Chapter 7
What Do We Know About *Blastocystis* Analyzing Research Studies with Statistical Methods

Kenneth Boorom

Abstract *Blastocystis* is the most commonly identified parasitic infection in stool samples from healthcare-seeking patients in many regions of the world. A collection of close to 1,000 studies exists on *Blastocystis*, with substantial contributions from researchers in the Middle East, Asia/Pacific Rim, Europe, and North America. In many cases, disagreements about *Blastocystis* appear to originate from difficulties in determining how decisions should be made from the existing database of studies. Although more studies will certainly be available in the future, it is possible to apply objective screening techniques to studies which can be expected to identify conclusions that are unlikely to be reversed. However, the conclusions identified with this technique are not necessarily the same conclusions described as certain by medical professionals. A better understanding of the meaning of statistical data obtained from populations would help reduce contradictory studies, and examples are provided of situations under which a pathogen like *Giardia intestinalis* will appear more often in healthy individuals. The role of *Blastocystis* in irritable bowel syndrome (IBS) is examined, and formulas presented to help understand the costs of blastocystosis, given research studies identifying the cost of IBS.

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

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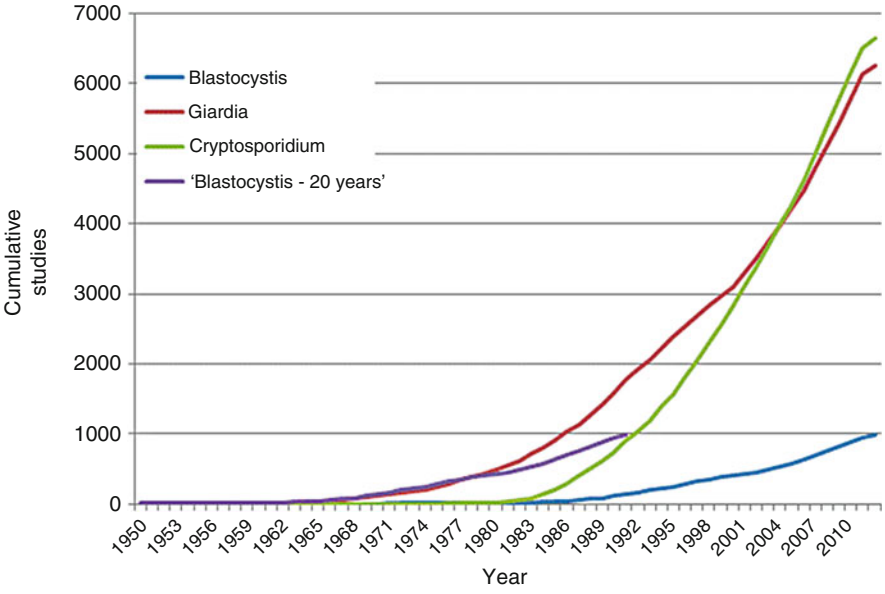

Fig. 7.1 Cumulative NIH indexed studies for three common intestinal protists. In terms of the total number of studies, *Blastocystis* spp. lags *Giardia intestinalis* by 30 years, and *Cryptosporidium* spp. by 20 years. Most of the controversy concerning *Blastocystis* infection occurred between 1986 and 1993, when there were very few studies on the infection available

7.1 Introduction

As of May 2012, the US National Institute of Health (NIH) Pubmed medical research database shows that 935 studies referring to *Blastocystis* have been cataloged. With close to 1,000 papers on the subject, it is likely that such a collection contains useful information, misleading information, as well as studies which contradict each other's findings. Given that this situation exists for *Blastocystis* (as well as most other microbes of clinical interest), how can we extract information that is needed from that database for clinical and research purposes? How can we use those studies to make decisions? Can we make decisions based on this quantity of studies?

