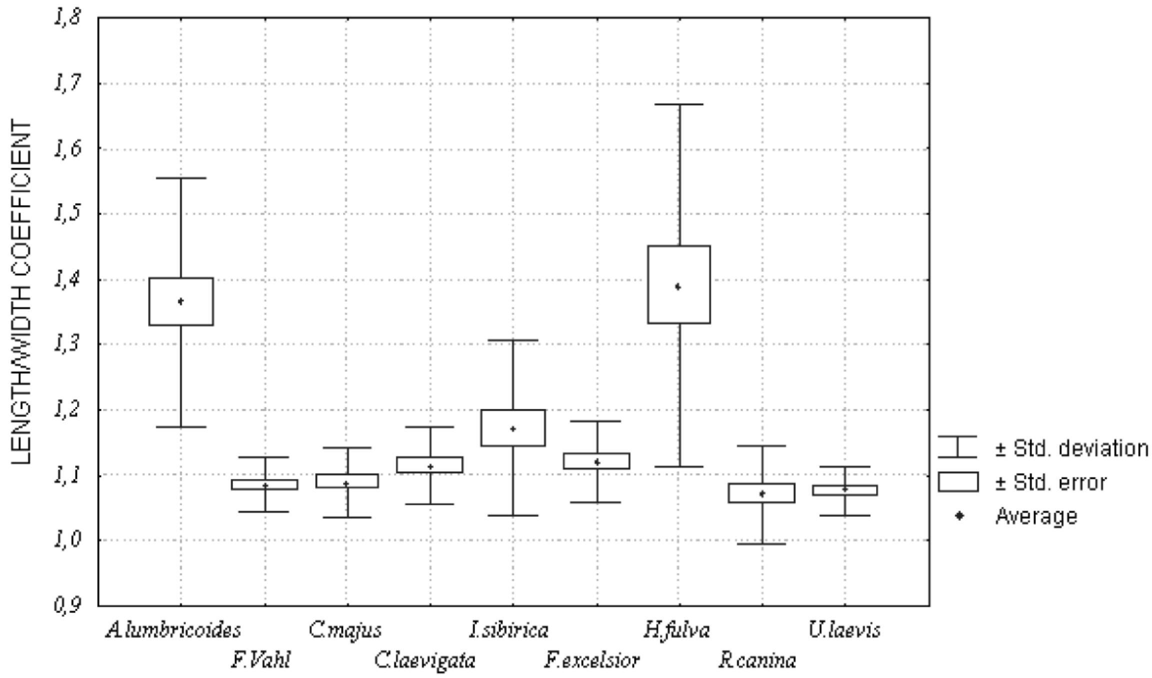


Fig. 2. The mean values [μm] of length/width coefficient of eggs of *A. lumbricoides* and selected pollen grains

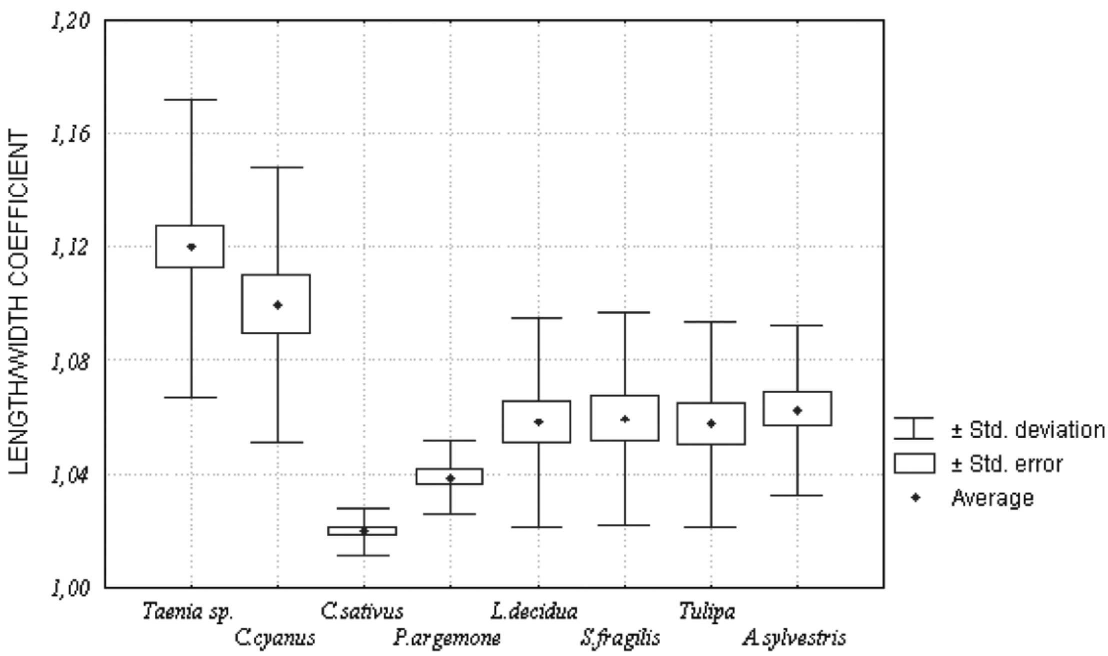


Figure 3. The mean values [μm] of lenght/width coefficient of eggs of *Taenia* sp. and selected pollen grains

