Chapter 8
Behavioral Decision Analysis and Pathogenicity: How Do We Decide What Makes Us Sick?

Kenneth Boorom

Abstract The previous chapter suggested ways to use statistics to understand conflicting study results and improve decision making, however, such approaches remain uncommon in the area of infectious diseases. This chapter examines the processes that are currently used to resolve controversies, and the criteria people use to judge pathogenicity. We examine methods by which a scientific controversy can be categorized and understood based on written statements by individuals involved. Existing published studies suggest that the idea of pathogenicity is strongly subjective, with significance variation from individual to individual. Considerations such as economic cost, perceived benefit, and conflict with religious and philosophical beliefs can influence researcher viewpoints. By understanding the particular interests and impacts such a decision makes on different interest groups, scientists can design studies and guide research efforts to avoid unproductive conflict with the medical community.

Keywords *Blastocystis* • Pathogenicity • Irritable bowel syndrome • Diarrheal diseases

8.1 Introduction

The last chapter focused on statistical ways to analyze medical literature to obtain conclusions, but in practice most decisions people make are not based on statistical analysis (Bazerman and Moore 2009). This chapter investigates the opinions people

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H. Mehlhorn et al. (eds.), *Blastocystis: Pathogen or Passenger?*,
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have formed about *Blastocystis*, and the factors that may have influenced those beliefs. The documents used to form this view consist of the following:

1. Medical studies and letters concerning *Blastocystis* published in peer reviewed journals.
2. Informational sheets and "fact sheets" on *Blastocystis* published by health organizations.
3. Correspondence from the US National Institutes of Health (NIH) and US Center for Disease Control to US Congressional Representatives and BRF concerning *Blastocystis* infection.
4. Internal correspondence between employees of the NIH concerning *Blastocystis* infection, which BRF obtained through the Freedom of Information Act (FOIA).
5. Public testimony provided by the State of Oregon's Epidemiology Office at a hearing of legislation to make *Blastocystis* a reportable infection in Oregon.
6. Funding history for research into *Blastocystis*, from 1985 to 2012, as obtained from the NIH's REPORTer database.
7. Journal articles and text books analyzing the phenomenon of scientific controversy.
8. Emails and letters from patients and physicians to BRF concerning *Blastocystis* infection.

Although many of these sources are from the USA, it is suggested the behavior is of general interest, because most of the published opposition to *Blastocystis* as a pathogen has come from the USA and other English-speaking countries (Chapter 7, Table 1.3). Most of the world's most influential medical journals (JAMA, Lancet, NEJM, Annals of Internal Medicine) are published in the USA or the UK, raising the possibility that medical opinion in those countries may have a disproportionate impact on global medical thought. Finally, the influence of medical information available through the internet is increasing in society, and the highest ranked documents concerning *Blastocystis* infection are commonly authored in the USA by the US Center for Disease Control and the Mayo Clinic, according to Google's search ranking results.

In its simplest form, assessing pathogenicity involves determining whether exposure to a microbe will result in illness in some patients who would not be sick if they were not exposed. But analysis of the process as a purely scientific endeavor can't account for the delays of decades between the first reports of illness associated with a microbe, and a consensus within the medical community that it should be classified as pathogenic (Table 7.8)

Over 15 years ago, researchers were already noting the slow rate of progress in this area:

> Despite more than 80 years of debate since the first accepted descriptions of the genus *Blastocystis*, not a single issue about this organism has been satisfactorily resolved. Substantial progress has been made in the description of the morphology of *Blastocystis hominis* during recent years, but structures and organelles present in the cell remain of unknown function. The method(s) of division is questioned. The pathogenicity of the organism has not been determined, and the significance of *B. hominis* in immunocompromised patients has not been ascertained. The necessity for treatment and the most appropriate chemotherapeutic strategies have not been defined. The taxonomy of the organism remains controversial.
>
> *Blastocystis Revisited* (Stenzel and Boreham 1996)

Considering the progress commonly seen in "hard sciences" (atomic physics, astronomy, electronics, aeronautics) over 80 years, it is difficult to attribute the delay associated with assessment of pathogenicity as stemming from purely scientific or technical barriers.

8.2 Cognitive Controversy Vs. Social Controversy

Disagreement about the significance of scientific data is not unique to *Blastocystis*, and sociologists have produced a body of literature that classifies controversies, and describes the interactions that occur in their formation and resolution. An awareness of concepts from this area of study can be valuable to researchers seeking to understand what may be seemingly irrational behaviors in the field of medical science.

Sociologists suggest that scientific controversies can be divided into two classes to facilitate analysis (Engelhardt and Caplan 1987). *Cognitive controversies* concern specific facts or scientific findings, while *social controversies* involve decisions about public policy. An example of a purely cognitive controversy might be the finding, reported by researchers in 2011, that neutrinos could be observed to travel faster than light (Cartlidge 2012). Because the outcome of the controversy would have little or no impact on any social policy, the controversy depended solely on the factual question as to whether neutrinos could travel at the proposed speed. A purely social controversy would be the disagreement about public funding of birth control information in predominantly Catholic countries. Because an abundance of information exists on the clinical efficacy and use of birth control methods, there are few factual questions that would be relevant to the resolution of this disagreement. Disagreements appear to stem solely from personal and religious beliefs.

Social controversies commonly involve a larger number of people, many of whom are not experts in a particular field, and who are participating due to an economic or personal interest in the debate's outcome. Social controversies can continue for decades or generations, because they involve competing interests and philosophical disagreements which are not resolved by the presence of new information. Cognitive controversies are often resolved in a few years. For example, the issue of faster-than-light neutrinos was explained in a few months once other groups tried to repeat the findings (Cartlidge 2012).

Which model best describes the controversies associated with assessment of pathogenicity? Although this may seem like a simple cognitive question, the timing of events suggests that the ability to demonstrate the pathogenicity of most enteric microbes predated the acceptance of pathogenicity by decades. For example, references to *Giardia intestinalis* pathogenicity date back to World War I, with a 1916 paper (Fantham and Porter 1916) noting that "While British troops were in Gallipoli, a number of men contracted various forms of dysentery or diarrhea, and severe cases occurred. Among the men invalided to England a number were found to be infected with Giardia (Lamblia)." In addition to reporting the illness, the authors of the paper performed an experimental animal infection on rats and kittens:

> From then till the forty-fourth day the feces were negative. On the forty-fourth day the feces were loose and diarrheic, and *Lamblia* cysts and flagellates appeared in them. The kitten showed signs of distress, howled, and refused food. Its coat became rough, and the animal shivered. On the forty-fifth, forty-seventh, and forty-eighth days some *Lamblia* cysts were present in the feces. The kitten seemed ill, vomited, sent up piteous cries, and the condition of the coat was bad. Violent diarrhea set in on the forty-eighth day, and the kitten died on the forty-ninth day after the first infective feed. At death it weighed 638 grams, its control weighing 997 grams.
>
> *The Pathogenicity of Giardia (Lamblia) Intestinalis to Men and to Experimental Animals* (Fantham and Porter 1916)

In the case of *G. intestinalis*, we can see that the technological means exist to demonstrate pathogenicity as early as 1916, but 40 years later, medical professionals still rejected the idea that it could cause epidemic illness. A paper reporting an epidemic of giardiasis in Portland, Oregon impacting over 50,000 individuals was rejected in the mid-1950s on the grounds that demonstration of *G. intestinalis* infection in a large portion of the population did not constitute proof of an epidemic (US_EPA 1979). Oregon's public health commissioner attributed the illness to an unknown virus and is quoted as such in the proceedings of the 1978 EPA Symposium on Waterborne Transmission of Giardia (US_EPA 1979). Those proceedings are now available online from the National Service Center for Environmental Publications, and they provide a fascinating look at the struggles scientists had in establishing *G. intestinalis* as a cause of disease (US_EPA 1979).

8.3 Resolution of Scientific Controversies

Most discussion of *Blastocystis* controversy has focused on specific findings concerning that infection. But the subject of *Blastocystis* controversy can be viewed in a larger context as just one of a number of scientific or medical controversies which can be found to exist at any point in history. In a text devoted to the subject, medical ethicist H. Tristam Engelhardt Jr. (Center for Ethics, Medicine, and Public Issues, Baylor College of Medicine) identifies five ways in which scientific controversies can be resolved (Engelhardt and Caplan 1987):

1. *Sound argument*: An argument is presented that is accepted by all parties and shows that the opposing view is incorrect.
2. *Consensus closure*: The opposing views have not been found to be incorrect, but the parties agree to end the controversy.
3. *Procedural closure*: The controversy is ended by a formal, governed effort which ends the discussion.
4. *Natural death closure*: The controversy has ended by the gradual fading away of interest.
5. *Negotiation closure*: The controversy has ended because a negotiated settlement has been reached which is acceptable to the opposing parties.

