ENTAMOeba HISTOLYTICA – THE VIRULENCE OF IMPORTED STRAINS

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Abstract. We tested the virulence of 15 strains of Entamoeba histolytica, imported to Czechoslovakia, by intracereal inoculation of laboratory rats. According to the scoring system of Neal, none of the 15 strains possessed the virulence index greater than 2. This indicates that all the organisms tested should be classified as avirulent. However, it should be noted that all the strains produced infection of the eecum and thus should be considered infective for rat. For 9 strains, isoenzyme patterns were determined for PGM, HK and ME. One imported strain, obtained from student from Congo, demonstrated isoenzyme patterns for PGM and HK indicated that the strain was virulent. This organism had the index of virulence 1.8 (virulent) in animal experiment; it was isolated from cysts of clinically asymptomatic patient. Examination of the rectal mucosa of the donor of the strain indicated typical chronic catarrhal proctitis of mild degree. Examination of the patient’s serum demonstrated the presence of anti E. histolytica antibodies by CIEP, while the ELISA test was negative. Twenty-one cyst carriers were examined by rectoscopy. Pathologic changes were observed in 20 of these, as follows: altered vascular structure (12×), roughened mucosa (12×), mucosal redening (10×), decreased glistening (7×), mucus in mucosa (5×), inflammatory pseudopolyps (3×), ulcers (2×), esophagitis (1×). Histological biopsies were obtained in 18 cases. One was considered normal. Remaining 14 biopsies exhibited following morphological changes: increased mucus secretion (8×), ulcers (7×), lymphocytic and plasmocytic infiltration (6×), lymphocytic and plasmocytic infiltration in addition to the presence of eosinophilic granulocytes (5×), presence of mucopurules (5×), hemorrhages (4×), increased vasculature (3×), lymphocytic and plasmocytic infiltration with presence of extremely abundant eosinophilic granulocytes (1×), erosive-ulcerative changes of mucosa (1×). The changes observed indicated chronic catarrhal proctitis with expression tol greater or less degree of signs of chronic catarrhal inflammation.

The occurrence of autochthonous invasive amoebiasis in countries of the temperate zone (Haas 1968; Knobloch et al. 1980; Vandeputte et al. 1980) stressed the significance of the import of virulent Entamoeba histolytica strains from the regions of endemic occurrence of this disease.

The problem of virulence or commensalism of E. histolytica has been studied since 1925, when Brumpt (1925) presupposed the existence of two morphologically similar but biologically different species: E. dysenteriae inducing the disease in man and E. dispar, nonvirulent for man. It was later demonstrated (Martinez-Palomo et al. 1973) that the virulent amoebae isolated from patients with invasive amoebiasis exhibit a higher degree of agglutination with concanavalin A than the amoebae of cyst carriers. Sargeaunt et al. (1978) found that the species of the genus Entamoeba have species specific enzyme profiles and that on the basis of profiles of four chosen enzymes the E. histolytica strains can be divided into more than 20 zymodemes, the pattern of malic enzyme being important for the assignment to this species. All hitherto known zymodemes have only one isoenzyme (ME) at the same position, which differs from the position detected in other Entamoeba species (Sargeaunt et al. 1982; Sargeaunt et al. 1982a, b). The amoebe isolated from patients with invasive amoebiasis can be assigned to 8 different zymodemes; other zymodemes are nonvirulent. Meza et al. (1986) using vertical polacrylamide gradient gel distinctly separated individual isoenzymes and found that asymptomatic carriers are parasitized.
mainly by amoebeae with avirulent zymodesmes. However, zymodesmes characterizing a virulent strain were detected in cyst carriers as well. Chadwick et al. (1985) observed pathological changes in intestinal mucosa induced by an amoebeae with nonpathogenic zymodesmes in an experiment on animals. Jackson et al. (1985) found specific antibodies in asymptomatic amoebeae carriers with virulent zymodesmes. Sepulveda and Martinez-Palomo (1984) proposed already earlier that not only the absence of clinical symptoms, but also negative rectosigmoidoscopy is necessary for the exact classification of an asymptomatic E. histolytica carrier.

