

Amebic Liver Abscess Diagnosed by Polymerase Chain Reaction in 14 Returning Travelers

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Abstract. Amebic liver abscesses (ALA) are not commonly described in travelers. The ALA diagnosis is usually based on serology and *Entamoeba histolytica* polymerase chain reaction (PCR) is a new tool. We retrospectively reviewed all ALA cases diagnosed by PCR on the liver abscess pus aspirate of patients admitted in French hospitals between 2007 and 2011. Fourteen cases (10 male, median age 48 years) were included. The median lag time between return and onset of symptoms was 23 days (interquartile range [IQ] 18–24). All patients had an elevated cardiopulmonary resuscitation level, and 11 had leukocytosis. The ALA was multiple in five patients, localized in the right lobe in 12, and higher than 5 cm in 11. Serology was initially negative in one patient, whereas PCR was positive. There was bacterial co-infection in one patient. The outcome was good. Liver puncture allows a rapid diagnosis of ALA with PCR and helps identify the association with a bacterial dual infection.

INTRODUCTION

Amebiasis is a parasitic disease caused by a protozoan, *Entamoeba histolytica*, which may cause acute diarrhea, colitis, and liver abscess.^{1,2} Whereas amebiasis is an important cause of acute diarrhea after travel, amebic liver abscess (ALA) is less commonly found in febrile returning travelers.³ In addition, ALA is difficult to differentiate from bacterial liver abscesses given that clinical, biological, and imaging characteristics are unspecific.

Diagnosis of ALA is usually based on a positive amebiasis serology but this serologic test lacks specificity because of the long-term persistence of positive serology in healthy patients exposed in endemic areas in patients with a previous history of amebiasis.⁴ It may also be caused by lack of sensitivity, because of delayed seroconversion leading to a delay or misdiagnosis.

Molecular biology has helped to fill this gap. Identification of *E. histolytica* by polymerase chain reaction (PCR) was first used in 1991.⁵ Since its discovery, PCR and then real-time PCR have been increasingly used for amebiasis diagnosis and showed to provide rapid, sensitive, and specific results, particularly in ALA.^{1,6} However, this method has mostly been evaluated in endemic countries,^{7–14} whereas the use of PCR in ALA in Western countries is less commonly described.¹⁵

We describe the epidemiological, clinical, biological, and radiological aspects of ALA diagnosed by *E. histolytica* PCR applied on the pus of liver abscess aspirate in returning travelers to France.

MATERIALS AND METHODS

We retrospectively reviewed all ALA cases diagnosed by *E. histolytica* PCR in the pus aspirate of liver abscess between January 2007 and August 2011. All returning travelers with positive PCR in ALA samples that were registered in the database of the Mycology and Parasitology teaching laboratory of the Pitié-Salpêtrière hospital were included. The samples were obtained from the four different teaching hospitals that belong to our university (Pierre et Marie Curie) in Paris: Pitié-Salpêtrière, Saint Antoine, Tenon, and Trousseau hospitals.

All patients with a diagnosis other than liver abscess, patients with positive PCR in a sample other than liver pus aspirate, and patients with positive amebiasis serology but without PCR performed on liver samples, were not included. Foreigners (i.e., persons living in endemic areas and visiting France for tourism or medical reasons) were not included to describe only ALA in travelers.

We evaluated the following variables: epidemiological data (age, gender, and citizenship), travel characteristics (purpose, destination, and duration), lag time between return from country of acquisition and onset of symptoms as well as the admission date, medical history and previous treatment, clinical presentation, biological results, and imaging features. Among the travelers, we distinguished the immigrants (foreign born living in France and returning from visiting friends and relatives), the expatriates (French born living in a tropical country for > 6 months), and tourists and humanitarian workers (French born visiting and working in a foreign country for < 6 months). Country of acquisition was defined as the last visited endemic area.

These variables were collected retrospectively in the medical charts. As far as ethical considerations are taken into account, the French National Commission on Informatics and Liberties authorizes the retrospective use of anonymous patient files on the site of patient care. All data were anonymized in a standardized case report form and entered into the database. Data were analyzed with Microsoft Excel version 12.2.8 (Microsoft Corp., Redmond, WA).

