Molecular Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* from Stool and Culture Samples Obtained from Polish Citizens Infected in Tropics and in Poland

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**Summary.** The examinations were carried out on stool and serum samples obtained from 38 Polish citizens infected with *E. histolytica sensu lato*. Prevalence of infection (according microscopic examinations) was 0.63% in the Polish who come back from abroad and about 0.19% in persons who did not leave our country. The investigations were performed with isoenzyme analysis and polymerase chain reaction with the use of specific (Psp, NPsp) and (p11+p12, p13+p14) primers. Serodiagnostic examinations were done with antigen produced from *E. histolytica* HK-9 axenic strain. The examinations revealed that in 31 cases the amoebae belonged to nonpathogenic *E. dispar*, in 4 to pathogenic *E. histolytica*, among this one indigenous case; and in two cases were mixed infections. In one case we neither got DNA amplification products nor cultured amoebae. However, from 25 persons we obtained amoebae cultures helping in identification the amoebae zymodemes. The prevailing zymodeme was nonpathogenic zymodeme I. The PCR results were in agreement with isoenzymatic and serodiagnostic examinations.

**Key words:** DNA isolation, *Entamoeba dispar*, *E. histolytica*, genotype, PCR, phenotype, restriction enzyme, serology, zymodeme.

**Abbreviations:** CIEP - counter immunoelectrophoresis test, GPI - glucose-phosphate isomerase E.C. 5.3.1.9., HK - hexokinase E.C. 2.7.1.1, IFA - indirect fluorescent antibody test, IHA - indirect hemagglutination test, ME - malic enzyme E.C. 1.1.1.40, PCR - polymerase chain reactions, PGM - phosphoglucomutase E.C. 2.7.5.1, RAPD - Random Amplified Polymorphic DNA assay.

**INTRODUCTION**

Very shortly after the first description of amoebic dysentery (Lösh 1875) it was noticed that clinical symptoms only occurred in some of persons infected with *Entamoeba histolytica*. Therefore as far back as in 1925 a concept of the existence of two morphologically identical amoeba species was formulated (Brumpt 1925). This idea, however, was not accepted then. It was not until 1993 that Diamond and Clark (1993) gave the ultimate redescription of *E. histolytica* species with morphologically identical cysts, leaving the name *E. histolytica* (Schaudinn, 1903) for the pathogenic strains and restoring the specific name *E. dispar* for nonpathogenic strains, previously proposed by Brumpt (1925).
The infection with *E. histolytica sensu lato* is one of the most common parasitic infections in humans worldwide. It is estimated that about 10% of the world population (Guerrant 1986) is infected with this parasite. Clinical symptoms are observed in 10% of the infected people only, which is related with *E. histolytica* infection, 90% of the cases are asymptomatic, which is most frequently attributed to *E. dispar* infection. Nonetheless, since in some *E. histolytica* infection cases the symptoms may not appear at a certain moment, the number of infections is expected to be markedly higher. People infected with *E. dispar* do not require medical treatment (WHO 1997). It is therefore of vital importance to differentiate these two amoebic species which are morphologically identical.

Hence, other methods that would not be based on the morphology of the parasite should be worked out to detect and/or differentiate amoeba species.

In view of the above, isoenzyme analysis, used already for 20 years (Sargeaunt et al. 1978, Farri et al. 1979), permit to differentiate *E. histolytica sensu lato* strains as pathogenic and nonpathogenic ones, at present identified with *E. histolytica* and *E. dispar*, respectively.


In addition, detection of amoebae coproantigens is very helpful; introduction of monoclonal antibodies specific for *E. histolytica* and *E. dispar* allows their differentiation (Haque et al. 1995, 1998, Jackson and Rawdin 1996, Mirelman et al. 1997).

The aim of the present study embraced: (1) molecular differentiation of *E. histolytica* and *E. dispar* in Polish citizens returning from tropical and subtropical countries, as well as in those who did not leave Poland or Europe and (2) the determination of the prevalence of *E. histolytica sensu lato* genotypes and phenotypes in Poland.