In order to determine the biological quality of E. histolytica strains imported to Czechoslovakia from endemic regions we have studied the virulence in an experiment on animals, by electrophoretic separation of enzymes, and by clinical examination of persons infected by this species. The test of electrophoretic enzyme separation is most frequently used for testing the virulence, though Miriman et al. (1986) demonstrated that this phenotypic characterization can be influenced by the change from an avirulent zymodesme to a virulent one and vice versa. The virulence test was used in the animal experiment because the conditions of this test are close to the effect of amoebeae in the intestine of man. The aim of the clinical examinations was to assess whether small changes exceeding the norm occur in the intestine when clinical symptoms of invasive amoebeae are absent.

MATERIALS AND METHODS

1. E. histolytica virulence test in animal experiment

Experimental animals. Non-line laboratory rats at the age of 21 days and weighing 23-30g originated from female rats supplied by VELAZ (Czechoslovakia). Coprological examinations were performed before the experiment and only E. maris-free animals were used. Each strain was tested on 26 animals on the average.

![Fig. 1. Isoenzyme patterns of E. histolytica strains imported to Czechoslovakia.](image)

E. histolytica strains. A total of 22 strains were tested: 12 strains were isolated from foreigners, 3 strains were isolated from Czechoslovak citizens after their long stay in tropical countries, 6 strains originated from the collection of Martinovsky Institute in Moscow, and one strain (HM-200) was obtained from the Institute of Parasitology, McGill University, Montreal and was cultivated in our laboratory during the experiments. The amoebeae were cultivated on liquid Jones' medium (Jones 1952) in a polyethylene culture with rice stalk, the strain HM-20 was grown axenically in TYI-S-33 medium (Dias and et al. 1978).

Inoculum preparation and inoculation. A large amount of concomitant bacteria was removed by repeated centrifugation and washing of 45-72-hour-old amoebeae cultures. The sediment containing amoebeae was diluted in a 3×10⁹ trophozoites/ml. Ether-anaesthetized animals were subjected to laparotomy and 0.2 ml of inoculum (6-10 thousand amoebeae) was introduced into the end of caecum under a slight pressure by means of a syringe with thin needle. The animals were fed with cow milk for two days p.i. and then with DOR-2B standard diet. Those which died within 48 h p.i. were not considered.

Evaluation of the test. The animals were killed by ether on day 7 p.i. After opening the peritoneal cavity, all changes characteristic for the digestive tube lining were observed. The large intestines and caeca were taken out, cut longitudinally and the intestine contents and changes in the intestinal wall were studied. The changes were classified after the original Neat's system (Neat 1951, Neat and Vinekar 1955) modified by workers of the Martinovsky Institute in Moscow, who evaluated besides the intestinal mucosa also the contents of the intestine (Gordeyeva, personal communication). Classification of the wall of caecum: 0 — without changes, 1 — locally thickened mucosa, 2 — moderate changes with scars smaller than 3 mm², 3 — mucosa ulceration on the area of 3-4 mm², 4 — massive ulceration. Classification of caecum contents: 0 — without changes, E. histolytica present, 1 — increased formation of light mucous of homogeneous consistency and containing amoebeae, 2 — presence of a darkly scarred mucous of granular consistency, 3 — of amoebeae, 4 — presence of dark bloody mucous, 5 — clusters of amoebeae with phagocytized erythrocytes in the dark mucous. Points for the intestinal wall and contents were counted and the mean virulence index was calculated from the infected animals for each strain. Strains with the mean index lower than 2 were regarded as avirulent, with the index of 2-4 as weakly virulent, and higher than 4 virulent. In addition to the virulence index also the infectivity index was calculated, i.e. the percentage of animals infected of the total number of inoculated animals. Histopathological examinations of the caecum performed after staining by PAS, Heidenheim haematoxyline and Crocott served for the detection of pathological changes. The virulence of the isolates proper was tested as soon as possible after the stable taking of the culture (16th passage). Exceptions were the strains 4 and 19 tested more than one year after isolation. Collection strains HM-200 to L (Table 1) were cultured in the respective medium for several years.