Certainly, there are fewer studies on *Blastocystis* than either *Giardia intestinalis* or *Cryptosporidium* spp., each of which has roughly 6,000 studies, or six times as many papers as *Blastocystis* (Figs. 7.1 and 7.2). Each year, about 350 new *G. intestinalis* papers are added to Pubmed's database. Each year a similar quantity of *Cryptosporidium* sp., research is added to that database, while 50–60 new studies on *Blastocystis* are added annually (Fig. 7.2). Physicians and researchers did make decisions concerning *G. intestinalis* and *Cryptosporidium* spp. when the collection of studies for those microbes consisted of only 1,000 papers, in the 1980s and

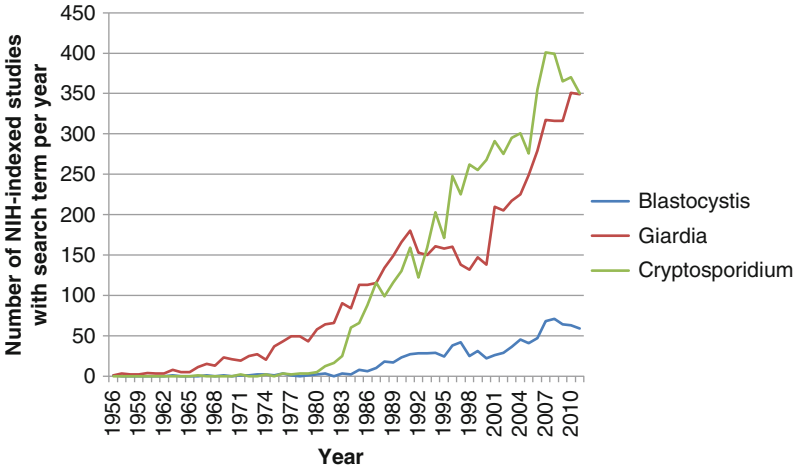

Fig. 7.2 Number of new NIH indexed studies published each year for three common intestinal Protists. The rate of publication for *G. intestinalis* and *Cryptosporidium* spp. is about 5–7 times higher than that for *Blastocystis*

Table 7.1 Comparison of the number of studies, the number of new studies, and NIH grant approval for three gastrointestinal protozoa

Metric	Selection criteria	*Blastocystis*	Giardia (Giardia : *Blastocystis*)	Cryptosporidium
Total studies published	Microbe name in title, abstract, or keyword	935	6,023	6,311
Total studies with microbe name in title	Microbe name in title	574	2,871	3,594
Average new studies published annually, 2009–2011	Microbe name in title, abstract, or keyword	56	310	325
Number of US National Institute of Health grants awarded 2000–2011[a]	Microbe name in title, abstract, or keyword	0	366	693

The total number of studies and the number of new studies for *G. intestinalis* and *Cryptosporidium* spp. outnumber *Blastocystis* by a factor of 5–7, and the finding is relatively invariant depending on the two selection criteria used for defining a microbe study *Blastocystis*.

[a]Search performed with NIH Project Reporter tool, 5/5/2012. http://projectreporter.nih.gov/reporter.cfm

1990s. There is little evidence that decisions made based on that smaller set of studies were egregiously wrong, or resulted in widespread harm or misdiagnosis of patients. As such, it is reasonable to suggest that we can use a database consisting of 1,000 studies to make responsible decisions concerning *Blastocystis* (Table 7.1).