In the last few years, a number of cognitive controversies in science have been resolved when other scientists have tried to repeat an experiment, failed, and sometimes succeeded in identifying an intervening variable that was present in the original research report, and lead to a faulty conclusion. For example, the phenomenon of cold fusion was not reproducible outside of the laboratories where it was discovered and may have been the result of measurement error (Huizenga 1992). When researchers were unable to reproduce the results linking xenotropic murine leukemia virus-related virus (XMRV) virus with Chronic Fatigue Syndrome (CFS), analysis by other labs showed that commercially available PCR reagents were commonly contaminated with the virus, which could have led to the original researcher's unreproducible findings (Zheng et al. 2011).

However, in most of the controversies surrounding gastrointestinal microbes, the resolution of the controversy did not coincide with a particular study or technique, or any action by the scientific community. Scientists had developed a model for experimental giardiasis as early as 1916 (Fantham and Porter 1916), and *Cryptosporidium spp*. was repeatedly identified as an agent of diarrhea in immunocompetent individuals in the mid-1980s (O'Donoghue 1985). During the 1980s, and 1990s, when cases were emerging by what could now be estimated in the millions (Kappus et al. 1994), scientific and medical organizations were largely silent on the issue of public response to *G. intestinalis* and *Cryptosporidium spp*. These included prestigious groups such as the American Medical Association, the National Academy of Sciences, the American Society of Microbiology, the US Center for Disease Control, the US NIH, and the Infectious Disease Society of America (IDSA).

In fact, it was the US EPA which took action unilaterally to organize researchers to identify and develop a response to highly publicized epidemics from drinking water contamination (Eisenberg et al. 2005; Matukaitis 1997; Smith and Wolfe 1980; US_EPA 1979). Actions by that organization, rather than a scientific or medical society, are credited by some researchers with recognition of the significance of the problem of *G. intestinalis* infection (Smith and Wolfe 1980). Giardiasis finally became a nationally reportable illness in the USA in 2002, 87 years after the 1916 publication of Fantham's animal model for the infection (Hlavsa et al. 2005).

8.4 Role of the NIH in *Blastocystis* Controversy

The US NIH is the world's largest funding body for medical research. In 2011, the agency's budget was $31 billion, which was distributed between internal research projects and over 40,000 external investigators (Crow 2011; Ginther et al. 2011). The NIH played an intriguing role in the controversy surrounding *Blastocystis*, in that the agency had a role in beginning the controversy, by funding a large part of the research into the organism in the 1980s. Afterwards, the NIH actions contributed to an end of research in the US, by making statements that discouraged researchers from investigating the infection.

Between 1976 and 1995, the NIH was the world's leading institution concerning research on *Blastocystis* infection (Table 8.1). All papers originating from the NIH labs published after 1986 identified *Blastocystis* as pathogenic (Table 8.1). NIH researcher Dr. Charles H. Zierdt authored several of the most frequently referenced paper of *Blastocystis* infection, including the second most frequently referenced review paper (Zierdt 1991a). He also contributed the first study to demonstrate that *Blastocystis* patients exhibited an elevated serum antibody response to *Blastocystis* antigen not seen in healthy controls (Zierdt et al. 1995). Dr. Zierdt performed many of the early studies which developed ways to culture the organism, as well as axenicizing isolates from patients into the collection of the American Tissue and Culture Collection (ATCC) (Jones 2008).[1]

Interestingly, the NIH appears to have reversed its position on funding of *Blastocystis* research after the 1990s. Communications from researchers and patients interacting with the agency indicate that the agency considered *Blastocystis* to be nonpathogenic, and ineligible for funding:

> When I've contacted the NIH about *Blastocystis* research, they've told me there is no evidence that *Blastocystis* can cause illness in humans.
>
> US Scientist to BRF, 2010 (name withheld)

The duality of the NIH's response is even more intriguing, as the agency's own Medical Terms Database (MESH) clearly identifies *Blastocystis* as a pathogen (Fig. 8.1). Interestingly. the entry also describes disabling illness, which is consistent with descriptions of illness provided by some patients, but had not been published widely in scientific literature.

8.4.1 Obtaining Data from the NIH: Methods and Results

The NIH's position on *Blastocystis* research appears to be a complex one, and one that varies with time. Understanding the agency's position further is a complex task, since the NIH does not publish planning or road-mapping documents for most of the infectious diseases studied at that agency, so one cannot point to a date where a specific planning document changed.

The NIH's grant award database provided one source of information. The NIH Reform Act, passed by the US Congress in 2006, required the agency to make its grant awards database accessible by the public. Queries to that grant database show that between 1995 and May 2012, the NIH approved a total of over 600 grants into *G. intestinalis*, *Cryptosporidium spp*., *and Entamoeba histolytica* with a cost of $100 million, but 0 grants were approved for *Blastocystis* research (Table 8.2).

[1] Cultures axenicized by Dr. Zierdt are still available from the ATCC and were used to develop the first real-time PCR test, which was used in the first US study to demonstrate that patients with symptoms attributable to irritable bowel syndrome or Gulf War Illness were infected with *Blastocystis*, which had not been previously detected in those patients (Jones 2008, 2009).

Table 8.1 NIH Papers on *Blastocystis* Infection

N	Date	Title	Journal
1	Oct 1995	Serum antibody detected by fluorescent antibody test in patients with symptomatic *Blastocystis hominis* infection	Recenti Prog Med
2	Feb 1995	Enzyme-linked immunosorbent assay for detection of serum antibody to *Blastocystis hominis* in symptomatic infections	Journal of Parasitology
3	Apr 1994	Lipid biosynthesis by axenic strains of *Blastocystis hominis*	Comp Biochem Physiol Biochem Mol Biol
4	Jun 1993	Antibody response to *Blastocystis hominis* infections	Annals of Internal Medicine
5	Jan 1993	Taxonomic status of *Blastocystis hominis*: reply	Parasitology Today
6	Jul 1992	Comparative analysis of lipid composition in axenic strains of *Blastocystis hominis*	Comp Biochem Physiol B
7	Mar 1991	Pathogenicity of *Blastocystis hominis*	Journal of Clinical Microbiology
8	Jan 1991	*Blastocystis hominis*—past and future	Clinical Microbiology Rev
9	Sep 1990	Magainin analogs effective against pathogenic protozoa	Antimicrobial Agents and Chemotherapy
10	May 1988	Biochemical and ultrastructural study of *Blastocystis hominis*	Journal of Clinical Microbiology
11	Jan 1988	*Blastocystis hominis*, a long-misunderstood intestinal parasite	Parasitology Today
12	Feb 1986	Cytochrome-free mitochondria of an anaerobic protozoan—*Blastocystis hominis*	Journal of Protozoology
13	May 1983	In vitro response of *Blastocystis hominis* to antiprotozoal drugs	Journal of Protozoology
14	Nov 1981	Generation time and growth rate of the human intestinal parasite *Blastocystis hominis*	Journal of Protozoology
15	Oct 1976	Ultrastructure and light microscope appearance of *Blastocystis hominis* in a patient with enteric disease	Zeitschrift für Parasitenkunde
16	Jun 1976	Endosymbiosis in *Blastocystis hominis*	Experimental Parasitology
17	Jun 1976	*Blastocystis hominis*: pathogenic potential in human patients and in gnotobiotes	Experimental Parasitology
18	Oct 1974	*Blastocystis hominis*: axenic cultivation	Experimental Parasitology
19	1974	Freeze-etch studies of the granular and vacuolated forms of *Blastocystis hominis*	Zeitschrift für Parasitenkunde
20	Nov 1973	Ultrastructure of *Blastocystis hominis*	Zeitschrift fur Parasitenkunde
21	Feb 1973	Studies of *Blastocystis hominis*	Journal of Protozoology
22	Nov 1967	Protozoan characteristics of *Blastocystis hominis*	American Journal of Clinical Pathology

The US National Institutes of Health was a major contributor to *Blastocystis* research until the mid-1990s, with over 22 articles published by Dr. Charles H Zierdt on the subject. All of the Zierdt's later papers identified *Blastocystis* as pathogenic. Z. Parasitenkd; now Parasitology Research