<p>| Table 1. Infectivity and virulence of E. histolytica strains in an animal experiment |
|---------------------------------|-------------|-------------|-------------|-------------|</p>
<table>
<thead>
<tr>
<th>Strain</th>
<th>Geographic origin</th>
<th>Form of amoebeae</th>
<th>Infectivity %</th>
<th>Virulence</th>
<th>Antibodies in host</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Czechoslovakia (Vietnam)</td>
<td>carrier</td>
<td>11.1</td>
<td>0.0</td>
<td>IHA 128, IFAT 32, CIEP neg.</td>
</tr>
<tr>
<td>10</td>
<td>South Africa</td>
<td>carrier</td>
<td>7.7</td>
<td>0.0</td>
<td>IHA 64, IFAT 32, CIEP neg.</td>
</tr>
<tr>
<td>11</td>
<td>Iraq</td>
<td>carrier</td>
<td>14.3</td>
<td>0.0</td>
<td>IHA 128, IFAT 32, CIEP neg.</td>
</tr>
<tr>
<td>33</td>
<td>Guinea</td>
<td>carrier</td>
<td>13.8</td>
<td>1.7</td>
<td>ELISA neg.</td>
</tr>
<tr>
<td>07</td>
<td>Ethiopia</td>
<td>carrier</td>
<td>15.0</td>
<td>1.0</td>
<td>ELISA neg.</td>
</tr>
<tr>
<td>MGA</td>
<td>Afghanistan</td>
<td>carrier</td>
<td>10.0</td>
<td>0.2</td>
<td>CIEP neg.</td>
</tr>
<tr>
<td>MGB</td>
<td>Afghanistan</td>
<td>carrier</td>
<td>13.3</td>
<td>0.8</td>
<td>IFAT 80, CIEP neg.</td>
</tr>
<tr>
<td>MGC</td>
<td>Guinea-Bissau</td>
<td>carrier</td>
<td>8.0</td>
<td>0.0</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>PA</td>
<td>Czechoslovakia</td>
<td>carrier</td>
<td>6.7</td>
<td>0.2</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>MGE</td>
<td>Vietnam</td>
<td>carrier</td>
<td>10.0</td>
<td>0.2</td>
<td>IFAT 80, CIEP neg.</td>
</tr>
<tr>
<td>MG</td>
<td>Angola</td>
<td>carrier</td>
<td>5.6</td>
<td>0.0</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>MGF</td>
<td>Czechoslovakia (Brazil)</td>
<td>carrier</td>
<td>6.7</td>
<td>0.0</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>V</td>
<td>Palestine</td>
<td>carrier</td>
<td>6.7</td>
<td>0.2</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>276</td>
<td>Venezuela</td>
<td>carrier</td>
<td>13.3</td>
<td>0.2</td>
<td>ELISA neg., CIEP neg.</td>
</tr>
<tr>
<td>015</td>
<td>Congo</td>
<td>carrier</td>
<td>56.6</td>
<td>1.8</td>
<td>IFAT 80, CIEP neg.</td>
</tr>
<tr>
<td>BM 200</td>
<td>Mexico</td>
<td>amoebic dys.</td>
<td>80.0</td>
<td>2.9</td>
<td>ELISA neg., CIEP +</td>
</tr>
<tr>
<td>A</td>
<td>Kaerelia</td>
<td>amoebic dys.</td>
<td>12.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>Turkmenistan</td>
<td>amoebic dys.</td>
<td>7.1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>Azerbaizhan</td>
<td>amoebic dys.</td>
<td>62.5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>BN</td>
<td>USSR (India)</td>
<td>amoebic dys.</td>
<td>66.7</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Vietnam</td>
<td>amoebic dys.</td>
<td>17.9</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>Vietnam</td>
<td>amoebic dys.</td>
<td>21.1</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>
2. Determination of *E. histolytica* virulence by electrophoretic separation of enzymes

Thin-layer starch gel electrophoresis was carried out after Wrazek and Cullford (1966) and Sargeaunt et al. (1978). A lysate from amoebae was prepared as follows. Polycetin-stained smears of *E. histolytica* were washed 4× with saline and centrifuged at 350 g for 10 min at 20 °C. After washing the sediment was resuspended in the same volume of distilled water and enzyme stabilizers were added (dithiothreitol, N-aminoacetic acid, EDTA) to final concentration of 1 mM. The sample was incubated for 30 min at 4 °C and 2× frozen to −20 °C and thawed. The lysate was then centrifuged at 15,000 g for 30 min at 4 °C. The supernatant was kept at −70 °C or in liquid nitrogen. The same method was used for the preparation of the lysate from concomitant bacterial microflora in order to differentiate the bacterial and amoebic enzymes. Lysate from axenically growing HK-9 strain belonging to cytopathic type I was used as standard. The acrylamide electrophoretic mobility of phosphoglucomutase (PGM, hexokinase (HK)) and malic enzyme (ME) was observed.