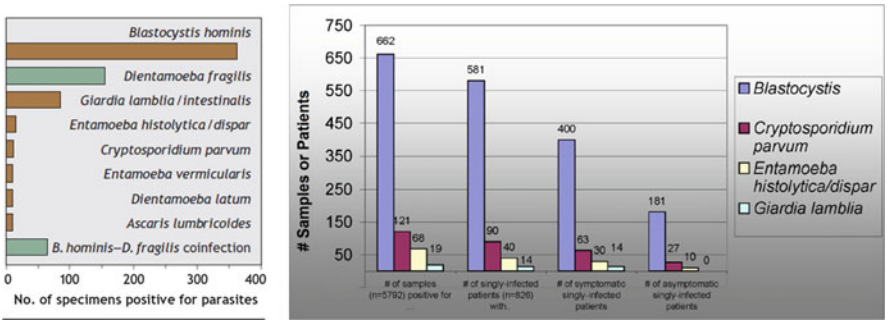

Fig. 7.3 *Blastocystis* infection dominates stool samples submitted by patients to clinical laboratories in most developed countries. Results from stool examination of patients at a regional Canadian laboratory in 2005 and a US study of samples collected from all 50 states in 2000

7.2 Should We Make Any Decisions About *Blastocystis*?

Before examining ways of making decisions, it may be worth acknowledging two contrarian arguments:

1. Sufficient studies do not exist to make a decision with confidence, so no decision should be made.
2. The existence of conflicting studies on *Blastocystis* pathogenicity proves that the microbe could be only marginally pathogenic, and as such, it warrants no attention.

In examining the first argument, it should be noted that in developed countries, symptomatic *Blastocystis* mono-infections now outnumber *G. intestinalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* mono-infections combined in parasitological samples from symptomatic patients (Fig. 7.3). That is, a physician ordering such examinations for patients with gastrointestinal illness will need to make some kind of decision as to whether to prescribe antimicrobial treatment, pursue further testing, or to diagnose the patient with irritable bowel syndrome (IBS). Because physicians cannot delay decisions about patients for several years, physicians will make decisions in these situations using the available information. As such, the choice presented to us is not between a decision and no decision, but between a decision based on current scientific studies, or a decision based on cultural beliefs, notes from an old parasitology course, or other less desirable sources. Even if we could prohibit clinical decision making for *Blastocystis*, it would still be necessary to make decisions about whether research should be performed.

In addressing the second argument, it is noted that evidence of *Blastocystis* nonpathogenicity has come almost exclusively from statistical analysis of population studies, and the statistical methods used in those studies are not guaranteed to prove pathogenicity, only correlation. A pathogen can become uncorrelated with illness when other factors, such as innate or acquired immunity, enter into the population and confound the study results. Additionally, as noted later in the chapter, the presence of other microbes that produce symptoms equivalent to

blastocystosis will confound a population study if those patients so-infected are also *Blastocystis*-negative.

One interesting example of this comes from studies relating infection with *Helicobacter pylori* and gastric cancer. Researchers used two different immunoassays to assess *H. pylori* infection, namely a CagA assay and a whole cell *H. pylori* assay were used to investigate cancer risk. The population consisted of 181 gastric cancer patients and 193 cancer-free controls. Taken individually, a positive finding for either of these immunoassays showed no statistically significant correlation with gastric cancer originating in the cardia, and no correlation with gastric cancer originating elsewhere ($p = 0.07, 0.11$). However, when the patient populations for both cancers were combined, a statistically significant relationship was formed with the whole cell response ($p = 0.03$). Furthermore, when the two immunoassays were combined, a statistically significant relationship was found between a positive antigenic finding, and both types of gastric cancer individually, and both types combined ($p = 0.02$, $p = 0.01$, $p = 0.003$, respectively).

In the case of *H. pylori* and gastric cancer, we can see that a serious illness can be correlated with a microbe, even though some study constructions do not show a correlation. This addresses the second argument, mainly that if all studies do not show a microbe is correlated with an illness, then the microbe must be a mild pathogen. It may be worth noting that in the case of *Blastocystis* studies, many of the most frequently cited studies, especially the ones that produced conflicting information, did not specify how patients were divided into symptomatic and asymptomatic groups with enough detail to allow us to replicate them. That is, we do not know how the symptoms typically found in this patient population, such as diarrhea, vomiting, nausea, fatigue, abdominal pain, and constipation would translate into an assignment to the symptomatic group or asymptomatic group. This is particularly problematic for enteric protozoal infections, as individuals with diarrhea commonly constitute only a third of the symptomatic group (Qadri et al. 1989; US EPA 1979).

