5. Roges G, Edlinger E: Immunoenzymatic test for Q fever. Diagnostic Microbiology and Infectious Diseases 1986, 4: 125–132.
6. Behymer DE, Ruppanner R, Brooks D, Williams JC, Franti CE: Enzyme immunoassay for surveillance of Q fever. American Journal of Veterinary Research 1985, 46: 2413–2417.
7. Field PR, Hunt JG, Murphy AM: Detection and persistence of specific IgM antibody to *Coxiella burnetii* by enzyme-linked immunosorbent assay. A comparison with immunofluorescence and complement-fixation tests. Journal of Infectious Diseases 1983, 148: 477–487.
8. Peter O, DuPuis M, Peacock MG; Burgdorfer W: Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody test for detection of *Coxiella burnetii* antibody. Journal of Clinical Microbiology 1987, 25: 1063–1067.
9. Cowley R, Fernandez F, Freemantle W, Rutter D: Enzyme immunoassay for Q fever: comparison with complement fixation and immunofluorescence test and dot immunoblotting. Journal of Clinical Microbiology 1992, 30: 2451–2455.
10. Roges G, Edlinger E: Immunoenzymatic test for Q-fever. Diagnostic Microbiology and Infectious Diseases 1986, 4: 125–132.
11. Uhaa IJ, Fishbein DB, Olson JG, Rives CC, Waag DM, Williams JC: Evaluation of specificity of indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. Journal of Clinical Microbiology 1994, 32: 1560–1565.
12. Döller G, Döller PC, Gerth HJ: Early diagnosis of Q fever: detection of immunoglobulin M by radioimmunoassay and enzyme immunoassay. European Journal of Clinical Microbiology 1984, 3: 550–553.
13. Peter O, Dupuis G, Bee D, Luthy R, Nicolet J, Burgdorfer W: Enzyme-linked immunosorbent assay for diagnosis of chronic Q fever. Journal of Clinical Microbiology 1988, 26: 1978–1982.
14. Tellez A, Martin A, Anda P, de la Fuente L, Benitez P, García C, León P: Study of *Coxiella burnetii* human and animal seroprevalence in a rural population in Madrid community. European Journal of Epidemiology 1989, 5: 444–446.
15. Ruiz-Beltrán R, Herrero-Herrero JI, Martín-Sánchez AM, Martín-González JA: Prevalence of antibodies to *Rickettsia conorii*, *Coxiella burnetii*, and *Rickettsia typhi* in Salamanca province (Spain). Serosurvey in the human population. European Journal of Epidemiology 1990, 6: 293–299.

Evaluation of an Antigen-Capture Enzyme Immunoassay for Detection of *Entamoeba histolytica* in Stool Samples

T. Jelinek, G. Peyerl, T. Löscher, H.-D. Nothdurft

In order to identify the prevalence of *Entamoeba histolytica* in tourists with diarrhoea returning from countries of the developing world, sensitivity and specificity of a commercially available enzyme immunoassay (EIA) kit for the detection of *Entamoeba histolytica* coproantigen in stool were evaluated. Five hundred seventy-seven specimens from 469 patients were examined by microscopy and EIA. Sixty-two specimens from 49 patients were considered positive for *Entamoeba histolytica*. Compared with microscopic examination of stool samples, the EIA was found to be slightly more sensitive (90.3% vs. 87.1%) and was 97.7% specific for *Entamoeba histolytica*.

Diagnosis of intestinal amoebiasis is based on the demonstration of *Entamoeba histolytica* in stool or in biopsy of mucosal tissue but is labour and time intensive and depends on the skill of an experienced microscopist (1,2). It has recently been suggested that there are two distinct species of *Entamoeba histolytica* that are morphologically identical (1). *Entamoeba dispar*, the more prevalent of the two forms, appears to be associated solely with an asymptomatic carrier state. The pathogenic species, now referred to as *Entamoeba histolytica* sensu strictu, appears to have the capacity to invade tissue and cause symptomatic disease. Microscopical examination of a single stool specimen yields a sensitivity of 50 to 70%. At least three separate stool samples are required for a sensitivity of 90% (1). It is essential to have permanent stains of fresh or fixed faecal specimens in addition to a saline wet mount of stool (1). Specimens

Department of Infectious Diseases and Tropical Medicine, University of Munich, Leopoldstr. 5, 80802 Munich, Germany.