3. Clinical examination of *E. histolytica* cyst carriers

Of the 118 foreigners carrying *E. histolytica* cysts, 21 were complexly clinically examined. At the time of examination the patients did not mention either present or previous symptoms which might have been associated with *E. histolytica* infection. The rectoscope was carried out before treatment in 21 persons (Table 2). The following criteria were used for the normal appearance of mucosa: light or dark pink colour, the coloration may be even non-homogeneous; smooth and glossy, light reflex undamaged; vascular pattern well marked; no erythema in the vicinity of visible vessels; mucosa not bleeding either spontaneously or after touching; no pathological contents in lumen. The appearance of mucosa was normal in one person. The following pathologies were found in 20 persons: altered vascular structure (18×), roughened mucosa (12×), mucosal reddening (10×), decreased glistening (7×), mucus in mucosa (5×), inflammatory pseudopolyp (2×), ulcers (2×), and enanthema (1×).

Bioplastic samples of rectal mucosa from 15 persons were histologically examined and onychomycosis was normal. The 14 remaining samples exhibited these changes: increased mucus secretion (8×), edema (7×), lympohytic and plasmocytic infiltration (6×), lymphocytic and plasmocytic infiltration in addition to the presence of eosinophilic granulocytes (6×), presence of mucophages (5×), haemorrhages (4×), increased vascularity (3×), lymphocytic and plasmocytic infiltration with presence of extremely abundant eosinophilic granulocytes (1×), erosive-ulcerative changes of mucosa (1×). The detected changes indicated a chronic catarrhal proctitis sometimes with more or less expressed signs of chronic catarrhal inflammation (Table 2): edema, congestion, increased number of lymphocytes, plasma cells, eosinophilic granulocytes in mucosal strata, and increased formation of mucus. The results of biochemical and haematological screening of all infected persons were within the range of normal values. Also the circulating immune complexes were not increased.