The median and interquartile ranges (IQ) were used for most of the continuous variables. Some of the latter were categorized following the laboratory cut-off values and dichotomized because of the small sample size. Clinical and biological data were defined or categorized according to the definition of

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sepsis¹⁶: temperature > 38.3°C, heart beats rate > 90/minute, systemic blood pressure < 90 mm of Hg, white blood cell count (WBC) > 12.10⁹/L, tachypnea > 20/min, thrombocytopenia < 100.10⁹/L. More than three liquid stools per day defined diarrhea. Hepatic cytolysis was defined by the rise of alanine aminotransferase (ALAT) and/or aspartate aminotransferase (ASAT) up to twice the normal values. Cholestasis was defined by the rise of alkaline phosphatase and/or gamma-glutamyl transpeptidase (GGT) superior to twice the normal values. Abdominal ultrasound (US) or computed tomography (CT) scan was performed to characterize the number and size of the abscesses. The latter were split into two classes: < 5 cm and greater than or equal to 5 cm as this size has been considered as an indication for aspiration of ALA.¹⁷

Amebiasis serologic tests were performed in the Parasitology and Mycology laboratories of the Pitié-Salpêtrière (eight patients) or Saint Antoine (six patients) hospitals, depending on where the patient was admitted. In Pitié-Salpêtrière hospital, three methods were used: indirect immunofluorescence assay (IIF) (slide Amoeba-Spot IF, BioMérieux, Marcy l'Etoile, France; conjugate anti-IgG, A, M, Biorad, Marnes la Coquette, France), indirect Hemagglutination test (IHA) (Amebiasis Fumouze, Levallois-Perret, France), and electrosyneresis performed with *E. histolytica* antigen (SR²B, Paris, France). The IIF was considered as positive if antibodies were greater than or equal to 1/100; IHA was considered as positive if greater than or equal to 1/320; and electrosyneresis, a qualitative test, was considered as positive if there was at least one band. In St. Antoine Hospital the methods used were Bichro-Latex Amibe (Fumouze, Levallois-Perret, France), a qualitative agglutination test of antibody-sensitized latex particles, and ELISA RIDASCREEN *E. histolytica* IgG, (R-Biopharm GmbH, Darmstadt, Germany). The latter test was considered as positive if > 1.1, negative if < 0.9, and equivocal if 0.9–1.1.

All the patients underwent either pus aspiration of liver abscess through radiological exploration or surgical drainage of abscess. All the pus samples were sent to the laboratory of the Pitié-Salpêtrière hospital for PCR analysis. The DNA was extracted using QIAmp DNA mini kits spin columns (Qiagen).

Before 2009, the method performed for amplification and detection was a conventional PCR described by Acuna-Soto and others¹⁸ in 1993 using Mastercycler gradient thermal cycler (Eppendorf). The amplification reactions were performed using 10 µL of DNA extract in a volume of 40 µL reaction mixture that contained a 1× of master mix from Applied Biosystems, 29.25 µL of H₂O, 3 µL of MgCl₂ (25 mM), 1 µL each of forward and reverse primer (0.5 µM) and 1,75 U of Taq polymerase. The primers for *E. histolytica* and *E. dispar* were described by Acuna-Soto and others (24) (Table 1). The thermal cycling conditions consisted of 1 cycle of 4 min at 95°C, 30 s at 55°C, and 30 s at 72°C followed by 41 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C and last cycle of 30 s at 95°C, 30 s at 55°C, and 5mn at 72°C. After 2009, the method performed for amplification and detection was a real-time PCR using a TaqMan 7500 Fast Real-Time PCR System (Applied Biosystems) in under 40 minutes.^{11,19} The amplification reactions were performed using 5 µL of DNA sample in a volume of 20 µL of a mixture that contained a TaqMan Fast Universal PCR Master Mix (2×) (Applied Biosystems), internal positive control (Applied Biosystems), and the primers for *E. histolytica* and *E. dispar*:

TABLE 1

Clinical, biological, and radiological categorical variables of 14 returning travelers with amebic liver abscess diagnosed by polymerase chain reaction (PCR)

Variables	Number of patients
Clinical picture	
Time from clinic onset to admission > 7 days	7/14
Time from the last travel to endemic area > 2 years	4/14
Reason of consult	
Fever	14/14
Diarrhea	5/14
Abdominal pain	11/14
Heart rate ≥ 90/min	8/13
Temperature > 38°C	13/14
Chills	7/14
Diarrhea	3/14
Anorexia	3/11
Asthenia	5/11
Hepatomegaly	2/14
Right hypochondrial pain	12/14
Defense and/or contracture	5/14
Biology	
WBC > 12.10 ⁹ /L	11/14
Platelet count < 100.10 ⁹ /L	0/13
Hemoglobin < 10 g/dL	2/13
CRP > 100 mg/L	14/14
Fibrinogen ≥ 7 g/L	7/8
SGOT and/or SGPT ≥ 2N	2/13
APL > 120 IU/L	8/12
GGT > 30 IU/L	11/13
Cholestasis*	11/13
Characteristics of abscess in imaging studies (ultrasound or CT scan)	
Size ≥ 5 cm	11/14
Number of abscesses > 1	5/14
Localization	
Right lobe	12/14
Left lobe	1/14
Left and right lobes	1/14
Effusion	
Pleural	3/14
Peritoneal	3/14

WBC = white blood cells; CRP = C-reactive protein; SGPT/SGOT = serum glutamic pyruvic transaminase/serum glutamic-oxaloacetic transaminase; APL = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase; CT = computed tomography.

