FOLIA PARASITOLOGICA 37: 97–104, 1990.

AMOEBIASIS IN FOREIGN STUDENTS

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Abstract. A total of 2,883 foreign students at the age of 18—30 years were examined for amoebiasis after their arrival to Czechoslovakia. Stool examinations revealed the presence of *Entamoeba histolytica* in 112 of them (3.9%). Students from 38 countries were found to be infected with this parasite. In a set of 2,064 students from these countries *E. histolytica* prevalence in stool was 5.4%. There were greater differences in the prevalence between individual countries inside a geographical region than between individual geographical regions. The highest *E. histolytica* prevalence in stool was found in students from tropical and southern Africa (6.7% of 745 examined) and the lowest in students from South-eastern Asia (3.1% of 321 examined). In a simple cross-section study, antibodies against *E. histolytica* were detected by enzyme-linked immunosorbent assay (ELISA) in the sera of 1,001 persons. Antibodies were detected in 7.9% of students at the following titres: 1 : 200 in 4.5%, 1 : 600 in 1.5%, 1 : 1,800 in 1.9%. Antibodies occurred more frequently in students carrying *E. histolytica* cysts ($X^2 = 14.9$). Titre of ELISA antibodies in patients with confirmed liver abscess was higher than 1 : 1,800. Counterimmunoelectrophoresis (CIEP) test was used for serum examinations of patients who had been demonstrated by ELISA to be seropositive and of those carrying *E. histolytica* cysts. In a set of 170 patients CIEP antibodies were also more frequent in those carrying *E. histolytica* cysts ($X^2 = 26.95$). A comparison of the results of ELISA and CIEP tests in the same patients revealed that CIEP antibodies were more dependent on the actual parasitization with *E. histolytica* than ELISA antibodies. Sera of 81 foreign students from Africa and Latin America staying in Czechoslovakia for more than 3 years did not contain any antibodies against amoebae as it was detected by ELISA. An exception was one student in which the titre of 1 : 600 was demonstrated. In 3 patients, antiamoebic ELISA antibodies did not persist for more than three years after healing of amoebic liver abscess.

The majority of papers dealing with the import of tropical parasitoses into Czechoslovakia by foreign students concerned the prevalence of intestinal parasitoses and search for suitable diagnostical methods (Dufek et al. 1967, Dufek and Kalivoda 1969, Chmelíková 1971, Giboda et al. 1982).The prevalence of *E. histolytica* in stool and frequency of antiamoebic antibodies in sera of students are treated in the present paper.

MATERIALS AND METHODS

Examined set. Stool and sera of 2,883 foreign students at the age 18—30 years were examined in the years 1982—1987. Blood and stool were collected within three weeks after their arrival to Czechoslovakia. The students originated from 70 countries of all continents except Australia. At the time of collection or within 1 year after arrival none of the students suffered from clinically manifested invasive amoebiasis. In addition to this set, sera of 7 other patients with confirmed amoebic liver abscesses were tested for equilibration of seroreactions, positive controls and antibody persistence.
Examination of stool. Each stool sample was tested by three methods: floatation (Kozak and Magrová 1960), sedimentation (Hunter et al. 1948), and merthiolate-iodine-formaldehyde concentration method for the detection of protozoan cysts (Giboda and Kratochvíl 1970). If uninucleate amoeba cysts were found, the test was repeated in order to exclude possible *E. polecki* infection (Giboda et al. 1988). Some of the stool samples were examined repeatedly; stool samples of 810 students were examined also by cultivation.
Serological tests. Soluble antigen was prepared by centrifugation of HK-9 strain amoeba lysate and user for CIEP at the concentration of 2 mg protein/ml and for ELISA at the concentration of 4 μg/ml. Antibodies against surface antigens were sporadically examined by immunofluorescent antibody test (IFAT).

Table 1. Frequency of 112 *Entamoeba histolytica* infections in 2,064 foreign students

Country	No. of students	No. of infected students	Prevalence (%)
North Africa, Near and Middle East	396	25	6.3
Iraq	19	5	26.3
Jordan	34	3	8.8
Lebanon	33	4	12.1
Morocco	9	1	11.1
Palestine	17	2	11.8
Syria	109	3	2.8
Tunis	19	3	15.8
People's Democratic Republic of Yemen	125	2	1.6
Israel	31	2	6.5
Tropical and South Africa	745	50	6.7
Angola	161	9	5.6
Benin	18	1	5.5
Ethiopia	307	12	4.0
Guinea	34	3	8.8
South Africa	12	1	8.3
Congo	29	3	10.3
Madagascar	30	1	3.3
Seychelles	6	2	33.3
Zimbabwe	16	2	12.5
Zambia	27	8	29.6
Mali	6	1	16.6
Nigeria	36	2	5.6
Guinea Bissau	13	2	15.4
Sierra Leone	4	1	25.0
Sudan	40	1	2.5
Cape Verde Islands	6	1	16.7
South-east Asia	321	10	3.1
Kampuchea	68	4	5.9
Laos	154	4	2.5
Vietnam	99	2	2.0
Middle and East Asia	403	14	3.5
Afghanistan	183	12	6.6
North Korea	98	1	1.0
Mongolia	122	1	0.8
Indian Subcontinent			
Bangladesh	8	1	12.5
Central America	180	11	6.1
Cuba	50	4	8.0
Mexico	3	1	33.3
Nicaragua	101	4	4.0
Dominican Republic	5	1	20.0
Costa Rica	21	1	4.8
South America			
Venezuela	11	1	9.1
Total	2,064	112	5.4