In our previous article (Myjak et al. 1998) we presented the results of PCR amplifications directly from the faeces of the persons examined. In this work we compare the results obtained from the larger number of examinations both PCR directly from faeces and PCR and isoenzyme analysis from amoebae cultures in vitro.

### MATERIALS AND METHODS

#### Sample material

In the years 1995-1998, 4270 Polish citizens who returned from the tropical and subtropical regions were examined in the Institute of Maritime and Tropical Medicine, according scheme using since many years (Zwierz et al. 1975). Twenty-seven persons (0.63%) were infected with *E. histolytica s. l.* Amoebae were also found in 6 (0.19%) persons who did not leave either Poland or Europe. Samples from 5 infected persons were obtained from another laboratory. Stool and serum specimens from those patients were obtained and examined.

#### Cultivation of amoeba.

Three kinds of medium were used: (1) liquid medium PAHM (Myjak 1967), (2) bi-phasic Robinson’s medium (Robinson 1968), and (3) solid medium MA (Myjak 1971).

#### Serological tests.

Indirect hemagglutination test (IHA) and other tests were performed according to the standard procedure (Myjak et al. 1979) with an antigen obtained in our laboratory from *E. histolytica* HK-9 axenic strain.

#### Isonzymatic determination.

Isoenzyme analyses were performed when a stable and sufficient amoeba culture was obtained on PAHM or Robinson’s medium. The lysate of amoebae and isoenzymatic examinations of 4 enzymes (HK /E.C. 2.7.1.1./; PGM /E.C. 2.7.5.1.; GPI /E.C. 5.3.1.9/; ME /E.C. 1.1.1.40/); were performed using the method described by Sargeaunt et al. (1978) and Farri et al. (1979).

#### Polymerase chain reaction (PCR)

**Isolation of DNA.** DNA was isolated from: (1) the in vitro culture and, in some cases, from the stool, using a Genomic DNA Prep Plus kit (A&A Biotecno and/or comparator in the stool, using a Genomic DNA Prep Plus kit (A&A Biotechnology, Gdynia, Poland) (Myjak et al. 1997); (2) from cysts and/or trophozoites present in the stool, in accordance with the procedure described by da Silva et al. (1996), with the final step of DNA purification carried out by means of a DNA Clean-Up kit (A&A Biotechnology, Gdynia).

**Primers.** Specific primers used for *E. histolytica* (Psp5 and Psp3) and for *E. dispar* (NPsp5 and NPsp3) were described by Clark and Diamond (1992). Moreover, in the comparative studies, primers (p11+p12 for *E. histolytica* and p13+p14 for *E. dispar* respectively) complementary to the sequence of DNA encoding 30 kDa protein were used, described by Tachibana et al. (1991). For PCR fingerprinting, a 10-nucleotide RAPD 3 primer was used: 5'- GTA GAC CCG T.

**DNA amplification.** 2 µl of genomic DNA was added to the reaction mixture: 5 µl of PCR buffer (10 x concentrated), 5 µl of dNTP mixture (concentration of each dNTP was 2.5 mM), 2 µl of each primer (10 µM) and 33 µl of water. The mixture was supplemented with 1 U (1 µl) of Taq DNA polymerase (Shark-2, DNA - Gdańsk, Poland) or Ampli Taq® (Perkin-Elmer; 1,25 U = 0.25 µl).

Amplification was carried out in a GeneAmp PCR System 2400 (Perkin-Elmer) thermal cycler according to the following scheme: initial denaturation for 2 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, 1.5 min of annealing at 55°C, the extension for 2 min at 72°C, and the final extension step for 5 min at 72°C. All the PCR were carried out in a 50 µL volume.

For comparison, the amplifications with primers p11+p12 and p13+p14 were carried out according to the thermal profile of Tachibana et al. (1991). In RAPD-PCR, the amplification profile was as the profile with primers Psp and NPsp, but the annealing was performed for 1.5 min at 30°C.
Detection of PCR products and restriction fragments. Some of the amplification products with primers Psp and NPsp were additionally analysed following their digestion with the restriction endonuclease Sau96I (Promega), using the manufacturer’s recommended procedure. The specific PCR products and restriction fragments obtained by enzyme digestion were separated electrophoretically on a 2% agarose gel (Sigma A-9539) while obtained with RAPD 3 primer on 8% polyacrylamide gel using standard procedures. The gels were stained with ethidium bromide, visualised by UV and photographed.