*Cholestasis: APL > 120 IU/L and/or GGT > 30 IU/L; §2N: twice the normal.

Eh -196F (5'-AAA TGG CCA ATT CAT TCA ATG A-3')
 Ed-185F (5'-GTA TTA GTA CAA AGT GGC AAT TTA TGT-3')
 Ehd-294R (5'-CAT TGG TTA CTT GTT AAA CAC TGT GTG-3') and the probe Eh/Ed-245/246 (6FAM)-AGG ATG CCA CGA CAA-(NFQ). The thermal cycling conditions were chosen according to TaqMan Fast protocol (Applied Biosystems) and it consisted of 20 s at 95°C followed by 50 cycles of 3 s at 95°C, and 30 s at 60°C. The detection and the data analysis were performed with TaqMan Fast 7500 software (Applied Biosystems) version 1.4.0.

The patients were evaluated for the efficacy and tolerance of the treatment that was based on a combination of antibiotic therapy by metronidazole and pus aspirate by liver puncture or surgical drainage. The minimum duration of follow-up was 8 weeks. Success was defined by the disappearance of clinical signs and the return of biological parameters within normal limits at 2 weeks.

RESULTS

During the study period, 14 ALA diagnosed by PCR on liver abscess samples were identified in travelers. Ten were men and 4 were women. The median age was 48 years (IQ 36–60). Ten patients were born in Europe: 3 in sub-Saharan

Africa, and 1 in North America. Nine were defined as tourists, 3 as VFRs (visiting friends and relatives), 1 as a French expatriate, and 1 as a humanitarian worker. Among the 14 patients, 7 were referred by the emergency department, 7 by their general practitioner. The most recently visited endemic countries were in Africa (6 cases: Mali in 2, Tunisia, Congo, Ivory Coast, and Senegal in 1 each), in Asia (6 cases: 3 in India and 1 in Cambodia, Vietnam, and Nepal), and in Latin America (2 cases: 1 in Uruguay and Guadeloupe, in the French West Indies). The median duration of travel was 30 days (IQ 18–34). Among the 10 patients who had traveled to an endemic area during the previous year, the median time between return and onset of the symptoms was 23 days (IQ 18–24). For the remaining 4 patients, the last travel in the endemic area occurred more than 2 years before. The median time between onset of the symptoms and admission was 8 days (IQ 5–15). Seven patients had received empirical antibiotic therapy for fever during or just after travel and 4 patients received ceftriaxone and metronidazole for undocumented liver abscess during the first days of hospitalization and before abscess puncture or drainage. The most common clinical signs at admission were fever, right hypochondrial pain, tachycardia, chills, and diarrhea (Table 1). Eleven patients had a sepsis. All patients had a biological inflammatory syndrome: C-reactive protein (CRP) level superior to 100 mg/L in 14 and white blood cells (WBCs) superior to $12.10^9/L$ in 11. Two patients had a hepatic cytolysis and 11 had a cholestasis. Abdominal US were performed in 13 patients and abdominal CT scan in all patients. Nine patients had a single abscess, whereas 5 had more than one abscess. Among these latter patients, 1 was found with multiple liver lesions, 2 with 5 lesions, 1 with 4 lesions, and 1 with 2 lesions. In 11 cases, the size of the biggest abscess was higher than 5 cm. In 12 cases, ALA was localized in the right lobe of the liver, in one case in the left lobe, and one abscess was localized at the limit between left and right lobes. There was an effusion of pleural and peritoneal liquid in 3 patients, respectively (Table 1).

Direct microscopic examination and cultures of the stool were performed in 10 and 5 patients, respectively, and all were negative. At admission, serology for amebiasis was positive in all patients except one for whom it became positive the following week. Two patients underwent surgical drainage. Nine patients had a percutaneous CT-guided liver abscess puncture and 3 patients had a US-guided puncture. Of the 14 patients, 11 underwent abscess drainage with a catheter during 5 days (IQ 5–8,3). The sample aspirate was purulent in 8 cases, hemorrhagic in 1 case, and “chocolate syrup”-like in 3 cases. Culture of the pus drained from the abscess and blood cultures were positive for *Staphylococcus aureus* in 1 patient. All patients were hospitalized and treated with intravenous and then oral metronidazole (1.5–2 g/day according to the weight) during 14 days (IQ 12–16). The median duration of hospitalization was 8 days (IQ 6–15). All patients were cured at 8 weeks.