Table 1: Comparison of microscopy and antigen-capture EIA for the detection of *Entamoeba histolytica* in stool samples (n = 469 patients).

	Method	All specimens (n = 577)	Initial specimens (n = 469)
Results of microscopy and EIA			
Microscopy positive, EIA positive		50	25
Microscopy positive, EIA negative		2	9
Microscopy negative, EIA positive		33	31
Microscopy negative, EIA negative		492	404
Sensitivity of EIA vs. microscopy[a]		50/52 (96.2 %)	25/34 (73.5 %)
Sensitivity of each method vs. all true positives[b]	EIA	56/62 (90.3 %)	43/49 (87.8 %)
	microscopy	54/62 (87.1 %)	44/49 (89.8 %)

[a]Values are number of tests positive by EIA or microscopy / number of tests positive by microscopy.
[b]Values are number of tests positive by EIA or microscopy / number of "true-positive" tests. This includes specimens positive for *Entamoeba histolytica* by microscopy as well as EIA-positive and microscopy-negative specimens from patients who delivered subsequently microscopy positive stool samples.

must be either evaluated immediately after production or fixed, since trophozoites may disintegrate after 30 min at room temperature (1). Still, false-negative results may arise due to poor sensitivity of microscopic methods and to intermittent low-level shedding of cysts and trophozoites in stool. Due to these difficulties, considerable effort has been invested in the development of new technologies for the easy detection of *Entamoeba histolytica* in stool. Enzyme immunoassay (EIA) technology offers the advantage of being rapid, cost effective, and easy to perform on single or multiple stool specimens (2). A variety of such tests have been described recently, each detecting different antigens of *Entamoeba histolytica* trophozoites and cysts (2–7).

In order to identify the prevalence of *Entamoeba histolytica* in travellers suffering from diarrhoea after their return from countries of the developing world, we evaluated the sensitivity and specificity of a commercially available EIA kit (ProSpect/*Entamoeba histolytica*, Alexon, USA) detecting *Entamoeba histolytica* in stool samples. This EIA is designed to detect *Entamoeba histolytica*-specific antigen with monoclonal antibodies produced to the cultured HK-9 strain (2).

Patients and Methods. From June to September 1995, 795 randomised patients who presented for various medical complaints were recruited from our outpatient clinic. All patients were German nationals returning from vacation trips abroad. The main symptoms were diarrhoea in 469 (59%) patients, fever in 167 (21%), and various skin problems in 83 (10.4%), while 76 (9.6%) presented with the wish for a medical post-travel check-up without complaining of any symptom.

After informed consent was obtained, 577 stool specimens from all 469 patients with diarrhoea as a main symptom were collected and processed. All stool samples were investigated for bacteria by culturing and for ova and parasites by direct microscopy and the formol-ether-concentration technique (8). Every slide was read for at least 10 min by two experienced microscopists before being considered negative. One part of every fresh stool sample was immediately stored at −20°C and tested later by the ProSpect EIA (Alexon, USA), according to the manufacturer's instructions, by one technician who was blinded to the results of microscopy. Results were obtained by use of a microplate reader (SLT-Labinstruments, Germany) with wavelength capability of 450 nm. Samples with an optical density (OD) of ≥ 0.05 were considered positive. If discordant results between microscopy and EIA were obtained, both tests were repeated.

Microscopy was defined as the gold standard for the final results: only samples positive for *Entamoeba histolytica* by microscopy were considered positive. The definition of true-positive samples, however, was extended to all samples of patients who produced at least one stool specimen positive by microscopy, even if they initially presented with a series of negative specimens. Statistical analysis was performed using the EPI-Info 6.0 (Centers for Disease Control and Prevention, USA, and World Health Organization, Switzerland) software package.