National Library of Medicine - Medical Subject Headings

2010 MeSH

MeSH Descriptor Data

Return to Entry Page

Standard View. Go to Concept View; Go to Expanded Concept View

MeSH Heading	Blastocystis hominis
Tree Number	B01.046.500.100.200.200.375
Annotation	infection: coordinate IM with BLASTOCYSTIS INFECTIONS (IM); coordinate with specific protozoan terms (ANTIGENS, PROTOZOAN, etc) if pertinent
Scope Note	A species of parasitic protozoa found in the intestines of humans and other primates. It was classified as a yeast in 1912. Over the years, questions arose about this designation. In 1967, many physiological and morphological B. hominis characteristics were reported that fit a protozoan classification. Since that time, other papers have corroborated this work and the organism is now recognized as a protozoan parasite of humans causing intestinal disease with potentially disabling symptoms.
Allowable Qualifiers	CH CL CY DE EN GD GE IM IP ME MI PH PS PY RE UL VI
Previous Indexing	Phycomyces (1971-1979)
Previous Indexing	Protozoa (1986-1991)
Previous Indexing	Zygomycotina (1980-1985)
History Note	92
Date of Entry	19910502
Unique ID	D016845

MeSH Tree Structures

Fig. 8.1 Entry for *Blastocystis* infection in the NIH's MESH Database, Retrieved July 2011. The entry illustrates the conflicting positions from that agency. Interestingly, the entry makes note of "disabling" symptoms, which have been frequently described to BRF in patient emails, but are generally undocumented in medical literature

The grant awards database made it possible to document an outcome (funding behavior), but it did not provide visibility into the thought process responsible for producing that outcome. Letters from BRF and US Congressman generated written responses from the agency. A 2008 response indicated *Blastocystis* was a special case, noting that a commitment of funding for clinical research was "infeasible," but researchers could try applying for grants (Fig. 8.1 and Supplemental files).

From 2009 to 2011, the NIH's position on the infeasibility of performing clinical *Blastocystis* research did not change, despite the emergence of a body of literature

Table 8.2 Comparison of the number of NIH grants and aggregate spending for selected enteric microbes from January 1995–May 2012

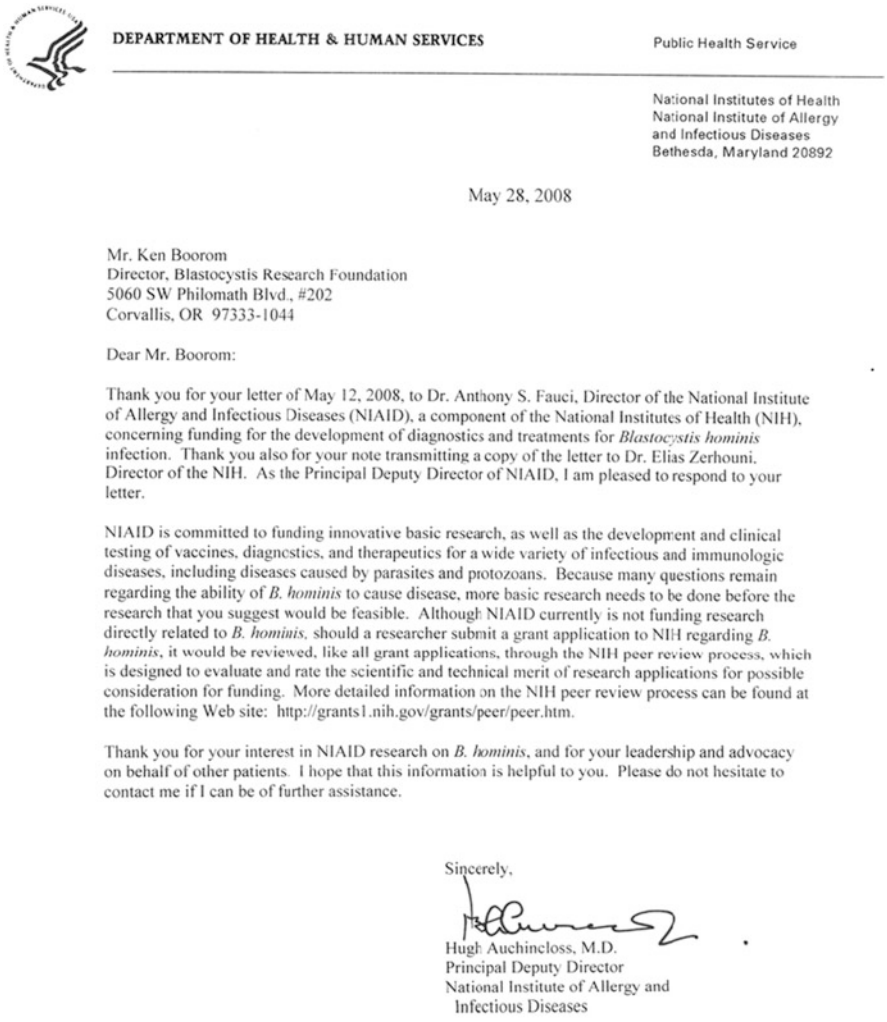
Organism	Number of NIH grants written with microbe term in title 1995–2012	Total dollar amount of grants (sum of primary project dollar column)	Frequency of detection in symptomatic patients based on 2000 US-wide survey (Amin 2002)	Percent of mono-infections that were symptomatic based on 2000 US-wide survey (Amin 2002)
Blastocystis sp.	0	$0	24 % (662/2,896)	69 %
Giardia intestinalis	176	$31,812,247	0.4 % (19/2,896)	100 %
Cryptosporidium spp.	267	$44,954,253	4.1 % (121/2,896)	70 %
Entamoeba histolytica	180	$24,080.646	2.3 % (68/2,896)	75 %

published internationally, which became progressive more detailed about how it was causing illness, and how to develop animal models. As international research groups had clearly made a different decision about *Blastocystis* research, we wanted to understand why the NIH's decision differed. Mainly, why would different scientific organizations draw very different conclusions, given the same body of research study available? What were the criteria by which the NIH had determined should be treated specially? Why was the funding level remaining at zero for so many years in a row? And why did researchers indicate that the NIH had advised them against applying for grants?

In 2011, BRF filed a FOIA request with the agency to obtain documents relating to the decision making process concerning *Blastocystis*.[2] The FOIA request asked for information similar to that requested in a November, 2011 letter from US Congressman Kurt Schrader to the NIH Supplementary files. The NIH responded to that letter, but the response did not answer the questions posed (Supplementary files). The FOIA request specified the following documents:

1. Written documents which describe the process by which NIAID reviews medical literature to assess the public health significance of specific microbes.
2. Documents from meetings held in 2008, 2009, 2010, and 2011 where the NIAID reviewed the public health significance of *Blastocystis* and/or its policies and research activities related to *Blastocystis* infection. The request included emails, agendas, meeting notes, and handouts.

[2] The intention in filing the FOIA request was not to be disrespectful of the institution or its staff members. Rather, BRF noted that each US University-based research effort that we had worked to organized came to an end when it was determined that the NIH would not provide funding. At the same time, we saw groups emerging in Mexico, Europe, and Asia, many of which did receive national funding. Why was the NIH's position different? What can we learn about the decision making process from the differences? We also felt that this was a legitimate public policy question, since this infection was present in 10–15 % of the US population, with about 70 % of mono-infections being symptomatic by some analyses (Amin 2002).



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892

May 28, 2008

Mr. Ken Boorom
Director, Blastocystis Research Foundation
5060 SW Philomath Blvd., #202
Corvallis, OR 97333-1044

Dear Mr. Boorom:

Thank you for your letter of May 12, 2008, to Dr. Anthony S. Fauci, Director of the National Institute of Allergy and Infectious Diseases (NIAID), a component of the National Institutes of Health (NIH), concerning funding for the development of diagnostics and treatments for *Blastocystis hominis* infection. Thank you also for your note transmitting a copy of the letter to Dr. Elias Zerhouni, Director of the NIH. As the Principal Deputy Director of NIAID, I am pleased to respond to your letter.

NIAID is committed to funding innovative basic research, as well as the development and clinical testing of vaccines, diagnostics, and therapeutics for a wide variety of infectious and immunologic diseases, including diseases caused by parasites and protozoans. Because many questions remain regarding the ability of *B. hominis* to cause disease, more basic research needs to be done before the research that you suggest would be feasible. Although NIAID currently is not funding research directly related to *B. hominis*, should a researcher submit a grant application to NIH regarding *B. hominis*, it would be reviewed, like all grant applications, through the NIH peer review process, which is designed to evaluate and rate the scientific and technical merit of research applications for possible consideration for funding. More detailed information on the NIH peer review process can be found at the following Web site: http://grants1.nih.gov/grants/peer/peer.htm.