7.3 What Kind of Decisions Need to Be Made Concerning *Blastocystis*?

Although the discussion concerning *Blastocystis* is often portrayed as "pathogenic versus nonpathogenic," the actual decisions that concern the microbe can span a much larger range. Examples of decisions that face institutions and individuals include:

1. If *Blastocystis* is found in a patient who is symptomatic, what actions should be taken?
   (a) Should additional testing be performed?
   (b) If additional testing is negative, should treatment be attempted?
   (c) Should the patient be informed about the infection?
   (d) If the patient is informed, what additional information should be provided?

2. If researchers studying psychosomatic illness perform a study on patients with "irritable bowel syndrome," should those researchers required to test patients for parasitological infections first?
   If patients in such a study are positive for *Blastocystis*, should they be informed of the infection?
   Is it acceptable to require neuro-gastroenterologists (NGs) to inform IBS patients of *Blastocystis* infection if those researchers disagree with the emphasis placed on the germ theory in identifying gastrointestinal illness, for example as noted in introduction to the Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders (Drossman 2006).
   If NG's must test IBS patients for infectious diseases, should microbiologists be required to test diarrheal patients for things of interest to NG's, such as early life exposure to emotional stress or traumatic instances of material separation? (Drossman 2006).
3. Should this country have a *Blastocystis* research effort?
4. Should lettuce or spinach contaminated with *Blastocystis* cysts be fed to humans?
5. If a food service worker at an elementary school develops chronic diarrhea, and tests positive for *Blastocystis*, should that worker be informed of the infection?
   If a particular physician opposes classification of *Blastocystis* as a pathogen, is it acceptable for that physician to diagnose such an individual with IBS and refer them to psychotherapy?
6. If some physicians believe that the food service worker should be informed of the infection, but other physicians indicate that no information should be provided, is that an acceptable situation?
7. Can an employer require a food service employee to be treated for Blastocystis infection if they have been shown to have infected others through food handling practices?
   What if the employee's physician opposes classification of Blastocystis as a pathogen?

7.4 What Is a Metastudy?

Scientists often perform studies on populations of patients, but how do we perform a study on a population of research papers? Unfortunately, there are few standards or consensus decisions for examining a collection of studies and drawing a conclusion.[1] The Cochrane Reviews may be the most familiar example of a

[1] In 2010, BRF submitted an early version of our metastudy on *Blastocystis* research to a journal, and that paper consisted mostly of a list of studies finding *Blastocystis* pathogenic or nonpathogenic. A reviewer rejected the paper on the basis that scientific findings are largely the result of researchers repeating studies until they obtain the result they want, and as such surveying papers or tabulating study results was of no value. While this is an interesting idea, it creates a kind of philosophical crisis, in that we would then be unable to use any scientific study result, if such results are fabrications of researchers.

methodological approach to decision making, but historically these have been limited to decision making about the efficacies of treatment, rather than assessment of clinical significance of microbial infections.[2]

One approach to decision making involves performing a "metastudy" and using statistical methods similar to those used in patient population studies. A metastudy analyzes research findings in the same way that a clinical study analyzes patients:

1. An initial selection criteria is applied to the database of studies. For example, a search term like *Blastocystis* might be provided to a search engine like the NIH's Pubmed database.
2. From the studies returned in step #1, further selection criteria are applied to eliminate studies that do not address the question being examined. For example, a metastudy on antibiotic resistance might discard studies which did not examine this particular phenomenon.
3. One or more study parameters are quantified and examined for correlation. For example, a study that investigates whether physical therapy is valuable in the rehabilitation of hamstring injuries might record the number of weeks each study noted until return to normal function, along with the type of physical therapy (if any) provided.