Results and Discussion. Five hundred seventy-seven specimens from 469 patients were examined by microscopy and EIA. Sixty-two specimens from 49 patients were considered positive for *Entamoeba histolytica*. Twenty-five of the initial specimens were positive by microscopy and EIA (Table 1), and 404 were negative by both methods. Nine specimens were positive by microscopy and negative by EIA; these were considered false negative in the EIA. Thirty-one specimens were positive by the EIA and negative by microscopy; these were considered false positive in the EIA. Twenty-two of the 31 patients remained symptomatic and presented again for control examination, when they delivered up to three stool samples. Subsequent microscopy produced positive results in 19 of these patients: eight became positive with the second sample and 11 with the third. Three patients remained negative in one subsequent examination and were lost to further follow-up. Therefore, the samples of 12 patients had to be considered false positive in the EIA.

Defining each microscopy result per specimen as the gold standard, the EIA reached a sensitivity of 73.5% against microscopy. By using our definition for true-positive patients (a single positive specimen in row of negatives defines a patient as infected with *Entamoeba histolytica*), however, the sensitivity of the EIA increased remarkably (87.8%). The sensitivity of microscopy of single specimens was similar, at 89.8%. Somewhat different results were obtained for the evaluation of all 577 samples (Table 1): the sensitivity of EIA versus microscopy was 96.2%, of EIA versus all true-positive results 90.3%, and of microscopy versus all true-positive results 87.1%. By this definition, the positive predictive value of microscopy versus all true-positive results had to be 100%, while its negative predictive value was 98.5%. By contrast, the EIA reached a positive predictive value of 82.4% and a negative predictive value of 98.8%.

Cross-reactions of the EIA with other antigens were not observed. Of the 469 patients with diarrhoea, 119 (25.4%) had at least one parasite in addition to *Entamoeba histolytica* in their stool. No stool sample from any of these patients was positive in the EIA.

East and West Africa were the most frequently visited regions in the infected group: 13 (26.5%) of 49 patients had travelled to East Africa and 12 (24.5%) to West Africa, respectively. The next most visited regions were India (9 patients, 18.4%) and Southeast Asia (5 patients, 10.2%). Patients without amoebiasis had travelled less frequently to East Africa and West Africa (8.6% and 9.3%, respectively) and more frequently to India and Southeast Asia (19.2% and 20%, respectively).

Compared with microscopic examination of stool samples, the EIA was found to be slightly more sensitive (90.3% vs. 87.1%) and 97.7% specific for *Entamoeba histolytica* at an OD of ≥ 0.05 (Table 1). The technical properties of an EIA permit many specimens to be processed and read by a single technician without considerable variety in quality of performance in a short period of time. These findings correspond with previous studies (2). Therefore, the EIA is potentially more suitable than microscopy in certain settings, especially in epidemiological surveys and in follow-up examinations of patients known to be *Entamoeba histolytica* positive.

Ten of the 49 patients positive for *Entamoeba histolytica* presented with additional symptoms apart from diarrhoea, ranging from fever to nausea, vomiting, and joint pain. However, only three patients (6.1%) presented with dysenteric symptoms, which are assumed to be typical of invasive amoebiasis. These data show that amoebiasis should be considered in travellers with all types of diarrhoea who are returning from endemic areas, especially East Africa and West Africa, which were both significantly more frequently visited in the positive group compared to the negative group.

The examination of more than one stool specimen slightly improved the sensitivity of the EIA (Table 1). It seems advisable, therefore, to retain the practice of examining three stool samples before considering a patient to be uninfected. This is emphasised by the follow-up of 31 patients who had stool samples that were initially negative by microscopy but positive by EIA. Twenty-two of these patients presented again for control examinations and delivered up to three stool samples. Subsequently, microscopy produced positive results in 19: eight patients became positive with the second and 11 with the third sample. Three patients remained negative in one subsequent examination but were lost to further follow-up.

The results of this and other studies (2) show that a microscopy-negative but EIA-positive patient should be regarded and treated as infected with *Entamoeba histolytica*. The assay could become a useful epidemiological tool, and, in addition, could be helpful for the confirmation of clinically suspected amoebiasis and the monitoring of treated patients.