Thank you for your interest in NIAID research on *B. hominis*, and for your leadership and advocacy on behalf of other patients. I hope that this information is helpful to you. Please do not hesitate to contact me if I can be of further assistance.

Sincerely,

Hugh Auchincloss, M.D.
Principal Deputy Director
National Institute of Allergy and
Infectious Diseases

Fig. 8.2 Letter from the NIH indicating that it was not pursuing any *Blastocystis* research, but that it would consider grants

3. Responses by the Laboratory of Parasitic Diseases to inquiries outside of the NIAID into whether *Blastocystis* should be considered a pathogen, and into any aspect concerning the scientific research or clinical status or *Blastocystis* infection including email responses and written responses to physicians, public health professionals, researchers, and patients.

The FOIA request produced only two email communications (Figs. 8.3, 8.4, 8.5). We found no evidence of any organized discussion or review of *Blastocystis* literature, or of any meetings conducted from 2008 to 2011 to discuss the issue of *Blastocystis* funding or pathogenicity (Table 8.3).

Schmidt, Beth (NIH/NIAID) [E]

Subject: FW: AFC referral- blastocystis

From: Tom Nutman <tnutman@niaid.nih.gov>
Date: Mon, 22 Nov 2010 16:24:11 -0500
To: JeanAnne Ware <jeananne.ware@nih.gov>
Cc: Kathryn Spates <spatesk@mail.nih.gov>
Subject: Re: AFC referral- blastocystis

I think you can pass this along to Kate tomorrow. We will likely not see the patient in that they have 2 non-pathogens and no eosinophilia. If they feel they must treat the B. hominis - now considered a fungus and not a parasite -- they should consider using metronidizole 750 mg TID x 10 d or Trimethoprim/sulfa 1 DS po BID x 7d. We would not treat this patient, however. Tom

On 11/22/10 4:20 PM, "JeanAnne Ware" <jeananne.ware@nih.gov> wrote:

Dr. Nutman,

We received some lab results on a patient from AFC whom they are referring for blastocystis hominis in the stool, trichrome stain showing few b. hominis, dientamoeba fragilis (there is just one O &P result). Aside from that, the patient does not have eosinophilla and we not have any other clinical information besides labs. Would this patient be appropriate for screening, or is there this anything else you'd like me to do with this, i.e. contact the provider with any guidance, or pass this along to Kate for any additional screening? (she is gone for the day so I am picking up the incoming labs).

JeanAnne
JeanAnne Ware, CRNP
Nurse Practitioner
Laboratory of Parasitic Diseases
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Thomas B. Nutman, M.D.
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Fig. 8.3 Decision as to whether the NIH Laboratory of Parasitic Diseases would consider seeing a patient with *Blastocystis* spp. infection as well as *Dientamoeba fragilis* infection. The response from the Deputy Chief indicates that the laboratory considers both to be nonpathogenic. The response notes that *Blastocystis* has been reclassified as a fungus, and is no longer a parasite

From: Allen, Andrew [mailto:aallen@jcvi.org]
Sent: Tuesday, June 08, 2010 1:48 AM
To: Giovanni, Maria (NIH/NIAID) [E]; Wang, Lu (NIH/NHGRI) [E]
Subject: Blastocystis - biomedical relevance

I just wanted to touch base with you again concerning a possible Blastocystis project.

I have spoken to many people in the scientific community who view this as an extremely high priority project. This includes a community of domestic and international physicians who continue to be extremely frustrated by lack of treatment as well as prominent scientists within the pathogen genomics community that readily acknowledge a major knowledge gap when it comes to Blastocystis. Blastocystis is widely recognized by the international parasite research community as a highly problematic emerging pathogen. This evident from many conversations I have had with different researchers as well as an objective surrey of the recent literature.

Blastocystis is classified as a pathogen by the CDC. In fact, Blastocystis is the single most common eukaryotic parasite in the intestinal tract of humans. Blastocystis infection is having a very significant impact on public health world-wide. There is highly significant biomedical relevance for development of genome-enabled approaches to the study Blastocystis. Long term Blastocystis infection is common and methods for diagnosis and treatment remain rudimentary at best.

Considering the biomedical importance Blastocystis and the existing knowledge-gap, I wondered if there is anything I can do in order to attempt to initiate a GSC project on Blastocystis.

Thanks very much.

Sincerely, Andy

Andrew E. Allen, Ph.D.
Associate Professor
J. Craig Venter Institute
Dept. of Microbial and Environmental Genomics
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San Diego, CA 92121
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Fax: 858-200-1881

Fig. 8.4 An inquiry from a US researcher to the NIH about the possibility of funding a research project into Blastocystis infection. Internal discussion of the inquiry at the NIH appears in Fig. 8.5

The FOIA documents more closely reflected the information we received from researchers. Those emails indicated that staff members considered the question of whether the infection was pathogenic to be resolved in favor of nonpathogenicity, and did not wish to revisit the issue (Figs. 8.3, 8.4, 8.5).

8.4.2 Analysis of Specific Responses from the NIH

Figure 8.3 provides additional visibility into the views concerning Blastocystis pathogenicity. This correspondence contains several notable elements:

1. Fig 8.3 *"They have 2 nonpathogens. . .they should consider using metronidazole . . . or Trimethoprin/sulfa"*: The author suggests that both *Blastocystis* and *Dientamoeba fragilis* are nonpathogenic, but antimicrobial treatment is suggested. This statement may illustrate the complexity of defining the term "pathogenic."

Hall, Lee (NIH/NIAID) [E]

From: Hall, Lee (NIH/NIAID) [E]
Sent: Tuesday, June 08, 2010 9:59 AM
To: Giovanni, Maria (NIH/NIAID) [E]
Subject: RE: Blastocystis - biomedical relevance

Maria,
This is not a high priority.

Although *Blastocystis spp.* have a wide geographic distribution, it is not clear that they are in fact "true" pathogens - perhaps just a low grade pathogen, or just part of the normal gut flora. Clinical symptoms attributed to *B. hominis* infection include the usual panoply of GI complaints, e.g., diarrhea, bloating, flatulence, cramping. The evidence that *B. hominis* is responsible for disease is based largely on case reports and uncontrolled or retrospective studies of patients with non-specific GI symptoms. Neither the identification of *B. hominis* in stool or the number of organisms in stool has been correlated with symptoms, however. Some observational studies and small clinical trials of antibiotic therapy have suggested improvement of symptoms with disappearance/reduction in *B. hominis*, but since the antibiotics themselves are broad spectrum (e.g., Bactrim, metronidazole, nitazoxanide), it is hard to attribute the effects specifically due to antibiotic activity on *B. hominis* and not on other intestinal microbes. In any event, blastocystosis is often considered to be self-limited, and many physicians opt not to treat *B. hominis* when it is found in the stool even when signs/symptoms are present unless there is some underlying condition, e.g., immunocompromised hosts.

Happy to discuss further if you want.

Lee

From: Giovanni, Maria (NIH/NIAID) [E]
Sent: Tuesday, June 08, 2010 8:04 AM
To: Hall, Lee (NIH/NIAID) [E]
Subject: FW: Blastocystis - biomedical relevance

I may have already asked you this but is this something of importance to pursue. He is very persistent.

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Fig. 8.5 An internal NIH communication discussing a researcher's inquiry as to whether the NIH would fund a proposed *Blastocystis* research project. The position of the NIH staff member is consistent with communications made to BRF by patients and researchers, which indicate that agency believes no evidence exists that the infection could cause illness in healthy humans

Mainly, some definitions of the term would suggest that if an antimicrobial treatment is prescribed for a microbial infection with the intention of relieving patient symptoms, then the microbe in question would be considered a pathogen.