**DISCUSSION**

All of the 15 strains of *E. histolytica* imported into Czechoslovakia were classified as avirulent on the basis of the virulence test on animals, though the amoebae of all strains survived for 7 days in the caecum and were therefore infective. Since all strains originated from asymptomatic cyst carriers, the results of the virulence test on animals were in agreement with the clinical state of the hosts — straining mucus. Their classification as avirulent zymodemes was in correlation as well. A single exception was the strain 515, which was classified as avirulent in the animal experiment, but according to its enzyme pattern belonged to virulent strains. The strain was isolated from a patient from Congo during his hospitalization for *Plasmodium falciparum* infection. In addition to slight changes in rectal mucosa (Table 2, patient No. 21) and CIEP antibodies in the serum, no other clinical symptoms casually related with *E. histolytica* infection were found in him. This result, though observed in one case only, documents the complicated classification of strains to virulent and avirulent...
<table>
<thead>
<tr>
<th>Country</th>
<th>Year of birth</th>
<th>Diagnosis (by admission)</th>
<th>Rectoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afghanistan</td>
<td>1959</td>
<td>cyst carrier</td>
<td>roughened mucosa, decreased glistening, altered vascular structure, small amount of mucosa</td>
</tr>
<tr>
<td>Cuba</td>
<td>1989</td>
<td>cyst carrier</td>
<td>several small polyp-like structures (2 up to 1 mm)</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1968</td>
<td>cyst carrier</td>
<td>mucosa slightly roughened, 3 ulcerations (2 up to 1 mm) immediately above rectal canal, others 7 cm from anus</td>
</tr>
<tr>
<td>Mongolia</td>
<td>1968</td>
<td>cyst carrier</td>
<td>normal mucosa</td>
</tr>
<tr>
<td>Laos</td>
<td>1965</td>
<td>cyst carrier</td>
<td>mucosa slightly roughened, glistening, vascular structure less distinct</td>
</tr>
<tr>
<td>Cape Verde Islands</td>
<td>1962</td>
<td>cyst carrier</td>
<td>focus of roughened mucosa (2 cm), other parts of mucosa normal</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1966</td>
<td>cyst carrier</td>
<td>mucosa roughened, decreased glistening, vascular structure diffusely widened off, mucosa in part of mucosa</td>
</tr>
<tr>
<td>Zambia</td>
<td>1966</td>
<td>cyst carrier</td>
<td>50% of mucosa surface reddened, decreased glistening; exanthema: red maculae (2 up to 1 mm), sometimes in clusters</td>
</tr>
<tr>
<td>Yemen</td>
<td>1963</td>
<td>cyst carrier</td>
<td>mucosa roughened, sometimes distinctly rough, decreased glistening, vessels numerous</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1964</td>
<td>cyst carrier</td>
<td>mucosa roughened, markedly increased number of vessels</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1965</td>
<td>cyst carrier</td>
<td>focus of reddening with uneven to rough surface, decreased glistening</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1966</td>
<td>cyst carrier</td>
<td>focus of reddening with uneven to tough surface; a polyp attacked at 8 cm (2 1 mm)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1963</td>
<td>cyst carrier</td>
<td>diffuse reddening, vascular structure almost disappearing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histological examination</th>
<th>Circulating antibodies</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>biotic examination not done</td>
<td>-- neg 32 128</td>
<td>light catarhal proctitis</td>
</tr>
<tr>
<td>lymphocytic and plasmocytic infiltrations; single eosinophilic granulocytes; increased mucous secretion; slight edema, congestion</td>
<td>neg -- -- --</td>
<td>light catarhal proctitis</td>
</tr>
<tr>
<td>thin lymphocytic and plasmocytic infiltration; single eosinophilic granulocytes; increased mucous secretion</td>
<td>neg neg 20 --</td>
<td>focal proctitis with small ulcerations</td>
</tr>
<tr>
<td>mucozas of normal histological structure</td>
<td>neg -- -- --</td>
<td>cyst carrier</td>
</tr>
<tr>
<td>mostly plasmocytes, less lymphocytes in inflammatory infiltration; single eosinophilic granulocytes at sites of more pronounced inflammatory changes; small clusters of mucophages; increased mucous secretion; edema, congestion</td>
<td>1,800 neg --</td>
<td>diffuse light catarhal proctitis</td>
</tr>
<tr>
<td>lymphocytic and plasmocytic infiltration; focal mucophages; group of cells from fat connective tissue in superficial layer of mucosa; edema, small haemorrhages</td>
<td>neg -- -- --</td>
<td>diffuse catarhal proctitis</td>
</tr>
<tr>
<td>biotic examination not done</td>
<td>-- neg -- --</td>
<td>diffuse catarhal proctitis</td>
</tr>
<tr>
<td>focal thin lymphocytic and plasmocytic infiltration in stroma; single eosinophilic granulocytes, diffusely increased mucous secretion</td>
<td>neg -- -- --</td>
<td>chronic catarhal diffuse proctitis</td>
</tr>
<tr>
<td>thin lymphocytic and plasmocytic infiltration in mucus stroma</td>
<td>neg -- -- --</td>
<td>light chronic catarhal diffuse proctitis</td>
</tr>
<tr>
<td>diffuse, mostly lymphocytic infiltration of mucosa with plasmocytes; single mucophages; small haemorrhages</td>
<td>600 posit. --</td>
<td>light chronic diffuse catarhal proctitis</td>
</tr>
<tr>
<td>focal thin lymphocytic and plasmocytic infiltration, slightly increased mucus production; edema in upper layer of mucosal stroma</td>
<td>200 posit. --</td>
<td>focal chronic catarhal proctitis</td>
</tr>
<tr>
<td>lymphocytic and plasmocytic infiltrations, single mucophages; marked production of mucous, oedema</td>
<td>1,800 posit. --</td>
<td>light chronic focal catarhal proctitis</td>
</tr>
<tr>
<td>focal lymphocytic and plasmocytic infiltration in upper layer of mucosal stroma; mucophages scarce; slight oedema</td>
<td>200 posit. --</td>
<td>chronic catarhal diffuse proctitis</td>
</tr>
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Table 2 (continued)

