Amoebiasis – what’s the problem?

TON POLDERMAN

Amoebiasis

Amoebiasis has been known for a long time as a common, invasive and potentially fatal protozoal infection with a cosmopolitan distribution. It is caused by Entamoeba histolytica, a small protozoan that lives first of all in the human large intestines. There the mobile ‘trophozoites’, (the ‘histolytica’-stages) invade the intestinal wall and a dysentery is the prominent clinical consequence. The patient produces a bloody and dirty diarrhoea. He can be really ill. Often periods of invasive disease are of an intermittent nature and interspersed with periods of months or even years of unapparent, non-invasive infection. Sometimes the amoebic trophozoites do not only enter the intestinal wall but they may pass it and be swept into the liver. An amoebic liver abscess may develop. This may happen a few weeks after the initial intestinal infection but it can happen with a delay of many years as well. An amoebic liver abscess can develop rapidly; if untreated it is fatal. It is, therefore, not surprising that amoebiasis earned a reputation as one of the great tropical infections and often it was referred to as the second most important protozoal infection, second only to malaria. Transmission is through the faecal-oral route: the trophozoites lose motility, get rounded and form a solid wall. These ‘cysts’ are excreted with the stools and if ingested by another host, they give rise to a new infection.

Diagnosis

Classically the laboratory diagnosis was based on the demonstration of the actively moving amoebic trophozoites, characterized by several morphological features and by the presence of erythrocytes of the human host. This is not easy: it requires microscopic examination of very fresh stool samples, i.e. not older than about 1 hour. In the classical approach of a small rural hospital such diagnosis could be done; in modern times, however, this is not easily organized. Moreover, the actively moving invasive trophozoites
should be differentiated from the more sluggish non-invasive ones. As a result, over time, diagnosis has gradually shifted from recognition of the characteristic trophozoites to stool examination to detect the cysts. That is easier because there is no need to examine very fresh stools; the presence of cysts is less intermittent, routine concentration methods can be used, and differentiation from the non-pathologic related species is based on morphological characteristics of the cysts and is considered fairly simple. In changing the approach in diagnosis, another major change was concurrently taking place: from detection of the acutely disease-causing phase of the parasite to demonstration of the mere presence of a potentially invasive but presently non-pathogenic phase. In fact, this was a misleading change of focus!

**Invasive and non-invasive species of Entamoeba**

For a long time clinicians and parasitologists have wondered why infection resulted in disease in some patients but not in others. Differences in nutritional status of the host, in the presence of other underlying diseases as well as in that of the strain of the parasite were thought to be responsible. In the seventies of last century iso-enzyme analysis showed consistent strain differences between parasites that proved invasive and those that remained non-pathologic. Later, in the nineties, molecular analysis demonstrated that amoebae excreting ‘characteristic’ four-nucleated cysts with the stools of infected humans differ genetically and significantly and a commensal, non-pathogenic species *Entamoeba dispar* was differentiated from the pathogenic species *Entamoeba histolytica*. (e.g. Aguirre et al., 1994; Verweij et al., 2000). Characteristic erythrocyte-engulfing vegetative stages are not produced by the commensal species but the cysts and non-invasive trophozoites of both species are morphologically identical and cannot be differentiated microscopically. It became clear that the common presence of *E. histolytica*-like parasites in temperate zones, like The Netherlands, was in fact due to *E. dispar*, and not to *E. histolytica sensu stricto*. The epidemiological consequences of that observation, however, required time to be fully understood.
Amoebiasis as a public health problem

In many tropical countries amoebiasis is recognised as an important cause of dysentery and diarrhoea, and metronidazole—the drug of choice to treat amoebiasis—is widely available over the counter, anywhere in Africa and elsewhere. In the process of giving advice to tourists and other travellers to the tropics, amoebiasis plays a central role as well: the advice to avoid eating fresh salads, tomato’s etc, is largely based on the notion that this is the best way to avoid amoebic dysentery. Indeed, amoebiasis is a well-accepted component of health risks in a great number of tropical areas and we developed ways of dealing with that risk.