2. Fig 8.3 *Blastocystis is not a parasite and has been reclassified as a fungus*: We were unable to find any reference to *Blastocystis* being reclassified from a parasite to a fungus in recent reviews of *Blastocystis*, or in common archives of medical literature. All recent papers continue to refer to *Blastocystis* as a parasitic infection, and none note any status as a fungus (Boorom et al. 2008;

Table 8.3 Institutional Affiliation of Selected Researchers co-authoring papers on *Blastocystis*

Agency	Author (year of publication)	Title
US National Institute of Health	Zierdt (1991a)	*Blastocystis Past and Future*
US Center for Disease Control	CDC (2000)	*Blastocystis Fact Sheet, 2000–2010*
US Center for Disease Control	CDC (2010)	*Blastocystis Fact Sheet, 2010-present*
US Center for Disease Control	Boorom et al. (2008)	*Oh my aching gut: irritable bowel syndrome*, *Blastocystis*, and *asymptomatic infection*
China Center for Disease Control	Boorom et al. (2008)	
US Air Force	Jones et al. (2009)	*Association of Blastocystis subtype 3 and 1 with patients from an Oregon community presenting with chronic gastrointestinal illness*
US Department of Agriculture	Santin et al. (2011)	Development of a new PCR protocol to detect and subtype *Blastocystis spp*. from humans and animals
World Health Organization (Coordinating Center)	Parkar et al. (2010)	Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers

Papers on the infection are commonly published by researchers at national and regional public health organizations and hospitals, as well as national universities. Within the USA, the NIH elected to discontinue research participation after the mid-1990s, but scientists from other federal agencies have published papers on the subject

Stensvold et al. 2010; Tan et al. 2011). The statement illustrates the difficulty associated with developing consensus around technical aspects of *Blastocystis* infection. Variations in viewpoints among individuals may develop spontaneously, and do not appear to originate from any medical studies.

3. Fig 8.3 *D. fragilis is nonpathogenic*: The statement was intriguing, as most recent papers from national health services in Canada, the UK, and large hospitals in Australia identify *D. fragilis* as pathogenic, and suggest that symptoms would be largely similar to those seen in other intestinal parasitic diseases (Lagace-Wiens et al. 2006; Windsor 2007; Windsor and Macfarlane 2005). Reviews noting diagnosis and treatment of patients are commonly published in mainstream clinical journals, such as the American Journal of Tropical Medicine and Hygiene (Stark et al. 2012) and Clinical Microbiology Reviews (Johnson et al. 2004).

Figures 8.4 and 8.5 illustrate an inquiry to the NIH concerning funding of a *Blastocystis* research grant. In Fig. 8.4, the researcher notes the trend of *Blastocystis* research, and the development of a consensus of researchers that the microbe is pathogenic. In Fig. 8.5, the discussion at the NIH concerning this grant suggests that no funds would be made available for a project investigating *Blastocystis* infection (Table 8.4).

Table 8.4 *Blastocystis* Firsts, 1990–2012

Item	Author (year)	Country
First studies to examine antimicrobial resistance	Silard and Burghelea (1985)	Romania
	Zaman and Zaki (1996)	Pakistan
First study to recommend that *Blastocystis* patients be diagnosed with IBS	Markell and Udkow (1986)	USA
First study to suggest that *Blastocystis* infection was the underlying cause of irritable bowel syndrome	Hussain et al. (1997)	Pakistan
First mouse model	Moe et al. (1997)	Singapore
First studies to suggest some *Blastocystis* isolates are associated with acute infection, while others may be associated with chronic infection	Lanuza et al. (1999)	Italy
	Stensvold et al. (2011)	Denmark
First study to report that common clinical diagnostic techniques fail to identify most *Blastocystis* infections	Leelayoova et al. (2002)	Thailand
First detailed study of a *Blastocystis* epidemic	Nimri (1993)	Jordan
First long term study (2 years) of *Blastocystis* immune response in patients with and without gastrointestinal symptoms	Kaneda et al. (2000)	Japan
First study to report a difference in *Blastocystis* antigen immune response between symptomatic and asymptomatic hosts	Mahmoud and Saleh (2003)	Egypt
First placebo controlled trial for treatment of *Blastocystis* infection with antimicrobials	Nigro et al. (2003)	Italy
First study to report death in animal models following experimental infection of *Blastocystis* from symptomatic humans	Yao et al. (2005)	China
First major analysis of *Blastocystis* genotypes from multiple countries	Noel et al. (2005)	France
First large epidemiological study of *Blastocystis* infection	Amin (2005)	USA
First large scale population study of *Blastocystis* using a reliable diagnostic technique and sub-typing of isolates	Li et al. (2007a)	China/Japan
First comparison of conventional diagnostics, stool culture and PCR detection for identification of *Blastocystis* infection in stool samples	Stensvold et al. (2007a)	Denmark
First international agreement on naming conventions for *Blastocystis* isolates	Stensvold et al. (2007b)	Denmark
First studies to investigate waterborne transmission of *Blastocystis*, including molecular epidemiology	Li et al. (2007b)	China
	Leelayoova et al. (2008)	Thailand
	Eroglu and Koltas (2010)	Turkey
First real-time PCR test for *Blastocystis* infection	Jones Ii et al. (2008)	USA
First genotyping of *Blastocystis* infection in cancer patients	Tan et al. (2009)	Malaysia
First study to provide details (molecular weights of antigens) for *Blastocystis* immune response in symptomatic humans	Hegazy et al. (2008)	Egypt

(continued)

Table 8.4 (continued)

Item	Author (year)	Country
First study to perform experimental animal infection with multiple *Blastocystis* isolates that were sub-typed	Hussein et al. (2008)	Egypt
First systematic review of *Blastocystis* literature	Boorom et al. (2008)	USA + ten other countries
First detailed clinical reports of *Blastocystis* in producing skin rash	Katsarou-Katsari et al. (2008)	Greece Turkey
First sub-typing of *Blastocystis* infection in an irritable bowel syndrome (IBS) patient as well as a "Gulf War Illness" patient	Jones et al. (2009)	USA
First study to genotype *Blastocystis* in inflammatory bowel disease patient	Dogruman-Al et al. (2010)	Turkey
First study to report multiple immunosuppressive properties of *Blastocystis* secretory substances	Chandramathi et al. (2010a)	Malaysia
First study to report that *Blastocystis* (and E. histolytica) are associated with relapsing symptoms in inflammatory bowel disease patients	Yamamoto-Furusho and Torijano-Carrera (2010)	Mexico
First study to identify a diagnostic test whose results differ in both symptomatic *Blastocystis* patients, and in experimentally infected rats	Chandramathi et al. (2009, 2010b)	Malaysia
First study to report that *Blastocystis* down-regulated nitric oxide production	Mirza et al. (2011)	Singapore
First complete genome sequencing of *Blastocystis*	Denoeud et al. (2011)	France
First study to report that DNA extraction kits have greatly varying performance level in their ability to produce DNA for *Blastocystis* detection	Yoshikawa et al. (2011)	Turkey/Japan
First study to identify a host genetic trait which is responsible for mediating symptoms in *Blastocystis* infection	Olivo-Diaz et al. (2012)	Mexico

Participation of laboratories in Asia, the Middle East, and most recently Europe and Mexico has been essential for the development of *Blastocystis* research. The lack of US participation after 1995 is notable

8.5 Blastocystis and IBS in Medical Studies

Published medical studies provide another avenue for understanding how views about pathogenicity can vary between individuals, and how those views are formed. The two studies by Dr. Edward Markell, provide a good reference point, as Dr. Markell's 1986 paper, "Blastocystis: Pathogen or Fellow Traveler" remains one of the ten most cited *Blastocystis* studies. The abstract of the paper describes his conclusions as follows:

> To investigate this possibility, we identified 148 persons whose stools contained this organism. Of this number, 32 had at least 6 stool examinations performed. Twenty-seven of the 32 persons were later found to have greater than or equal to 1 recognized pathogens—*Entamoeba histolytica*, *Giardia lamblia* or *D. fragilis*—and, after receiving appropriate therapy,

> became asymptomatic. The *B hominis* infection, however, was unaffected by therapy. Five persons with only *B. hominis* infection were treated with iodoquinol without effect; these persons fulfilled the medical criteria for irritable bowel syndrome. We believe that when an apparently symptomatic *B. hominis* infection responds to therapy, the improvement probably represents elimination of some other undetected organism causing the infection.
>
> *Blastocystis: Pathogen or Fellow-Traveler* (Markell and Udkow 1986)

We can identify several concepts about pathogenicity from this statement:

1. *Pathogenicity can be determined by response to antimicrobial treatment*. That is, the lack of symptomatic improvement following treatment with iodoquinol shows that a microbe is nonpathogenic. Subsequent in vitro studies showed the iodoquinol had little activity against *Blastocystis* infection (Dunn and Boreham 1991; Mirza et al. 2010). The idea that pathogens must be treatable is also noted in a 1990 letter to the Journal of Clinical Microbiology concerning *Blastocystis* pathogenicity (Rosenblatt 1990), and also in a 2010 review on the treatment prescribed for *Blastocystis*, with the title, "Eradication of Blastocystis carriage with antimicrobials: reality or delusion" (Stensvold et al. 2010).
2. *If a patient has symptoms meeting the criteria for irritable bowel syndrome, microbial causes can be eliminated.*
3. *The drop-out effect is not a concern in studies of this nature*. Most patients (116/148, 78 %) dropped out of Dr. Markell's study. Of the original 148 study participants, only 32 completed the study. In the paper text, Markell noted that he communicated the results of the testing with patients while the study was being conducted, causing patients with *Blastocystis* mono-infections to drop out: "In others [leaving the study], the patient was reassured by initial examinations which were negative for recognized pathogens, and did not return." (Markell and Udkow, 1986, p. 1024) Markell did not perceive this drop-out effect to be incompatible with the study's conclusion that symptomatic *Blastocystis* mono-infections were uncommon.
4. *The appearance of another pathogen during the study proves that the original infection was not the cause of illness in the patient*. Markell collected six samples over a period of 11 months, according to the paper.
5. *At the end of Markell's study, he was left with a population where 15.6 % (5/32) of the Blastocystis patients were symptomatically mono-infected*. This level of symptomatic mono-infection is thought to prove that *Blastocystis* does not cause of illness.