RESULTS

Isoenzyme analyses. E. histolytica sensu lato was detected in the stool samples from 37 persons. In 28 out of those 37 cases (75.7%), amoebae were found in cultures, from which 25 were stable (67.6%), thus enabling isoenzyme analyses to be performed. From among these 25 isolates, 3 were classified as pathogenic and 22 as nonpathogenic according to the obtained zymodemes (Table 1).

Results of serological tests. All sera collected from persons infected with E. dispers were IHA negative except for two with lower IHA titre (1:81-1:243). Five of 6 sera from persons infected with E. histolytica were IHA positive (Table 1), CIEP and IFA positive.

PCR determinations

PCR determinations from short-lived culture. The PCR was carried out with DNA isolated from a short-lived culture (1-2 subculture) of amoeba (n = 28). In two persons E. histolytica was detected, one was suspected of a mixed invasion of E. histolytica and E. dispers, and in 25 cases E. dispers was diagnosed (Table 1).

PCR results obtained directly from the clinical samples. Positive DNA amplification results were directly obtained in 36 (97.3%) stool samples out of 37 persons (with E. histolytica s. l. found in stool) examined. In 3 persons, E. histolytica was detected. DNA amplifications with E. histolytica and E. dispers primers were achieved in two cases, whereas in 31 persons, only with E. dispers primers were detected (Table 1, Fig. 1A).

In patient (No. 340) with amoeba detected in histological preparations of the intestine, the DNA template isolation from these samples following standard removal of paraffin, xylene and alcohol was unsuccessful.

In one case examined, indicating dysenteric amoebiasis at first, E. dispers was recognised by PCR and isoenzymatic examinations, while IHA was negative.

Therefore, the stool was cultivated on bacterial media and the Shigella bacteria then harvested, turned out to be the cause of the dysentery.

Digestion of PCR products with Sau96I. In the samples of amplified DNA, 23 of the samples investigated (stool, culture) were additionally verified by digesting the PCR products with restriction enzyme Sau96I. As the result of enzymatic digestion of the PCR products obtained from 14 clinical samples examinations, two bands of about 740 and 140 bp were displayed, characteristic of E. dispers, whereas in two samples (and in two control samples of E. histolytica HK-9 and 200:NIH), of the electrophoretic patterns displayed, there was only one nondigested band of 876 bp, characteristic of E. histolytica (data not shown). In three samples from two patients (Nos. 376 and 388) in which DNA amplifications were positive with two pairs of primers, digestion of PCR products with restriction enzyme Sau96I gave one 876 bp band with E. histolytica primer product and two bands (about 740 and 140 bp) with E. dispers primer product, which illustrated mixed infections (Fig. 1B).

Identity between E. dispers isolates. Two E. dispers isolates (Nos. 324, 325) were collected from a married couple, No. 325 from the wife who did not leave Poland. Both isolates belonged to nonpathogenic zymodeme I (Table 1). The electrophoretic profiles obtained with RAPD 3 primer for above two isolates were identical, so, these isolates were considered to represent the same strain. The bacteria isolated from their culture gave different profiles, although some of the bands showed the same size as that obtained with amoeba (Fig. 1C).

The same RAPD-PCR method was successfully used for genotyping of another E. dispers and E. histolytica isolates. The results show that isolates were genetically unrelated regarding the number of bands and the size of DNA fragments (Fig. 1C for E. dispers No 328 isolate).