A metastudy differs from a review in that the studies being examined are selected using a process which can be applied by other researchers in such a way that the findings would be repeatable. Reviews can largely be the product of a specific researcher, and as such, can be skewed in favor of the author's particular view, and are therefore not repeatable. The lack of repeatability can be seen by comparing different reviews on the same subject.

7.5 The First *Blastocystis* Metastudy

By 2008, the NIH's Pubmed server database had a total of 670 studies on *Blastocystis*, but nobody had ever performed a metastudy on this group of studies. Moreover, it appeared that some public agencies were engaging in a very selective process when identifying which studies were deemed relevant concerning *Blastocystis* significance. At BRF, we organized a diverse group of 11 *Blastocystis* researchers from 9 countries to examine this collection of research and also to examine the relationship between *Blastocystis* and IBS (Boorom et al. 2008). In terms of researcher participation and number of countries represented, this study may be the largest published on either *Blastocystis* or IBS (Table 7.2).

[2] We contacted the Cochrane Reviews group about this possibility, but they indicated that such a study would likely be a low priority, because *Blastocystis* infection was not viewed as germane to global world health goals.

Table 7.2 Co-authors of a metastudy of *Blastocystis* research published in 2010 in BMC Parasites and Vectors

No.	Name	Affiliation	Country
1	Kenneth Boorom	Blastocystis Research Foundation, Corvallis, OR	USA
2	Huw Smith	Scottish Parasite Diagnostic Laboratory Glasgow	UK
3	Laila Nimri	US Center for Disease Control and Jordan University of Science and Technology Atlanta, GA	Jordan
4	Eric Visclgliosi	Pasteur Institute, Lille, France	France
5	Gregory Spanakos	National School of Public Health Athens	Greece
6	Unaiza Parkar	WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary and Biomedical Sciences, Murdoch University	Australia
7	Lan-Hua Li	Department of Preventative Medicine Weifang Medical University	China
8	Xiao-Nong Zhou	China National Institute of Parasitic Diseases	China
9	Ulgen Ok	Department of Parasitology Celal Bayer University	Turkey
10	Saovanee Leelayoova	Phramongkutklao College of Medicine Bangkok	Thailand
11	Morris Jones	US Air Force Travis Air Force Base, CA	USA

We invited researchers from nine countries to participate in order to minimize the impact of cultural beliefs on the study's findings

For each of the *Blastocystis* studies examined, we collected data about sample size, study approach (animal studies, population studies, treatment studies), the year of the study, etc. We also recorded the country where the study was performed. We then sought to determine if any of those factors were related to findings of pathogenicity.

At the time, a line of investigation had suggested that certain *Blastocystis* subtypes may be pathogenic or nonpathogenic, and this was responsible for disagreement among researchers. We were, expecting to see some kind of geographical clustering, but as happens sometimes in such studies, our actual findings were very different and potentially more valuable:

1. We analyzed 680 studies, 102 of which provided a finding concerning pathogenicity or nonpathogenicity. We excluded review studies, and letters to the editor, surveys of enteric protozoa which did not report symptoms, etc. (Fig. 7.4).
2. Within the 102 studies, we found 16 studies identifying *Blastocystis* as nonpathogenic, and 86 studies identifying it as pathogenic (Table 7.3).
3. All (16/16) studies identifying *Blastocystis* as nonpathogenic were conducted on individuals from more affluent countries (Europe, US, and Australia). Half of the 16 studies (8/16) identifying *Blastocystis* as nonpathogenic were performed in the USA before 1994. When studies performed outside of the USA, or after 1994 were considered, 93 % (79/85) of such studies identified *Blastocystis* as pathogenic.