The issue as to whether asymptomatic carriers can be used to prove that a microbial infection is nonpathogenic repeatedly appears in the literature concerning *Blastocystis* infection. The argument is that since all enteric protozoal infections have high rates of asymptomatic carriers, this would not be the best criteria to use in "proving" that a suspected enteric protozoan is nonpathogenic. Dr. Charles H. Zierdt's response to the issue of asymptomatic carriers was as follows:

> Dr. Rosenblatt states: "Some patients with diarrhea have the organism in their stool and some do not; some asymptomatic patients have it in their stool and some do not." What a beautiful description of the presence of *Entamoeba histolytica*!
>
> *Blastocystis pathogenicity* (Zierdt 1991b)

The issue as to whether *Blastocystis* patients (and patients with other microbial infections) should be diagnosed with IBS continues to be controversial in the medical community. One group of scientists indicates that such infections should be excluded before diagnosing patients with IBS, publishing a 2007 review article entitled, "*Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis*" (Stark et al. 2007). However, other researchers argue the microbial causes for gastrointestinal illness have been given too much emphasis in the medical community. Dispensing an infective diagnosis for diarrhea based solely on the presence of an infection, be it bacterial or protozoal, is not appropriate because infections appear in asymptomatic patients:

> For example, an individual with a bacterial gastroenteritis or other bowel disorder who has no concurrent psychosocial difficulties and good coping skills may not develop the clinical syndrome (or be aware of it) or if it does develop, may not perceive the need to seek medical care. Another individual with coexistent psychosocial comorbidities, high life stress, abuse history, or maladaptive coping, may develop a syndrome (e.g., postinfectious irritable bowel syndrome [IBS] or dyspepsia), go to the physician frequently, and have a generally poorer outcome."
>
> *The Functional Gastrointestinal Disorders and the Rome III Process* (Drossman 2006)

This text appears in the introduction to the Rome III Process document, published in the journal *Gastroenterology*. The document is an international standard for the diagnosis of functional gastrointestinal disorders. According to the text, the paradigm shift began thirty years ago, and was produced as a result of centuries-long unjust exclusion of a type of research by the medical community, a process which is remedied with the development of new paradigms:

> The first event began 3 decades ago with a paradigm shift that moved away from conceptualizing illness and disease based on a 3-century-old reductionistic model of disease in which the effort was to identify a single underlying biological etiology to a more integrated, biopsychosocial model of illness and disease. The former disease-based model had its roots with Descartes' separation of mind and body and at the time was a concept that harmonized prevailing societal views of separation of church and state. What resulted was permission to dissect the human body (which was previously forbidden), so disease was defined by what was seen (i.e., pathology based on abnormal morphology). This approach led to centuries of valuable research producing effective treatments for many diseases. The concept of the mind (i.e., the central nervous system [CNS]) as being amenable to scientific study or as playing a role in illness and disease was marginalized, however. The mind was considered the seat of the soul, not to be tampered with.
>
> *The Functional Gastrointestinal Disorders and the Rome III Process* (Drossman 2006)

The viewpoint that individuals control their response to infectious diseases is not a new idea, and was promoted widely in the teachings of Mary Baker Eddy, the founder of the Christian Science movement in the USA, which advocates prayer for the treatment of infectious diseases (Swan 1983). Advocates of the idea point to the presence of asymptomatic carriers of infectious diseases like *Vibrio cholerae*. The paradigm can be extended to other diseases, such as tuberculosis. The following appeared in a journal of the largest charity in the UK devoted to patients with irritable bowel syndrome (IBS):

> We have known since the birth of civilization how people weakened by malnutrition and poverty are particularly susceptible to illness. The same applies to people undermined by

> emotional distress. The dramatic potential of the Tubercle bacillus to devastate the health of young men and women crossed in love is a dramatic theme of many novels of the nineteenth and early twentieth centuries.
>
> Nick Reed, MD, Issue 69, *Gut Reaction*, *Journal of the Gut Trust*

It is difficult to resolve this controversy between microbiologists and bio-psycho-social (BPS) scientists because of the different methods used by each group. Specifically, BPS faults the use of reductionism in the medical community, a process which typically involves narrowing possible causes with laboratory experiment, until the smallest set of factors is identified which can reproduce the disease. BPS scientists emphasize "multi-determinism," and believe many factors influencing symptoms in infectious diseases cannot be clearly quantified, and have unfairly been excluded from the medical process. These influences might include traumatic experiences early in life, maternal separation, the cumulative effect of emotionally stressful life events, etc. BPS scientists may also draw on discussion of novels or seventeenth century European philosophers, while microbiologists emphasize repeatable clinical and laboratory studies.

8.6 Prevalence as a Test for Pathogenicity

Measurements of the prevalence of *Blastocystis* have also been cited in conjunction with discussion of pathogenicity. In 2006, I worked with an Oregon State legislator, Representative Sarah Gelser, to bring a bill, HB2699, before the legislature to make *Blastocystis* a reportable infection (Fig. 8.6).[3] The bill was opposed by the State Epidemiologists who cited the prevalence of the infection as proof that it could not cause disease:

> Based on the number of studies we have seen or read, about 10–20 % of the population in the world, and the USA, may actually carry Blastocystis hominis. That would equal about 700,000 individuals in Oregon carrying the organism, walking around with Blastocystis hominis, and not having any symptoms at all. So that is very telling of the likelihood of the organism and the possibility of causing disease.
>
> Dr. Emilo deBess, Testimony to State of Oregon Health Policy Committee, March 2007

A counter-point to the argument that prevalent microbes can not be pathogenic was suggested in a 2010 review on *Blastocystis*, which noted that the prevalence of chronic gastrointestinal illness (IBS) generally tracked the prevalence of

[3] The logic was that physicians already recognized *Blastocystis* as a pathogen by their actions. The common practice, as communicated to me by interviews with multiple physicians and gastroenterologists, was to treat *Blastocystis* when it was found in symptomatic patients. That is, laboratories looked for the organism in stool samples, physicians diagnosed patients with blastocystosis, and treated the infection. We had detailed testimony from patients or their guardians who had been seen repeatedly by physicians associated with the major hospitals and clinics in the region, and had been diagnosed and treated for *Blastocystis* infection. All physicians and board certified gastroenterologists who we contacted in the area supported passage of the bill, and provided signed letters supporting it.

Fig. 8.6 The author testifying to the State of Oregon Health Policy Committee in support of making *Blastocystis* a reportable infection in Oregon

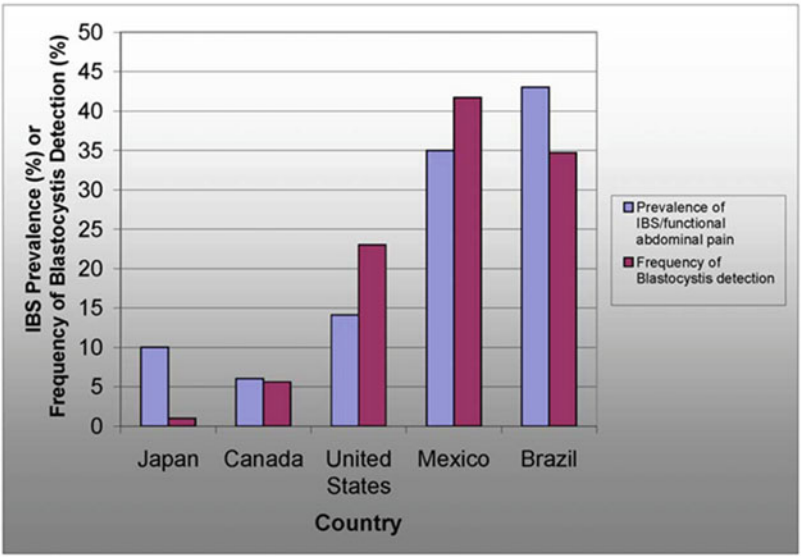

Fig. 8.7 Comparison of the prevalence of irritable bowel syndrome (IBS) or chronic abdominal pain in surveys from various countries to the frequency of detection of *Blastocystis* infection at clinical laboratories. The high prevalence of both the patients and the illness may contribute to opposition to pathogenicity, some individuals suggest that an infection which is highly prevalent is unlikely to be pathogenic

Blastocystis infection in a number of developed nations (Fig. 8.7) (Boorom et al. 2008). The review also noted that in Egypt, 21 % of apparently individuals have been found to carry *E. histolytica*. In a study of a slum in Brazil, 11 % carried *E.*

histolytica. In both cases, ELISA assays were used to differentiate *E. histolytica* from *E. dispar*, and the number reported is the prevalence of *E. histolytica*.