<table>
<thead>
<tr>
<th>Country</th>
<th>Year of birth</th>
<th>Diagnosis (by admission)</th>
<th>Rectoscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnam</td>
<td>1964</td>
<td>cyst carrier</td>
<td>diffuse reddening with uneven to rough surface, vessels numerous</td>
</tr>
<tr>
<td>Cambodia</td>
<td>1966</td>
<td>cyst carrier</td>
<td>diffuse reddening with wiped off vascular structure; several small ulcerations (≥ 1 mm)</td>
</tr>
<tr>
<td>Cambodia</td>
<td>1967</td>
<td>cyst carrier</td>
<td>diffuse reddening, vascular structure sometimes increased, flattening decreased, small amount of mucous present</td>
</tr>
<tr>
<td>Guinea-Bissau</td>
<td>1957</td>
<td>cyst carrier</td>
<td>mucosa surface normal; small amount of mucous present</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>1964</td>
<td>cyst carrier</td>
<td>focus of redness (≥ 2 cm), without vascular structure, mucous present, 5-7 cm from anus</td>
</tr>
<tr>
<td>Korea</td>
<td>1969</td>
<td>cyst carrier</td>
<td>focus of slightly roughened surface with decreased flattening</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1984</td>
<td>cyst carrier</td>
<td>focus of redness, roughened surface, decreased vascular structure</td>
</tr>
<tr>
<td>Congo</td>
<td>1987</td>
<td>cyst carrier</td>
<td>diffuse reddening, roughened surface, vascular structure partly wiped off</td>
</tr>
</tbody>
</table>

Histological examination

<table>
<thead>
<tr>
<th>Circulating antibodies</th>
<th>ELISA CIEP IFAT IHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>focal thin lymphocytic and plasmocytic infiltration in mesosalpinx str.</td>
<td>1,800 pos. ---</td>
</tr>
<tr>
<td>thin lymphocytic and plasmocytic infiltration; single eosinophil granulocytes; increased mucosal production; slight edema, congestion</td>
<td>--- neg. 128 neg light chronic --- catarhal proctitis</td>
</tr>
<tr>
<td>biopptic examination not done</td>
<td>--- --- ---</td>
</tr>
<tr>
<td>biopptic examination not done</td>
<td>--- neg. 16 neg --- catarhal proctitis</td>
</tr>
<tr>
<td>biopptic examination not done</td>
<td>--- neg. --- --- catarhal proctitis</td>
</tr>
<tr>
<td>dense mixed inflammatory infiltration with prevailing eosinophilic granulocytes; numerous superficial erosions to ulcerations of mucosa</td>
<td>--- --- --- --- intermediate chronic catarhal proctitis</td>
</tr>
<tr>
<td>lymphocytic and plasmocytic infiltration; single eosinophilic granulocytes, mucous production slightly increased</td>
<td>--- --- --- --- chronic catarhal proctitis</td>
</tr>
<tr>
<td>biopptic examination not done</td>
<td>--- neg. pos. --- catarhal proctitis</td>
</tr>
</tbody>
</table>

Conclusion

The biological properties are responsible for discrete tissue changes and also for the presence of CIEP antibodies in the case of strain 515. The virulence can be one of the diverse properties. The present study on the virulence of imported *E. histolytica* strains into the temperate zone countries shows some new views for the evaluation of the biological properties of *E. histolytica*, particularly in relation to discrete changes on rectal mucosa and interpretation of the given sympotm. It shows also the complexity of an objective estimation of the real risk of introduction of the viral strains into the territory.

**ENTAMOEBA HISTOLYTICA — ВИРУЛЕНТНОСТЬ ИМПОРТИРОВАННЫХ ШТАММОВ**

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Резюме. Изучены вирulence 15 штаммов *E. histolytica*, импортированных в ЧССР, при помощи опыта с лабораторными крысами. Ни у одного штамма средний индекс вирulence по системе Нива не был выше 2. Это значит, что все импортированные штаммы были классифицированы как невирулентные. Однако, все штаммы переносили в слое кишечных и были инфекционными для животных. У 7 штаммов *E. histolytica* было определено индекс вирulence 10. Оно является основание для снятия штаммов 515, импортированных в ЧССР, с учетом введения штаммов, что его средний индекс вирulence в опыте с животными...
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Fig. 1. Edematous caecal mucosa of rat infected with an avirulent strain of *Entamoeba histolytica*. (PAS, 250×.)

Fig. 2. Caecal crypts of rat with a trophozoite of *E. histolytica*. The other amebae in caecal contents. (PAS, 600×.)

Fig. 1. Caecal mucosa of rat infected with a virulent strain of *E. histolytica*. A trophozoite penetrating through the epithelium. Light inflammatory cellulization in the stroma. (PAS, 300×.)

Fig. 2. Subepithelially localized trophozoite of *E. histolytica* in edematous caecal stroma with inflammatory infiltration. (PAS, 400×.)