ELISA. The method of sandwich antibody titration with soluble antigen bound to solid phase was applied using peroxidase conjugate SwAHuIgG/Px (Sevac, Praha) and orthophenylendiamine as chromogen. Extinction was read on MICROELISA READER MR 580 (Dynatech). False positivity caused by nonspecific binding of immonoglobulins to polysterene plates was eliminated by saturating the sera with specific antigen; cross-reactions were eliminated by immunoblotting (Ditrich et al. 1988).

CIEP. Barbiturate buffer (pH 8.6) was used. Nonspecific precipitates mentioned in other papers (Blumengranz et al. 1981, Chan and Folds 1981) were eliminated by repeated examination of sera.

IFAT. The method was described in the paper by Ambroise-Thomas and Truong (1972). The source of antigen was mostly an axenic HK-9 strain of amoeba; polyxenic strains isolated in our laboratory were also successfully used (Ditrich et al. 1988).

RESULTS

Stool examinations. *E. histolytica* was demonstrated in 112 (3.9%) students. The students originated from 38 countries. In a set of 2,064 students from these countries, *E. histolytica* prevalence increased to 5.4% (Table 1). There were differences in the prevalence between individual geographical regions and between individual countries.

Antibodies against *E. histolytica*. In 1986—1988, a simple cross-section study using ELISA was performed (Molineaux 1981) in order to detect the frequency and level of antiamoebic antibodies in 1,001 foreign students (Table 2). Antibodies were detected in 7.9% of students at the following titres: 1 : 200 in 4.5%, 1 : 600 in 1.5%, and 1 : 1,800 in 1.9%. Students from South-east Asia exhibited the highest seropositivity. ELISA antibodies occurred more frequently in carriers of *E. histolytica* cysts ($t^2 = 14.9$ — the difference is detectable at 0.001 probability level) (Table 3).

CIEP test was used primarily for the examination of persons seropositive in ELISA and carrying *E. histolytica* cysts (Table 4). Therefore their number was reduced to 170. The character of antibodies detected by ELISA was verified in this way. CIEP is more suitable for the detection of active amoebiasis (Patterson et al. 1980,

Table 2. Results of serological examinations of 1,001 foreign students in 1986—1988 using ELISA with HK-9 antigen and SwAHuIgG/Px conjugate

Geographical region	South Europe	North Africa, Near and Middle East	Tropical and South Africa	Indian sub-continent
Titre	n = 19	n = 145	n = 341	n = 18
Negative	19	139	305	16
1 : 200	0	5	23	0
1 : 600	0	1	8	0
1 : 1,800	0	0	5	2
GMRT (from posit.)	0	240	346.4	1,800

Geographical region	South-east Asia	Middle and East Asia	Central America	South America
Titre	n = 292	n = 74	n = 78	n = 34
Negative	284	73	73	31
1 : 200	11	1	3	2
1 : 600	4	0	1	1
1 : 1,800	11	0	1	0
GMRT (from posit.)	600	200	386.6	288.4

Walsh 1986). The criterion of antibody distribution in carriers and non-carriers of *E. histolytica* was used also in this case. It has been demonstrated that CIEP antibodies occurred more frequently in *E. histolytica* carriers ($t^2 = 26.95$ — the difference is detectable below 0.001 probability level).