Measurement of the prevalence of the infection in specific populations has also been cited as a way to determine pathogenicity of *Blastocystis*. Markell and Udkow's second study, published in 1993, noted that the infection was found at a similar prevalence in symptomatic and asymptomatic individuals:

> There was no statistically significant difference in prevalence between the two study groups (11.5 % vs. 12.8 % for asymptomatic and symptomatic respectively, P=0.435, Fisher's one-tailed test)
>
> *Blastocystis hominis: Prevalence in Asymptomatic versus Symptomatic Hosts* (Udkow and Markell 1993)

However, it has been noted that Markell also published two papers noting that there was no correlation between the presence of *E. histolytica* and *G. intestinalis* and symptoms in his study groups (Boorom et al. 2008).

> Of the health fair group, however, 44 (42 %) responded "no" to all nine questions [concerning gastrointestinal symptoms]. Of the 44, 12 (27 %) were infected with potential pathogens. No correlation between symptoms and the presence or absence of infection with *E. histolytica*, *G. intestinalis* could be detected.
>
> *Intestinal parasitic infections in homosexual men at a San Francisco Health fair*, (Markell et al. 1983)
>
> The prevalence of Entamoeba histolytica (28.6 %) was similar to that reported in other studies, whereas that of Giardia lamblia was lower. Infection with E. histolytica was correlated significantly with a prior history of syphilis or gonorrhea ($P < 0.0001$), with the number of sexual partners in the preceding 12-month period ($P < 0.0001$), and with the reported frequency of oral–anal sexual contact ($P < 0.001$). Giardial infection was also significantly related to oral–anal sex ($P < 0.001$). No relation was seen between the presence or absence of gastrointestinal symptoms and infection with pathogenic protozoa.
>
> *Intestinal protozoa in homosexual men of the San Francisco Bay area: prevalence and correlates of infection* (Markell et al. 1984)

Markell's last study equated attention to *Blastocystis* with dereliction of the physician's professional responsibility:

> Reconciling even potential pathogenicity with the essentially equal prevalence of this organism in symptomatic and asymptomatic persons...seems difficult. Furthermore, focusing attention on *B. hominis* may stifle further investigation to the possible detriment of the patient or may prompt treatment that is both unnecessary and potentially dangerous.
>
> Blastocystis hominis: Prevalence in Asymptomatic vs. Symptomatic Hosts (Udkow and Markell 1993)

We do not have an explanation as to why investigating *Blastocystis* would cause such harm, while investigating *G. intestinalis* and *E. histolytica* would presumably be acceptable, even though as Markell noted, there was similarly no correlation between any of these infections and symptoms in his study populations. That is, we have an example where the identical finding is interpreted in different ways by the same researcher, depending on the objectives of the paper.

Why was every intestinal protozoal infection uncorrelated with symptoms in Markell's studies? Studies of intestinal protozoal infections in humans are, at best, partially controlled studies. Both Markell and a *Blastocystis* review paper suggested

the possibility that patients who show symptoms are more likely to seek medical treatment, which can skew results of population studies (Boorom et al. 2008; Markell et al. 1983). That is, if all the symptomatic patients seek and receive treatment, we are left with asymptomatic patients for epidemiologists to study. Besides patient behavior, acquired immunity can influence expression of symptoms. Studies citing a lack of correlation between intestinal protozoal infections and symptoms are often conducted on individuals with frequent exposure to the infections (Pickering et al. 1984). The results from detailed study of individuals in endemic settings suggests the possibility that in cases of frequent exposure, most of the infections are asymptomatic, and this process may maintain immunity at a level such that the infection does not develop into severe illness (Haque et al. 2006). This pattern is very different from some other diseases (i.e., HIV infection, tuberculosis) where individuals who are most frequently exposed also have the highest morbidity.

Other confounding factors include the presence of an undetected pathogen like *Cryptosporidium* spp. in the microbe-free symptomatic population (Chapter 7, Section 7.13); the presence of innate immunity (Duggal et al. 2004; Haque et al. 2002); and the manner in which the physician classifies a patient as symptomatic or asymptomatic (Janoff et al. 1990). Markell was apparently aware of the influence of acquired and innate immunity in mediating symptoms of parasitic infections, as he noted both in his text on medical parasitology, but they were not noted as possible factors in influencing the outcome of *Blastocystis* population studies (Markell and Voge 1976).

8.7 Other Tests for Pathogenicity

Additional tests for pathogenicity have been proposed. One author, writing a letter to the editor in the Journal of Clinical Microbiology in 1990, cited five criteria:

> Markell and Udkow comment on the "guilt by association" phenomenon which has grown up around this organism in the recent literature. Stated another way, simply repeating continuously the statement that *B. hominis* is a pathogen will not make it so. In addition to the fact that there is no clear segregation of this organism between symptomatic and asymptomatic persons, we need to remember the following: (1) Koch's postulates have never been satisfied (there is no reproducible model of experimental infection due to *B. hominis* only), (2) no pathologic evidence of or immunologic response to "infection" has been demonstrated in humans, (3) no mechanisms of pathogenicity, such as toxin elaboration, attachment to intestinal mucosa, or invasiveness, in humans have been described, (4) no antimicrobial agent which is uniquely active against *B. hominis* has been shown to reliably eradicate both the organism and the diarrhea, and (5) there has never been a point-source outbreak of gastroenteritis in which epidemiologic evidence suggested that *B. hominis* was the cause.
>
> *Blastocystis Pathogenicity* (Rosenblatt 1990)

In this case, the author suggests that a combination of laboratory studies (animal models, immunologic response, and pathogenesis) should be used in conjunction

with an epidemiological criterion of the point-source outbreak, and the clinical criteria of a drug that acts against the microbe.

Additional criteria were cited by Dr. Emilio deBess, in testimony from the State of Oregon Epidemiologist's Office in testimony concerning legislation making *Blastocystis* a reportable infection (Supplemental files).

1. We asked the CDC. They have an epidemiologist section where they deal with things like *Giardia* and other parasites, and again they claim there is no convincing evidence at this point that would make us think that *B. hominis* is an organism that could actually cause an illness or disease.
2. One of the interesting facts about this particular organism or parasite is that when it was actually fed to animals that were bacteria free, it did not cause disease that is one of the basis by which we actually look at how a bacteria or parasite works.
3. As I said, the AIDS and HIV era did not put Blastocystis hominis forward as an organism that could actually cause illness in people who are immune compromised.
4. There are few lines of evidence in regards to blastocystosis but one of them in 2000 has to do with a Swedish study, and they actually found more Blastocystis hominis in well people than sick people. And that is kind of a landmark article.

– Dr. Emilio deBess, DVM
Oregon State Epidemiologist's Office
April 2006

As an illustration of the difficulty in developing consensus on the idea of pathogenicity, Dr. deBess' four criteria form almost a disjoint set with respect to Rosenblatt's criteria. Dr. deBess introduces a new criterion for establishing a pathogen—mainly, it should be shown to be problematic in HIV patients. Also, while Dr. Rosenblatt limited his criteria to the existence of medical studies, Dr. deBess introduces a criterion for opinion polling, in that a specific group should agree that the organism is pathogenic. While Rosenblatt's criteria required that specific studies exist, Dr. deBess criteria include what might be called "lack of existence" criteria. That is, an organism should not be considered pathogenic if specific studies exist which would conflict with the idea of pathogenicity, such as the Swedish study, or the animal study.