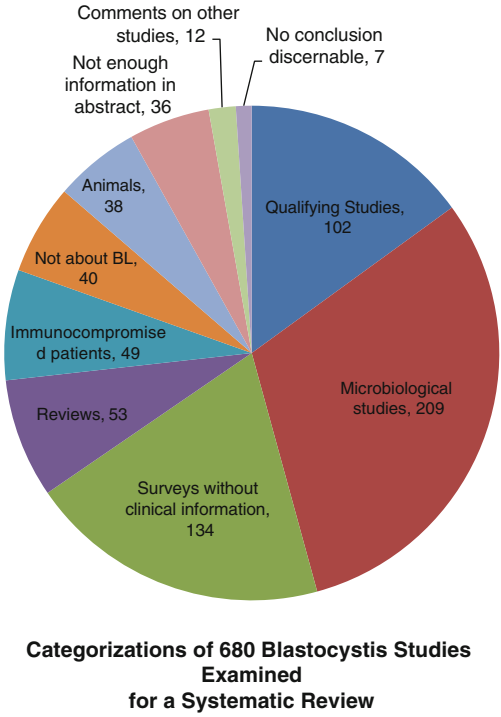

Fig. 7.4 Analysis of NIH-Indexed *Blastocystis* Studies as of January 2008. Qualifying studies were those related to infection in immunocompetent individuals where the researcher reported a finding concerning pathogenicity based on a scientific investigation

4. Overall, 44 % (8/18) of the studies performed in North America before 1994 identified *Blastocystis* as nonpathogenic, while 93 % (79/85) of the studies performed after 1994 or outside of North America identified *Blastocystis* as pathogenic.
5. In almost all studies (15/16) concluding that *Blastocystis* was nonpathogenic, the author had identified a specific property of *Blastocystis* infection thought to be incompatible with pathogenicity. For example, the presence of symptomatic and asymptomatic individuals in the same family was thought to be impossible if *Blastocystis* were a pathogen (Senay and MacPherson 1990).

We mapped researcher findings for those studies conducted in the Western Hemisphere, because most of the disagreement appeared to originate in this region (Fig. 7.5). While some clustering existed, we could not explain the divergence of opinion in terms of the spread of different *Blastocystis* subtypes, primarily because of item #5 above. Mainly, researcher finding varied not because *Blastocystis* varied, but because researchers themselves differed.

The most fascinating example of this involved one study published in 1993 by a pair of California physicians (Dr. Edward K. Markell and Dr. Udkow) that noted *Blastocystis* presence was uncorrelated with symptoms in patients from Oakland, California (Udkow and Markell 1993). This paper became one of the five works selected by the Center for Disease Control for that group's *Blastocystis* Fact Sheet, which was distributed from the CDC's web site for over 10 years, from 2000 to 2010 (CDC 2000) (Table 7.4)

Table 7.3 In a survey of 680 *Blastocystis* studies published before January 2008, we identified 16 that found *Blastocystis* to be nonpathogenic, and 86 that found it to be pathogenic. Studies finding *Blastocystis* to be nonpathogenic are shown in this table.

No.	Pubmed ID	Title	Author	*n* Subjects	Year	Location
1	2889924	Lack of serum immune response to *Blastocystis* hominis	Chen		1987	USA
2	3766850	*Blastocystis* hominis: pathogen or fellow traveler?	Markell	148	1986	USA
3	3055191	*Blastocystis* hominis: an organism in search of a disease	Miller		1988	USA
4	8463612	*Blastocystis* hominis in inflammatory bowel disease	Nagler	12	1993	USA
5	2596457	Questionable clinical significance of *Blastocystis* hominis infection	Sun	6,262	1989	USA
6	2229995	Frequency of recovery of *Blastocystis* hominis in clinical practice	Zuckerman		1990	USA
7	13677378	Clinical characteristics and endoscopic findings associated with *Blastocystis* hominis in healthy adults	Chen	292	2003	Taiwan
8	10414382	Prevalence and clinical relevance of *Blastocystis* hominis in diverse patient cohort	Cirioni O.	1,216	1999	Italy
9	9158042	Epidemiologic survey of *Blastocystis* hominis infection in Japan	Horiki	6,476	1997	Japan
10	8545396	*Blastocystis* hominis: a common commensal in the colon. Study of prevalence indifferent populations of Paris	Junod	7,677	1999	France
11	16105126	No correlation between clinical symptoms and *Blastocystis* hominis inimmunocompetent individuals	Leder	2,800	2005	Australia
12	2401797	*Blastocystis* hominis: epidemiology and natural history	Senay		1990	Canada
13	7578767	Is *Blastocystis* hominis a cause of diarrhea in travelers? A prospective controlled study in Nepal	Shlim	301	1995	Nepal
14	2218447	Etiology of diarrheal diseases in immunocompetent and HIV-positive patients	Steinman	206	1990	Germany
15	10816147	Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases	Svenungsson	1,053	2000	Sweden
16	8515120	*Blastocystis* hominis: prevalence in asymptomatic versus symptomatic hosts	Udkow		1993	USA