PCR sensitivity. The examinations were performed with DNA isolated from trophozoites from an axenic culture of E. histolytica strain HK-9 and polyxenic culture of E. dispers isolate No. 350. The PCR sensitivity was determined by DNA isolations from 1000, 100, 10, 5 and one amoebae followed by PCR amplification with E. histolytica (Psp5+Psp3) and E. dispers (NPsp5+NPsp3) specific primers. The analysis performed has shown that the amplification of amoebae DNA with specific primers for both amoeba species, a number of 5 trophozoites in the sample was sufficient (data not shown).
Table 1. Laboratory and clinical examinations of patients infected with *Entamoeba histolytica* sensu lato

<table>
<thead>
<tr>
<th>sample</th>
<th>number</th>
<th>n</th>
<th>microscopic</th>
<th>serology (IHA)</th>
<th>culture in vitro</th>
<th>zymodeme</th>
<th>PCR from:</th>
<th>symptoms</th>
<th>location / time</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>stool</td>
<td>culture</td>
<td></td>
</tr>
<tr>
<td>329</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>-</td>
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<td>E. d.</td>
<td>ND</td>
<td>none</td>
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</tr>
<tr>
<td>318</td>
<td>3</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. ?</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
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</tr>
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<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>South Africa, 77</td>
</tr>
<tr>
<td>337</td>
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<td>c</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>S. America, Africa, Asia, all time</td>
</tr>
<tr>
<td>327</td>
<td>2</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Cameroon, 82-95</td>
</tr>
<tr>
<td>362</td>
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<td>-</td>
<td>+</td>
<td>np. ?</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>UEA, 95</td>
</tr>
<tr>
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<td>3</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Zaire, 93-96</td>
</tr>
<tr>
<td>371</td>
<td>3</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
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</tr>
<tr>
<td>89</td>
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<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Thailand, 78</td>
</tr>
<tr>
<td>372**</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Turkey, Morocco, Mexico Gulf, 8.96-2.97</td>
</tr>
<tr>
<td>373**</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Poland</td>
</tr>
<tr>
<td>374</td>
<td>3</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. ?</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
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</tr>
<tr>
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<td>2</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>negative</td>
<td>ND</td>
<td>none</td>
<td>RSA, 91-97</td>
</tr>
<tr>
<td>386</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Poland</td>
</tr>
<tr>
<td>392</td>
<td>1</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Poland</td>
</tr>
<tr>
<td>403</td>
<td>1</td>
<td>c</td>
<td>1:81</td>
<td>-</td>
<td>ND</td>
<td>E. d.</td>
<td>ND</td>
<td>none</td>
<td>Libya, 96-98</td>
</tr>
<tr>
<td>397</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>E. d.</td>
<td>ND</td>
<td>none</td>
<td>America, all time</td>
</tr>
<tr>
<td>407</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. ?</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Congo, 73-98</td>
</tr>
<tr>
<td>409</td>
<td>1</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>India, 98 - 5 months</td>
</tr>
<tr>
<td>410</td>
<td>2</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. ?</td>
<td>E. d.</td>
<td>E. d.</td>
<td>none</td>
<td>Middle East, 7.97-5.98</td>
</tr>
<tr>
<td>414</td>
<td>2</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>E. d.</td>
<td>ND</td>
<td>none</td>
<td>Congo, 90-99</td>
</tr>
<tr>
<td>396</td>
<td>1</td>
<td>c, t</td>
<td>1:243</td>
<td>-</td>
<td>ND</td>
<td>E. h.</td>
<td>ND</td>
<td>none</td>
<td>Congo, 87-96</td>
</tr>
<tr>
<td>404</td>
<td>3</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Turkey, 98 - 2 weeks</td>
</tr>
<tr>
<td>395</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>E. d.</td>
<td>ND</td>
<td>n.d.c.</td>
<td>Egypt, 12.97</td>
</tr>
<tr>
<td>330</td>
<td>4</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Italy, 94, Spain, 95</td>
</tr>
<tr>
<td>324*</td>
<td>2</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Poland</td>
</tr>
<tr>
<td>326</td>
<td>2</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Cameroon, 90-95</td>
</tr>
<tr>
<td>328</td>
<td>1</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. XVII</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>West Africa, 4-9.95</td>
</tr>
<tr>
<td>383</td>
<td>3</td>
<td>c, t</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Cameroon, 92-96</td>
</tr>
<tr>
<td>402</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>E. d.</td>
<td>ND</td>
<td>n.d.c.</td>
<td>India, 98, 1 month</td>
</tr>
<tr>
<td>401</td>
<td>1</td>
<td>c</td>
<td>1:243</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Chad, 97</td>
</tr>
<tr>
<td>350</td>
<td>3</td>
<td>c</td>
<td>-</td>
<td>+</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>n.d.c.</td>
<td>Bahrain, 1-5.95</td>
</tr>
<tr>
<td>388</td>
<td>2</td>
<td>c</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>E. h/E. d</td>
<td>ND</td>
<td>n.d.c.</td>
<td>Rwanda, 80-97</td>
</tr>
<tr>
<td>384</td>
<td>2</td>
<td>c, t</td>
<td>1:729</td>
<td>+</td>
<td>p. II or XIV</td>
<td>E. h.</td>
<td>E. h.</td>
<td>n.d.c.</td>
<td>Cameroon, 89-97</td>
</tr>
<tr>
<td>351</td>
<td>1</td>
<td>c</td>
<td>-</td>
<td>+*</td>
<td>np. I</td>
<td>E. d.</td>
<td>E. d.</td>
<td>dysentery</td>
<td>Rwanda, 89-96</td>
</tr>
<tr>
<td>334</td>
<td>3</td>
<td>c, t</td>
<td>1:6561</td>
<td>+</td>
<td>p. XIV</td>
<td>E. h.</td>
<td>E. h.</td>
<td>liver abscess</td>
<td>Angola, Cameroon, Venezuela, Peru, 1995</td>
</tr>
<tr>
<td>340</td>
<td>2</td>
<td>1:6561</td>
<td>ND</td>
<td>negative**</td>
<td>ND</td>
<td>liver abscess, intestinal ulcers</td>
<td>Poland***</td>
<td>Poland ***</td>
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</table>
Molecular differentiation of *Entamoeba*