The abstract from an invited review on *Blastocystis* published in the January 2012 issue of *Clinical Infectious Diseases*, the journal of the Infectious Disease Society of America, provides some additional viewpoints on pathogenicity, and also shows how the requirements can potentially change over time:

> Parasites in the genus *Blastocystis* comprise several subtypes (genotypes) and have a worldwide distribution. In some surveys, these are the most common parasites found in human stool specimens. An emerging literature suggests that the pathogenicity of *Blastocystis* is related to specific subtypes and parasite burden, although even individuals with small numbers of cysts may be symptomatic. Some data suggest an association between infection with *Blastocystis* and irritable bowel syndrome. However, there anuare few clinical studies demonstrating a direct relationship between the presence of this parasite and disease, few animal models to explore this relationship, and no consensus as to appropriate treatment. We recommend that asymptomatic individuals with few cysts not be treated. However, those who have gastrointestinal or dermatologic signs and symptoms

> and many cysts in stool specimens may require treatment. Metronidazole is the drug of choice. Additional studies are required to determine pathogenicity and appropriate therapy.
>
> *Blastocystis: To Treat or Not To Treat* (Coyle et al. 2012)

In 2006, animal models had been identified only by a research team in Singapore (Moe et al. 1997). By 2012, a number of laboratories, primarily in the Middle East and China had published papers describing animal models for blastocystosis in mice and rats. While the body of research would have met Rosenblatt's criteria from 1990 for animal models, a new criterion is proposed by Coyle, mainly that many animal models should exist (Table 8.5).

At the same time, some researchers had reported that symptomatic experimental animal infection required inoculating animals orally with a large number of cysts, noting that small numbers of cysts did not produce symptomatic infection (Elwakil and Hewedi 2010). This adds a level of complexity if negative criteria are to be applied, such as that noted by Dr. deBess in 2006. Mainly, if researchers use a small number of cysts in inoculating animals, Dr. deBess' second criteria (or objection) will be present, mainly that studies exist which show that inoculating animals with the microbe does not produce illness.

Table 8.6 shows the diversity of opinion concerning pathogenicity that can exist among experts by comparing nine different criteria for pathogenicity cited by these five experts (Markell, Rosenblatt, deBess, Hall, Coyle). At most, different experts share only two of these nine properties, and in many cases, experts do not have any concepts in common. Pathogenicity appears to be highly subjective, and the discovery of new information does not necessarily improve the level of consensus between decision makers. This may explain why historically, decision making about many pathogens is seldom performed by organizations of physicians.

8.8 Other Methods for Making Medical Decisions

The slow pace of progress in the area of gastrointestinal protozoal infections may be due to the mechanism by which public policy decisions are made. Although medical studies go through a peer review process prior to publication, in many cases, the development of policy from medical studies is performed through a second review process (Table 8.7). Examples include the process of drug approval, which is performed by the Food and Drug Administration (FDA) in the USA. The process by which mental disorders are defined is performed by panels of experts convened every few years by the American Psychiatric Association.

In these cases, it is recognized that the second review process is needed, because a consensus does not develop in the community solely from the existence of more studies. The process of reviewing studies and making decisions requires resources and an environment which may not be present in the physician's office. It is not a process that happens spontaneously. The process is designed to prevent specific biases. For example, in drug approval, physicians who develop drugs may develop biases in favor of their use, so the FDA selects groups of individuals to review studies

Table 8.5 Comparison of criteria for pathogenicity from different experts, based on statements from those individuals. Between the five experts examined, at least nine different criteria for pathogenicity are suggested, but very few experts identify the same criteria for pathogenicity

Authority	Year	Prevalence in symptomatic and asymptomatic	Existence of animal models	Severe illness in AIDS patients	Success of iodoquinol treatment	Success of any treatment	Physician behavior (do they treat it?)	Number of animal models	Presence of epidemic	Must be present at low rate in general population
Markell	1986	Yes	No	No	Yes	Yes	No	No	No	No
Rosenblatt	1990	No	Yes	No	No	Yes	No	No	Yes	No
deBess	2009	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes
Hall	2010	No	No	No	No	No	Yes	No	No	No
Coyle	2012	No	Yes	No	No	Yes	No	Yes	No	No

Table 8.6 Number of times overlapping is observed in criteria for pathogenicity cited by experts, based on data from Table 8.5. Out of nine possible criteria only two criteria were identified by more than one researcher, suggesting that the idea of pathogenicity may be a strongly subjective concept.

	Markell	Rosenblatt	deBess	Hall	Coyle
Markell	–	1	2	0	1
Rosenblatt	–	–	2	0	2
deBess	–	–	–	1	2
Hall	–	–	–	–	0
Coyle	–	–	–	–	–

Table 8.7 Examples of groups that review medical studies regularly, and public policy decisions concerning those studies

Region	Group name	How often	Input	Output
USA	Food and drug administration (FDA)	Weekly–monthly	Medical studies testimony	Decisions concerning drug approval and warning labels
USA	Institute of medicine (IOM)	Several times/ year	Medical studies testimony	Written medical policy analyses, often for US Congress
USA	American Psychiatric association (APA)	Once every several years	Medical studies	Statistical manual of mental disorders (DSM)—mental disorders and diagnosis codes
Europe	European medicines agency	Weekly–monthly	Medical studies	Decisions concerning drug approval and warning labels for the EU

who are not involved in the drug's development. In writing the Statistical Manual of Mental Disorders (DSM), the American Psychiatric Association recognizes that different physicians may develop different ideas about how to classify mental disorders, and that there is a value in developing an industry-wide standard.

Why are such regulatory organizations necessary in medical science, and not in physics or mathematics? One reason may come from an examination of the number of individuals who have an interest in the outcome of a decision, and the process by which the "debate" is held. In the case of *Blastocystis* infection in the USA, there have been approximately five researchers who have pursued multiyear research projects concerning *Blastocystis* infection over the last 30 years. However, data from those research efforts impacts medical practice of virtually all physicians in the USA, who number over 600,000.

The decision making process is generally self-selective, with researchers choosing to publish, and individuals within the medical community choosing to object to such publication. If just one physician in 1000 objects to a type of research, individuals who object to the research will quickly out-number the parasitologists. In contrast, organizations that develop medical policies generally use an appointment process, where individuals are selected to serve on a committee which renders a decision.

The mechanics of this process might be seen in the recent controversy surrounding reclassification of Pluto from a planet to a small planetary body. Even though this proposal would have little tangible economic or social impact, the reclassification was vigorously opposed by many schoolchildren and teachers. State legislative bodies in the USA even passed laws declaring Pluto to be a planet. The final decision was made by the International Astronomical Union, and is a potential illustration of the necessity of decision making bodies in science. It is unlikely that publication of additional studies about the size of Pluto would have much effect in resolving the controversy.

8.9 Blastocystis Firsts and the Shift of Research East

In compiling a list of significant events in *Blastocystis* research (Table 8.4), it is intriguing to note the number of countries involved, and that regions in the Middle East and Asia are strongly represented in this list, while the USA and UK are virtually absent. In the development of an international standard for naming *Blastocystis* isolates in 2010, no US researcher participated.

It is suggested that this distribution differs from that seen in *G. intestinalis* or *Cryptosporidium spp.*, where US and UK teams made significant contributions. Researchers from the US and UK were the first to achieve a variety of milestones, such as the first animal models (Fantham and Porter 1916), the first studies of immunological responses in humans (Brown et al. 1973), leadership for the first full-genome mapping of *G. intestinalis* (Morrison et al. 2007) and *Cryptosporidium* spp.(Xu et al. 2004). In the case of *Blastocystis*, the US withdrawal from research has altered the landscape for this organism, and most advanced research is now performed in Asia, the Middle East, and more recently Europe and Mexico (Table 8.4).

The variation cannot be explained by the prevalence of the infection alone, since studies show that *Blastocystis* ranks as the most frequently identified parasitic infection in the US and UK, and is detected in over 20 % of individuals with gastrointestinal illness in studies from those countries (Windsor et al. 2007, Boorom et al. 2008). Given research trends noted earlier, some parallels may be drawn between the situation in *Blastocystis*, and the ban of US federal funding of many types of embryonic stem cell research, which was a response to objections from leaders of certain religious groups in the USA. The ban provides advantages to research centers in Asian countries, where similar objections do not exist (Walters 2004).

For researchers interested in future work, this suggests that the epicenter for this type of research will remain in the East, as the majority of advanced research will come from a few European countries (Denmark, Turkey, France, Greece) working with Asian and Middle Eastern groups. Those collaborations can already be seen in papers, such as a 2010 review paper with strong participation from researchers in these regions (Boorom et al. 2008), a collaboration between French and Egyptian researchers (Souppart et al. 2010), and a collaboration between Turkish and Japanese researchers (Yoshikawa et al. 2011).

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