A prospective research

In the year 2000 Amha Kebede, working at the Ethiopian Health and Nutrition Research Institute in Addis Ababa, started an investigation into the potential role of amoebiasis as a potentially dangerous opportunistic infection. The prevalence of amoeba-infections was considered high and in the context of a large HIV-AIDS project more information was required on the various opportunistic infections. A retrospective analysis of microscopic diagnostic laboratory findings, as reported in the annuals reports of Wonji Hospital, indicated that indeed ‘amoebiasis’ was the most common intestinal parasitic infection: ‘E. histolytica’ was demonstrated in 30% of the in- and out-patients, between 1988 and 1998 (Fontanet et al., 2000). Species-specific molecular analysis in a number of prospective studies carried out by Amha Kebede, and guided and supervised by Prof Beyene Petros of Addis Ababa University and our Department of Parasitology in Leiden, using PCR-SHELA first and real-time PCR later, gave rather unexpected results, requiring a very different interpretation of cases of chronic and episodes of recurrent diarrhoea. (Amha Kebede, 2005)

A better understanding of amoebiasis as a cause of diarrhoea and disease was desired by parasitologists. Patients suffering from dysentery in Ethiopia’s countryside had a somewhat different agenda: dysentery and diarrhoea are widely experienced as a burden and more effective and cheaper methods, not requiring several days of unpleasantly bitter Flagyl, were desired. For the Ministry of Health, for Departments like the Ethiopian Health and Nutrition Research Institute and for Programs dealing with patients with diarrhoea, information on the real causes to more effectively cope with the problem were of importance as well. Amha’s studies were, therefore, consid-
ered of relevance by all parties involved: by the patients, the Ministry, and researchers in Parasitology.

**Results and reading the findings at different levels**

In subsequent prospective studies consistent findings were recorded, as summarized in table 1.

The invasive *Entamoeba histolytica* appeared to be almost absent and virtually all cyst-excreters were shown to be infected with the non-invasive *E. dispar* (Kebede et al., 2004b). This was considered good news. Retrospective analysis of cases of liver abscesses possibly due to *E. histolytica* in the country’s main hospital confirmed that indeed liver-manifestations too, were in fact much rarer than believed hitherto (Amha Kebede et al., 2003; Amha Kebede, 2005). The parasitological project came to an end: the problem originally believed to be a major and common problem was shown to be rare, not requiring further in depth study.

The medical profession in Ethiopia was informed about Amha’s findings in various official and unofficial meetings and most explicitly so during the XXXIXth annual conference of the Ethiopian Medical Association. The reactions were quite different from those of the parasitologists. They reflected a feeling of deception and frustration: amidst a wide gamma of medical problems in the country, one was not pleased seeing one of the few certainties removed. While supported by a large body of laboratory-based and clinical evidence, despite Amha Kebede’s capacity to vividly describe the evidence obtained during years of field work in the country, and even with the supervision and support of the local supporting agencies and supervising professor, the findings were sceptically received and not accepted. Up until today, almost 8 years later, little has changed in the Medical Profession’s attitude towards amoebiasis in the country.

At the level of the individual patient, no change in acceptance and attitude towards ‘amoebiasis’ was to be expected. The fame of amoebiasis as an important diarrhoea-causing infection hasn’t faded and people continue to buy metronidazole when they feel like it. Indeed, doctors do not express any contradictory suggestions, if requested.
Interpretation and conclusion

As a parasitologist, co-supervising the project I saw the project ending with ambiguous feelings. The parasitological research project as such was very successful, giving a clear and unmistakable insight in the nature of amoebic infections in man, in Ethiopia. At the same time we had to conclude we failed to successfully transfer the message to the parties involved: to doctors and patients. How did this happen?

First we should conclude that a project like the present one was a research project and not a health project. That is the way it was set up. Patients were not asked for their view, nor were doctors in Health Centres and hospitals. They were simply told about the results. The real aims of the project got mixed up: was it a project on (amoebic) dysentery patients were suffering from, or was it a project on *E. histolytica* and *E. dispar*? Indeed, the question on the parasitological problem was solved but the diarrhoea remained and no clue was given how to deal with it more effectively. We, as parasitologists, should be aware of the implications of that choice and we should be modest in our expectations of the impact of the results of our research.