**PCR results obtained with *Entamoeba histolytica* (p11+p12) and *E. dispar* (p13+p14) primers.** The usefulness of these primers for DNA amplification was verified on the example of 5 *E. histolytica*, 11 *E. dispar* and one mixed isolates (No. 376). The products of similar size (100 and 101 bp) were obtained with each of these primers, which...
were complementary to the appropriate amoeba species DNA sequences, in the case of No. 376 only with *E. histolytica* primer (data not shown).

**DISCUSSION**

The investigations carried out have shown that the methods used to isolate DNA from amoeba in vitro culture as well as directly from the stool are suitable for the PCR amplification of amoeba DNA, i.e. for differentiation of *E. histolytica* and *E. dispar*. The second method seems to be more effective.

The investigations have also confirmed that PCR is superior to isoenzyme analyses, since no satisfactory amoeba cultures could be obtained from as many as 32% of the patients, thus making impossible the isoenzyme examinations to be carried out. Walderich *et al.* (1997) and Haque *et al.* (1998) did not manage to obtain amoeba cultures in 43% and 8% of persons examined respectively, which also did not allow isoenzymatic determinations and PCR. On the other hand culturing allows for the detection of additional invasions (Haque *et al.* 1998).

In the case of the occurrence of nondysenteric colitis most persons (10/13) were infected with *E. dispar*. According to criteria established recently (WHO 1997), other causes of clinical symptoms observed in those persons should be investigated. Anand *et al.* (1997) reports that in some cases initially suspected of amoebic nondysenteric colitis, the irritable bowel syndrome, requiring specific treatment, was diagnosed most frequently.

It is interesting that in two cases (Nos. 376 and 388) the examinations using primers Psp and NPsp revealed a mixed infection with *E. histolytica* and *E. dispar*. A restriction analysis of the PCR products digested with *Sau96I* proved this observation (Fig. 1B). In view of this, we postulate that in the case of doubtful results, the restriction analysis of the products obtained should be performed. In the case of the isolate (culture) No. 376, the PCR with primers p11+p12 and p13+p14 and isoenzymatic examinations displayed the infection with one amoeba species only, i.e. *E. histolytica*. These differences in results may be explained by the supposition that p11+p12 and p13+p14 primers did not amplify *E. dispar* DNA from this amoeba isolate. The fact that isoenzyme analysis detected *E. histolytica* only, not *E. dispar*, suggests that the amoebae were in insufficient amount and a small number of *E. dispar* cells were eliminated by *E. histolytica*. PCR from templates obtained from cultures several times showed mixed infections. However, after six months culturing the PCR results showed *E. histolytica* only, suggesting elimination of *E. dispar* from culture by *E. histolytica*.