DISCUSSION

To the best of our knowledge, this is the largest series of ALA cases imported into Western countries by travelers and diagnosed by PCR on *in situ* samples. Because PCR is more sensitive and more specific than serology,¹⁰ this study provides a clearer picture of ALA in returning travelers. Most of the

series of ALA have been reported in endemic countries, such as in Asia, where ALA was diagnosed by serology.^{20–22} However, in most recent years, three series of ALA diagnosed by PCR in ALA samples have been reported in Bangladesh and India.^{9,10} In contrast, there are only three case series of ALA performed in non-endemic countries: one in France where the diagnosis was based on serology²³ and two in Spain where the diagnosis was based on serology and PCR in abscess sample.¹⁵

The interest of this study was that its population was exclusively composed of travelers, mainly tourists and African migrants living in France. The latter were VFR returning to their country of origin in Africa, which can be explained by the fact that 41% of the migrants in France come from Africa.²⁴ In the three other case-series performed in non-endemic countries, a few patients did not travel but had sex with returning travelers or persons from endemic areas.¹⁵ Indeed, sexual relationships, particularly oro-anal sex, have been associated with the transmission of amebiasis.²⁵

In contrast to the studies performed in endemic countries, those performed in travelers in non-endemic countries provide a more accurate evaluation of the incubation time of ALA, which is unknown.^{1,26} Of importance, in our study the mean lag time between return and onset of symptoms was 23 days in 10 patients relating to a recent travel, providing an idea of the incubation time period of ALA. However, some patients may have been contaminated during a previous travel as we only took into account the last travel into endemic areas. In addition, ALA occurred more than 1 year after the last travel into an endemic country in one-third of the patients. This shows that the incubation period can also be prolonged.

As far as clinical presentation, biological results, and imaging features are concerned, patients' characteristics did not differ from previous studies. Clinical presentation of ALA is well characterized by the combination of fever and abdominal pain (most often in the right upper quadrant).^{21,22} The ALA is associated with an inflammatory blood syndrome with a high WBC level and elevated CRP. In most cases there is no hepatic cytolysis and cholestasis is more common.^{21,23} The CT scan is more efficient than ultrasounds to evaluate the number of abscesses, their size, and localization. Most patients with ALA present with a single large (> 5 cms) abscess located in the right lobe.^{21–23}

Direct microscopic examination for parasites in the stools and culture were always negative when it was performed, which is consistent with results of previous studies.²⁷ Diarrhea is a symptom described in 10% to 66% of patients with ALA.^{1,26,28} Three of our patients had diarrhea at admission and two underwent a colonoscopy, which showed erosive or ulcerative colitis (probably amebiasis, but with no microbiological proof).³

Serodiagnosis is considered as the gold standard^{1,29} for diagnosis of ALA but it lacks the sensitivity as illustrated in one of our cases where the diagnosis relied on positive PCR, whereas seroconversion for amebiasis was delayed. In addition, false-positive results are common in endemic areas, in particular in patients with a previous history of amebiasis.^{30,31} Therefore, PCR may be a very useful tool to diagnose ALA as illustrated in some case series where the sensitivity of PCR in the ALA sample ranged from 80% to 100%.^{9–11,15} In these studies, negative PCR could be explained by the fact that these patients were already treated with metronidazole at the time of liver abscess puncture, which can negative the PCR. Real-time PCR has also been evaluated in blood,

urine, and saliva to detect *E. histolytica* in the case of proven ALA with a sensitivity of 49%, 77%, and 69%, respectively, and 100% specificity,⁹ however we did not perform PCR in these samples.

Bacterial superinfection was found in one patient in this study, which had been scarcely described previously. It should be suspected in patients with uncertain diagnosis, such as those who have not responded to metronidazole therapy and lead to liver aspiration. This invasive procedure should also be performed in individuals with large left-lobe abscesses and severely ill patients with an imminent risk of rupture.²

This study presents some limits. Retrospective evaluation from clinical files may lead to information bias, especially for the clinical description and the lack of microbiologic data. There is an inclusion bias as all of our patients underwent abscess drainage or aspiration, which is not always necessary in ALA, particularly in the case of small abscesses. We thus may have selected the most severe cases. Finally, the sample size is quite small compared with series in endemic countries, where those that are imported are scarce.

CONCLUSION

Amebiasis should always be suspected in every case of liver abscess in travelers returning from endemic areas regardless of the time that has elapsed since their last travel. According to our results, *E. histolytica* PCR on liver abscess samples is an interesting tool for early diagnosis and adapted treatment.

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