A comparison of the results obtained by ELISA and CIEP tests in carriers and non-carriers of *E. histolytica* confirmed that the result of CIEP test is more dependent

Table 3. Geographical distribution of ELISA antibodies against *E. histolytica* antigen in 982 foreign students in 1986—1988

Region	Carriers of *E. histolytica*			Non-carriers of *E. histolytica*		
	No. examined	No. positive (%)	GMRT	No. examined	No. positive (%)	GMRT
North Africa Near and Middle East	7	1 (14.3 %)	200	138	5 (3.6 %)	249.1
Tropical and South Africa	13	3 (23 %)	865.3	328	33 (10.1 %)	329.6
Indian Subcontinent	1	0	0	17	2 (11.8 %)	1,800
South-East Asia	25	8 (32 %)	688.3	267	18 (6.7 %)	564.4
Middle and East Asia	2	0	0	72	1 (1.4 %)	200
Central America	6	0	0	72	5 (7.0 %)	387
South America	2	1 (50 %)	200	32	2 (6.2 %)	346
Total	56	13 (23.2 %)	—	926	66 (7.1 %)	—

Table 4. Geographical distribution of CIEP antibodies against *E. histolytica* in 170 foreign students in 1986—1988

Region	Carriers of *E. histolytica*			Non-carriers of *E. histolytica*		
	No. of students	No. positive	% positive	No. of students	No. positive	% positive
North Africa, Near and Middle East	7	3	42.9	13	0	0
Tropical Africa	10	9	90.0	52	2	3.8
Indian Subcontinent	0	0	0	2	1	50.0
South-East Asia	24	8	33.3	30	5	16.7
Middle and East Asia	1	0	0	11	1	9.1
Central America	3	0	0	9	2	22.2
South America	1	0	0	7	0	0
Total	46	20	43.5	124	11	8.9

on the recent parasitization than that of ELISA test (Table 5). Table 6 shows the levels of antiamoebic antibodies in patients with a confirmed amoebic liver abscess. The levels of ELISA antibodies in patients before treatment were always above the titre of 1 : 1,800. Long-term observations of antibody dynamics in three patients revealed that the antibodies did not persist for longer than three years. The levels of antiamoebic antibodies were detected by ELISA also in 81 foreign students after a long stay in Czechoslovakia (Table 7).

Table 5. Correlation of results of CIEP and ELISA tests in 177 persons without manifested amoebiasis

E. histolytica	ELISA (titre)	CIEP +	CIEP —
Carriers n = 39	negative	4	21
	200	3	5
	600	1	0
	1,800	5	1
Non-carriers n = 138	negative	4	70
	200	3	37
	600	0	12
	1,800	7	5

DISCUSSION

The demonstrated 5.4% prevalence of *E. histolytica* corresponds to the mean prevalence in countries with warm climate (Elsdon-Dew 1985, Jedlička 1985, Walsh 1986), but highly exceeds the prevalence in the population of our country. The geographical origin of the examined students influenced the total prevalence of *E. histolytica* in the set of students (Dufek et al. 1967, Dufek and Kalivoda 1969, Chmelíková 1971, Giboda et al. 1982, Jedlička 1985, Zítová 1985).

It is interesting from the viewpoint of epidemiology and biology that in the majority of geographical regions (five of seven) *E. histolytica*, as to its prevalence frequency, ranks fifth behind *E. coli*, *E. nana*, *G. lamblia*, and *E. hartmanni*.

The records of antibodies in foreign students without a manifested invasive amoebiasis have the character of a seroepidemiological study and the seropositivity is a "background noise" (Patterson et al. 1980, Elsdon-Dew 1985) of the circulation of invasive strains in the given geographical regions (Table 2).

The total seropositivity of 7.9% revealed by ELISA is in agreement with the data published in large statistical surveys (Patterson et al. 1980, Elsdon-Dew 1985, Walsh 1986) and reflects the world representation of the examined persons. The frequency of 18.2% seropositive persons in the group of cyst carriers is high. The higher percentage of CIEP seropositivity of the whole set was also due to the selection of the examined individuals. Persisting antibodies are supposed to be present in some of the seropositive patients after treatment or spontaneous healing of particularly intestinal invasive amoebiasis. An evidence of this is the level of ELISA antibodies in an active phase of liver amoebiasis (Table 6, patients Nos. 4, 5, and 6) in the convalescing persons (patients Nos. 1, 2, 3), as well as negative records of antiamoebic antibodies in persons from countries with endemic amoebiasis after three or more years' stay in Czechoslovakia (Table 7). A question remains how to explain

Table 6. Antibody levels in patients with liver abscess and antibody dynamics in treated persons

Patient	Illness	Treatment	Result of treatment	Serological examinations		
				ELISA	CIEP	IFAT
1. T.N.D. 29 years Vietnam	July 1984	metronidazole emetine chloroquine tetracycline	healed ad integrum	1/1,800 1/200	28 months after treatment + 36 months after treatment negative	1/320 negative
2. K.M. 27 years Czechoslovakia	January 1986	ornidazole	residual cyst in liver hilum (30×35 mm)	1/1,800 negative	10 months after treatment + 20 months after treatment negative	1/160 negative
3. R.P. 27 years Czechoslovakia	July 1985	metronidazole chloroquine ornidazole	healed ad integrum	1/1,800 negative	16 months after treatment + 27 months after treatment negative	1/160 negative
4. P.P. 32 years Czechoslovakia	March 1987	metronidazole chloroquine tetracycline	healed ad integrum	1/3,600	before treatment +	1/256
5. Benda 36 years	3 December 1987	metronidazole chloroquine	clinical improvement	1/6,400	serum collected January 19, 1988 +	1/5,120
6. K.M.L. 23 years Kampuchea	October 1985	surgical	?	1/7,200	serum collected during operation +	1/640
7. Contents of liver abscess	?	surgical	?	1/14,400	abscess contents taken during operation +	1/1,280