All studies identifying *Blastocystis* as pathogenic were performed on individuals from affluent countries. Most of the studies characteristics of *Blastocystis* infection which would seem to be unlikely in a pathogen. However, most of those characteristics had previously reported in *E. histolytica* and *G. intestinalis* patients

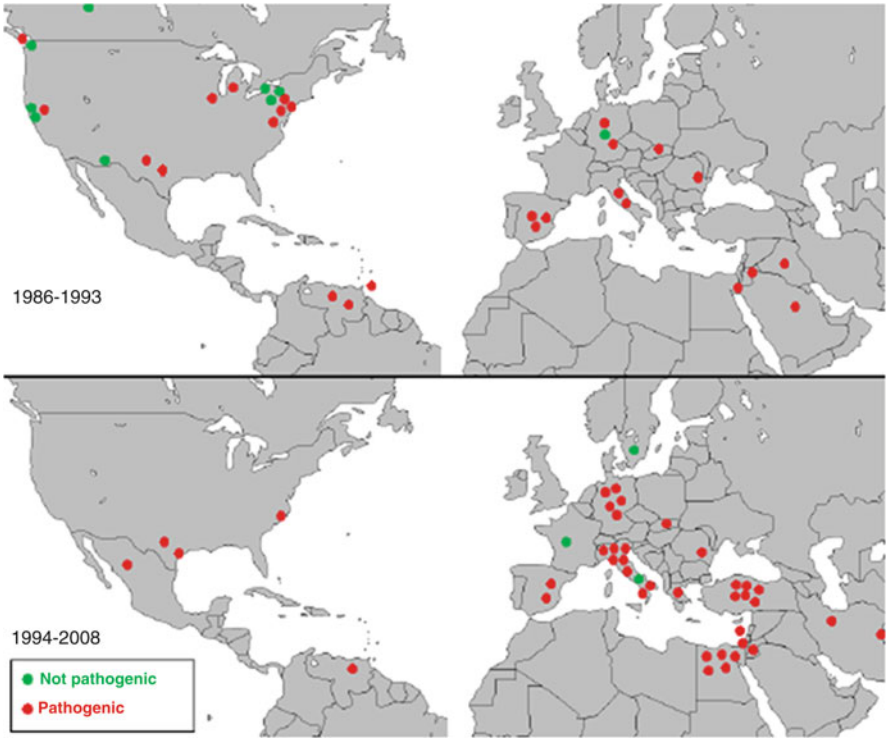

Fig. 7.5 Research conclusion in *Blastocystis* studies during two time periods. Most studies identifying *Blastocystis* as nonpathogenic were published between 1986 and 1993, and in those studies researchers generally identified characteristics of *Blastocystis* that were common to *G. intestinalis* and *E. histolytica*. After 1994, those studies became increasingly rare. Research came to a halt in the USA, possibly due to a policy change at the US National Institutes of Health, where staff members began informing potential *Blastocystis* researchers that no evidence existed to suggest *Blastocystis* was pathogenic