It was proved for the first time in Poland that a person (the wife of an infected man) who did not leave Poland herself was infected with *E. dispar* from the husband who previously travelled to different regions of the world. The identical nonpathogenic zymodeme (I) and the profiles of PCR products obtained with RAPD 3 primers (Fig. 1C) indicated the identity of these two isolates especially that the PCR profiles obtained for other *E. dispar* and *E. histolytica* isolates were different. Clark and Diamond (1993) performed similar investigations permitting the differentiation of *E. histolytica* isolates.

It is also worth noting the occurrence of the invasive amoebiasis (ulcer of the intestine, liver abscess) in a patient (No. 340) who did not leave the country but worked on a ship repaired in the Gdańsk shipyard and eat meals prepared by a Liberian cook on that ship. The patient was admitted to our clinic following the previous resection of the intestine fragment (amoeba trophozoites were detected in histological preparations) and institution of antiamoebic treatment. Hence, the stool examination (including PCR) in that period gave a negative result while the serological reactions were highly positive. This is one of several known cases of invasive amoebiasis in Polish citizens who did not leave the country (Jaroszewicz 1932, Rybicka-Stryjecka and Perlinska-Schneider 1967, Łachecki 1972). Twelve persons from the patient’s family and co-workers from shipyard were also examined but in none of the cases *E. histolytica* infection was detected.

The examinations carried out demonstrated (Table 1) that in Poland, among the imported and indigenous strains of *E. histolytica sensu lato*, *E. dispar* species occurred most frequently (90%), the phenotype of which, i.e. zymodeme I, was the most common one. *E. histolytica* occurred more rarely (15%), but also in persons who did not travel abroad. The results of isoenzymatic examinations, except one, were in good accordance with the PCR and serological tests. In that latter method, all but two sera from persons infected with *E. dispar* were serologically negative, and sera of 5 out of 6 persons infected with *E. histolytica* were positive in several tests.

Comparing our results with those reported by German authors (Walderich *et al.* 1997), a conclusion can be
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drawn that the ratio of *E. histolytica* to *E. dispar* infections was almost 3-fold lower in Poles than in Germans returning from areas endemic for amoebiasis. One possible explanation may be the time spent in the tropics. According to Walderich et al. (1997), the Germans staying in the tropics for a short time, not exceeding 3 months, were more frequently infected with *E. histolytica* than those stayed there for a longer time, while the Poles usually stayed in the tropics much longer, for several years. Also the incidence of *E. histolytica sensu lato* infection was lower in Poles (0.63% according microscopic examinations) than in Germans and foreigners coming from those regions. The reasons can be sought for in the choice of the study group. In Poland, all persons reporting after the return to the country are examined, irrespective of clinical symptoms. This is due to the control examinations, including parasitological ones obligatory, after the official stay in tropical countries. Moreover, the group of 0.63% of the Poles infected with *E. histolytica sensu lato* comprises persons who returned to the country, while in examinations of Walderich et al. (1997), 5.1% of infected cases pertained in 2/3 to Germans and in 1/3 to foreigners visiting Germany.

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REFERENCES


Anand A. C., Reddy P. S., Saiprasad G. S., Kher S. K. (1997) Does the group of 0.63% of the Poles infected with *E. histolytica* sensu lato comprise persons who returned to the country, while the Poles usually stayed in the tropics much longer, for several years. Also the incidence of *E. histolytica sensu lato* infection was lower in Poles (0.63% according microscopic examinations) than in Germans and foreigners coming from those regions. The reasons can be sought for in the choice of the study group. In Poland, all persons reporting after the return to the country are examined, irrespective of clinical symptoms. This is due to the control examinations, including parasitological ones obligatory, after the official stay in tropical countries. Moreover, the group of 0.63% of the Poles infected with *E. histolytica sensu lato* comprises persons who returned to the country, while in examinations of Walderich et al. (1997), 5.1% of infected cases pertained in 2/3 to Germans and in 1/3 to foreigners visiting Germany.

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REFERENCES


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