References

1. Ravdin J: Amebiasis. Clinical Infectious Diseases 1995, 20: 1453–1466.
2. Ungar BL, Yolken RH, Quinn TC: Use of a monoclonal antibody in an enzyme immunoassay for the detection of *Entamoeba histolytica* in fecal specimens. American Journal of Tropical Medicine and Hygiene 1985, 34: 465–472.
3. Das P, Sengupta K, Pal S, Das D, Pal SC: Biochemical and immunological studies on soluble antigens of *Entamoeba histolytica*. Parasitology Research 1993, 79: 365–371.
4. Haque R, Neville LM, Wood S, Petri Jr WA: Short report: detection of *Entamoeba histolytica* and *E. dispar* directly in stool. American Journal of Tropical Medicine and Hygiene 1994, 50: 595–596.
5. Jain U, Patel MT, Desai PK, Kaliraj P: Development of simple and stable ELISA for detection of copro antigen in intestinal amoebiasis. Indian Journal of Experimental Biology 1990, 28: 671–675.
6. Sharma M, Reed SL, Singh S, Talwar GP, Ghosh S: Characterization of monoclonal antibodies to conserved antigens of *Entamoeba histolytica* and *E. dispar* and development of a stool ELISA. Hybridoma 1994, 13: 123–130.
7. Vinayak VK, Kumar P, Punj V, Vashist N, Kauwar JR, Bhasin DK, Singh K: Detection of *Entamoeba histolytica* antigens in stool in amebiasis. Indian Journal of Gastroenterology 1993, 12: 77–79.
8. Garcia L, Bruckner D: Diagnostic medical parasitology. American Society for Microbiology, Washington DC, 1993.

Lack of Evidence of Nosocomial Cross-Infection by *Burkholderia cepacia* among Danish Cystic Fibrosis Patients

H.C. Ryley[1]*, B. Ojeniyi[2], N. Høiby[2], J. Weeks[1]

Burkholderia cepacia isolates from nine of the ten Danish cystic fibrosis (CF) patients known between 1975 and the present day to carry this organism were investigated. Eight distinct genotypes were found with polymerase chain reaction ribotyping and pulsed-field gel electrophoresis. The results indicate that there is little patient-to-patient cross-infection with *Burkholderia cepacia* within the Danish CF population, even though the majority of patients attend the same CF clinic on a regular basis.

Recently, it has been realised that primary acquisition of *Burkholderia cepacia* in cystic fibrosis (CF) patients may be associated with changes in clinical condition, ranging from asymptomatic carriage to rapid and fatal deterioration (1, 2). The possibility of severe deterioration coupled with the broad-spectrum antibiotic resistance of this organism and evidence of patient-to-patient cross-infection have had a profound effect on the clinical management and social life of such patients. Many CF centres and clinics have implemented segregation policies for attendees carrying this organism. The evidence that person-to-person transmission is an important mode of acquisition of *Burkholderia cepacia* is based on a number of epidemiological studies from the UK and North America (3–6). However, in at least one study no evidence of person-to-person transmission could be demonstrated (7).

Currently, in Wales, about 4% of CF patients carry *Burkholderia cepacia*, and up to 1994 all had been attending either the paediatric or adult CF clinics in Cardiff prior to detection of the organism. Within each clinic, one strain type was found in about 60% of infected patients, although the main strain types differed between centres and were also different from the so-called "epidemic" strain found in many infected patients attending other CF centres in the UK (8). Thus, although there are clinical benefits to be had from attending a CF centre, it appears that there might be an increased risk of acquiring *Burkholderia cepacia*.

In Denmark, most of the 330 patients with CF attend a clinic either in Copenhagen or in the Århus CF centre and undergo regular hospitalisation for antibiotic therapy if they are carrying *Pseudomonas aeruginosa*. The incidence of *Burkholderia cepacia* is reported as low, and this has been ascribed to the strict hygiene methods used within the clinics (9). In this study we characterised the strain types occurring among Danish *Burkholderia cepacia* isolates in an attempt to estimate the importance of nosocomial infection in the CF population carrying this organism.

[1]Department of Medical Microbiology, University of Wales College of Medicine, Heath Park, Cardiff CF44XN, UK.

[2]Department of Clinical Microbiology 9301, Rigshospitalet, Juliane Maries Vej 22, DK-2100 Copenhagen 0, Denmark.