Table 7. Antibodies in 81 foreign students (70 from Africa and 11 from Latin America) after a long stay in Czechoslovakia

Duration stay in Czechoslovakia	No. of students	Antibodies against *E. histolytica*
3 years	20	ELISA negative in all students
4 years	23	ELISA negative in all students
5 years	17	ELISA negative in all students
6 years	13	One student from Angola positive (1 : 600)
7 years	6	ELISA negative in all students
8 years	2	ELISA negative in all students

the higher positivity of *E. histolytica.* cyst carriers compared to non-carriers (4 times higher in CIEP and 3 times higher in ELISA) (Tables 3 and 4). A very probable explanation is the theory that *E. histolytica* strains imported by the students into Czechoslovakia consist of a population of biologically heterogeneous specimens. A minor proportion of the strain population may consist of invasive amoebae which may stimulate antibody formation, but the discrete changes in intestinal mucosa caused by them are insufficient for the propagation of manifested amoebiasis. Already Prodeus and Soloviev (1980) explained in this way the subdiagnostical antibody titres without illness in Soviet citizens staying in the tropics. Mirelman et al. (1986) also pointed out that a small number of invasive trophozoites may be present in the population of trophozoites of a non-invasive isolate. In their experiments these invasive trophozoites were responsible for the first successful axenization of a non-virulent strain.

These arguments are supported by our observations that discrete changes in the rectal mucosa are detectable also in cyst carriers, which will be the subject of the second part of our communication on the imported amoebiasis.

Acknowledgements. This work was partially supported by WHO-PDP/013/RB/84.310. We are indebted to Dr. P. Myjak of the Institute of Maritime and Tropical Medicine, Gdynia for kindly supplying us with antigen from HK-9 strain. Our thanks are due also to Dr. Lepš of the Biomathematical Laboratory, Biological Research Centre, České Budějovice for computing statistical tests and to physicians and nurses of language centres in Bohemia for their cooperation.

АМЕБИАЗ У ИНОСТРАННЫХ СТУДЕНТОВ

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Резюме. Обследовали на амебиаз 2883 иностранных студента в возрасте 18 –10 лет после их приезда в ЧССР. При исследовании стула было обнаружено присутствие *Entamoeba histolytica* у 112 студентов (3,9 %). Импорт *E. histolytica* был доказан у студентов из 38 стран. В группе 2064 студентов была обнаружена 5,4 % встречаемость *E. histolytica* в стуле. Были отмечены большие разницы во встречаемости между отдельными странами одной географической области, чем между отдельными географическими областями. Самая высокая встречаемость *E. histolytica* в стуле была найдена у студентов из тропической и южной Африки (6,7 % из 745 обследованных студентов) и самая низкая — у студентов из юго-восточной Азии (3,1 % из 321 обследованных лиц). При помощи теста энзиматической иммуноадсорбции (ELISA) определяли антитела к *E. histolytica* в сыворотках 1001 иностранного студента. Антитела встречались у 7,9 % студентов в следующих титрах: 1 : 200 у 4,5 %, 1 : 600 у 1,5 % и 1 : 1.800 у 1,9 %. Антитела встречались чаще у носителей цист *E. histolytica* ($\chi^2 = 14,9$). Титр антител ELISA у пациентов с абсцессом печени был выше, чем 1 : 1.800. С помощью встречного иммуноэлектрофореза (CIEP) исследовали прежде всего сыворотки студентов, положительно реагирующих в тесте ELISA и носителей цист

E. histolytica. Также здесь, в группе 170 студентов, антитела CIEP встречались чаще у носителей цист *E. histolytica* ($\chi^2 = 26{,}95$). Сравнением результатов тестов ELISA и CIEP было обнаружено, что антитела CIEP более зависимы от актуального паразитирования *E. histolytica*, чем антитела ELISA. В сыворотках 81 иностранного студента из Африки и Латинской Америки, пробывших в ЧССР дольше, чем 3 года, антиамебные антитела ELISA не встречались, за исключением одного лица с титром 1 : 600. У 3 пациентов после излечения амебного абсцесса печени антиамебные антитела ELISA непереживали больше 3 лет.

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Received 9 January 1989

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