free-living animals. Among all those objects, special consideration should be paid to plant cells and among them pollen grains. It is particularly visible while searching for geohelminths eggs in environmental research. Often only incubation of found objects and careful observations of objects' interiors allows proper identification. [5,10]. Professional literature gives examples of mistaking celery fibres for *Ancylostoma* and *Ascaris* and pear stone cells for *Taenia* eggs [9]. While identifying eggs of the tapeworm and giant roundworm extreme caution should be exercised not to make an error. For example the grains of pollen of the following popular plants: *Amarylis belladonna*, *Lilium candidum*, *Zinnia* sp., *Gladiolus imbricatus*, *Chrysanthemum leucanthemum* are similar to eggs

Table 3. Comparison of the length for pairs of parasites eggs and pollen grains. No significant differences were found.

Pairs of parasites eggs and pollens	Length of eggs [μm]±σ (median)	Length of pollen grains [μm]±σ (median)
Toxocara sp. and *Larix decidua*	75.2±2.24 (75.2)	74.3±5.22* (76.0)

*p=0.8973

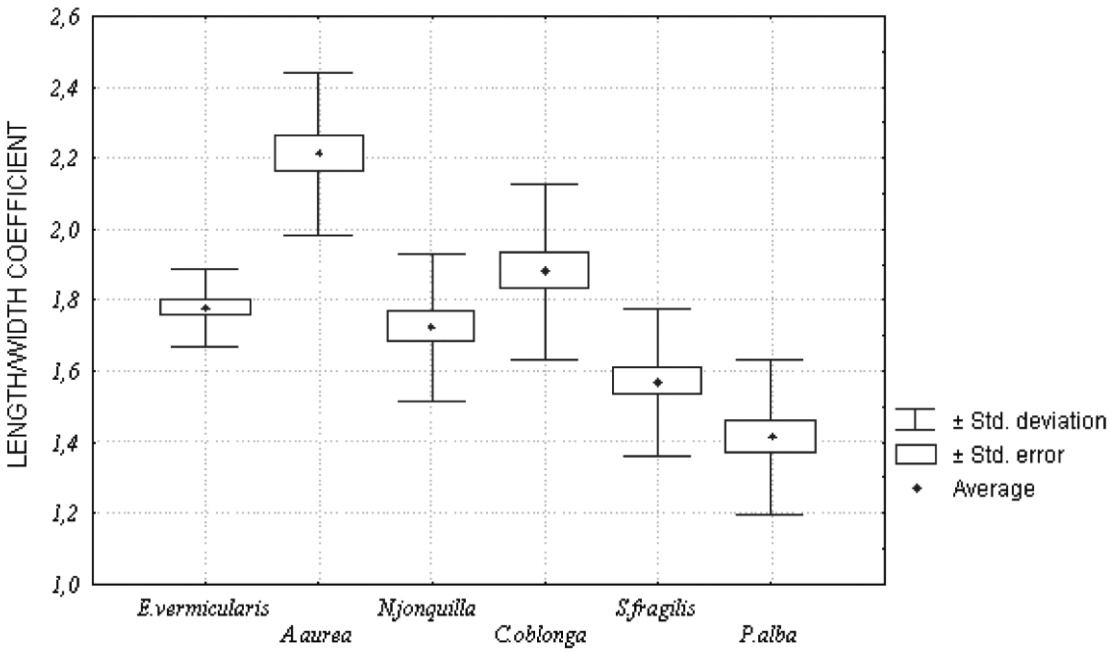


Fig. 4. The mean values [μm] of lenght/width coefficient of eggs of *E. vermicularis* and selected pollen grains

of the giant roundworm [3,5,6].

The microscopic analysis of the collected pollen grains of the plants which discharge a lot of pollen which are common in Lodz proved a certain similarity of some plant taxa to eggs of selected parasites. In most cases the measurements of morphometric features (perimeter, length, width) and the calculated length/width coefficient proved differences between the analyzed pollen and eggs. Having analyzed the detailed statistical data presented in this study it can be concluded that some of pollen grains and parasite eggs have common morphometric features.

On the basis of the study the following conclusions can be drawn:

The computer image comparative analysis of parasite eggs and plant pollen proved the existence of statistically significant differences between the particular morphometric features.

A routine direct microscopic observation of feces is performed with the naked eye which is not able to notice slight differences (up to a few μm in size), and therefore, despite lack of statistically significant differences, it is easy to make an error.

During a routine microscopic observation it should be remembered that the structure, shape or exine of many commonly occurring pollen grains are extremely similar to eggs of some parasites.

Both in the analysis of feces specimens and the evaluation of environmental samplings characteristic morphological features of eggs should be considered to facilitate differentiation between eggs and pollen grains.

Following the precautionary measures to avoid contamination of feces simples with plants pollens, quality control as well as reliability and awareness of risk eliminates danger of obtaining false positive results.

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