Second, we were insufficiently aware of the fruitful mutual understanding between lab-technicians and doctors. The doctor’s suspicion of a dysenteric patient having amoeba is easily supported by the lab-technician finding cysts or moving trophozoites of one sort or another in the patient’s stools, and the lab-technician on his turn sees his capacity to identify cysts and trophozoites – that are difficult to identify – confirmed by the doctor’s clinical diagnosis. Doctor and technician confirm each other.

Third, the process of mutual confirmation is greatly enhanced by the fact that the drug of choice for amoebiasis, metronidazole (*Flagyl*), has some antibiotic efficacy as well. It kills gram negative bacteria, incriminated in many diarrhoeas. Treatment with metronidazole did not result in curing amoebiasis because amoeba were not there, but it did have some effect on the intestinal flora. The effects of metronidazole were easily but mistakenly interpreted as a proof of a correct diagnosis of ‘amoebiasis’.

It can only be concluded, once again, that an even stronger interaction between biomedical research and social and patient-oriented approaches is essential. The research, although embedded in local Ethiopian structures and co-supervised by highly esteemed Ethiopian authorities, required a more robust integration in the local health structures to achieve a real impact for the patients involved. Further, non-parasitological research into the real nature of what is believed to be ‘amoebic diarrhoea’ needs to be done to achieve a real impact for the people involved.
Acknowledgements

This rather personal view on amoebiasis in Ethiopia could only mature thanks to all laboratory and field work carried out by Dr Amha Kebede in Addis Ababa and the continuous support, assistance, and technical leadership of Dr Jaco Verweij in Leiden.

References


1997 Diagnosis of *entamoeba histolytica* and *entamoeba dispar*. In Clinical specimens by Pcr-Shela. Archives of Medical Research, 28, S282-S284.


Kebede, A., Kassa, E., Ashenafi, S.Dorigo, W., Sanders, E., Polderman, A.M. & Petros, B.


Kebede, A., Verweij, J., Dorigo, W., Sanders, E., Messele, T., Van Lieshout, L., Woldemichael, T., Petros, B. & Polderman, A.M.


Kebede, A., Verweij, J.J., Petros, B. & Polderman, A.M.

2004b Short communication: Misleading microscopy in amoebiasis. Tropical Medicine and International Health, 9, 651-2.


Kebede, A.


Verweij, J.J., Blotkamp, J., Brienen, E.A.T., Aguirre, A. & Polderman, A.M.

2000 Differentiation of *entamoeba histolytica* and *entamoeba dispar* cysts using polymerase chain reaction on dna isolated from faeces with spin columns. European Journal of Clinical Microbiology and Infectious Diseases, 19, 358-361.
<table>
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<tr>
<th>Locality</th>
<th>Subjects</th>
<th>N</th>
<th>% positive in microscopy</th>
<th>% positive in E. histolytica-PCR</th>
<th>% positive in E. dispar-PCR</th>
<th>published</th>
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<tr>
<td>Wonji Hospital</td>
<td>Retrospective data hospital laboratory</td>
<td>154,425</td>
<td>30%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Amha Kebede, 2005</td>
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<td>Wonji/Metahara</td>
<td>Population sample</td>
<td>225</td>
<td>24.9%</td>
<td>0%</td>
<td>18.2%</td>
<td>Amha Kebede et al, 2004c</td>
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<tr>
<td>Wonji/Metahara</td>
<td>Diarrhoea patients, mainly adults</td>
<td>246</td>
<td>39.8%</td>
<td>0%</td>
<td>14.9%</td>
<td>Amha Kebede, 2005</td>
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<td></td>
<td>3 primary schools in different parts of the country</td>
<td>363</td>
<td>26.7%</td>
<td>0%</td>
<td>n.d.</td>
<td>Amha Kebede et al, 2004a</td>
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<td></td>
<td>3 different prisons In different parts of the country</td>
<td>409</td>
<td>32.5%</td>
<td>0%</td>
<td>n.d.</td>
<td>Amha Kebede et al, 2004a</td>
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<td></td>
<td>8 hospitals throughout Ethiopia</td>
<td>110</td>
<td>100%</td>
<td>2.7%</td>
<td>70%</td>
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