But the same property had been reported for *G. intestinalis* and *E. histolytica* in two papers studying patients in the same region a few years earlier:

> No correlation between symptoms and the presence of absence of infection <with Giardia lamblia and *Entamoeba histolytica*> could be detected
>
> –*Intestinal Parasitic Infections in Homosexual Men at a San Francisco Health Fair*, 1983
>
> 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*, 1984

Following the rules of logic, if correlation with symptoms in humans is to be used as a litmus test for pathogenicity, one would need to understand why *G. intestinalis* and *E. histolytica* were uncorrelated with symptoms in those regional

Table 7.4 Studies cited by the US Center for Disease Control *Blastocystis* Factsheet, 1991–2010

Albrecht H, Stellbrink HJ, Koperski K, et al. *Blastocystis hominis* in human immunodeficiency virus-related diarrhea. Scand J Gastroenterol 1995;30:909–914
Markell EK, Udkow MP. *Blastocystis hominis*: pathogen or fellow traveler? Am J Trop Med Hyg 1986;35:1023–1026
Miller RA, Minshew BH. *Blastocystis hominis*: An organism in search of a disease. Rev Infect Dis 1988;10:930–938
Udkow MP, Markell EK. *Blastocystis hominis*: prevalence in asymptomatic versus symptomatic hosts. J Infect Dis 1993;168:242–244
Zuckerman MJ, Watts MT, Ho H., et al. *Blastocystis hominis* infection and intestinal injury. Am J Med Sci 1994;308:96–101

The works of Markell and Udkow had an extraordinary influence on US thought, to the extent that their work was virtually the sole source cited for information on *Blastocystis* until 2010, when that agency replaced the fact sheet and updated the references. By 2010, the average age of the references in this list was 18.6 years. Copies of both fact sheets appear in the supplemental files to this chapter

studies. Without knowing why the lack of correlation existed for known pathogens, it is not possible to state that a lack of correlation proves nonpathogenicity.

In this case, it is likely that Dr. Markell was aware of the two papers noted above, as he was listed as the primary author for both of them. In reviewing Markell's other work, it appears that he never published a paper that identified a statistically significant relationship between any enteric protozoal infection and symptoms. So we can rephrase the scientific question to, "Why did all of Markell's studies show no correlation between *G. intestinalis*, *E. histolytica*, and *Blastocystis* and symptoms?" Identification of cases where the same property is interpreted differently depending on researcher goals suggests that researcher interpretation has played a large role in study outcome.

There are, in fact, many counterintuitive findings that exist for enteric protozoal infections. In our review paper, we included a collection of these in an appendix called "Characteristics of Known Enteric Pathogens and Diseases." A copy of that appendix is included in the supplementary material for this chapter.

7.6 Analyzing Blastocystis Controversy

Our systematic analysis showed that most of the written controversy about *Blastocystis* occurred in the late 1980s and early 1990s, when a number of US physicians were publishing papers on behavior in *Blastocystis* thought to be incompatible with pathogenicity. The controversy peaked between 1988 and 1990, with researchers trading angry letters in the Journal of Clinical Microbiology. That exchange appeared to be the origin of the notion of *Blastocystis* as a controversial pathogen (Markell and Udkow 1990a,b; Rosenblatt 1990; Zierdt 1991).

Dr. Edward K. Markell, a physician with a health maintenance organization (HMO) in Oakland, California, and coauthor of the venerable Markell's *Medical Parasitology*, became particularly well known for his efforts in this area. Dr. Markell passed away in 1998, and *Blastocystis* appears to have followed him to the grave:

> He strongly believed that Blastocystis was not a pathogen, and he led virtually a one-man campaign to draw attention to its coincidental association with disease.
> In Memorian, Edward K. Markell, Parasitology Research, 1999 (Editor 1999)

Markell's work can be shown to be extraordinarily influential on *Blastocystis* opinion in the USA, to the extent that US federal policy from the mid-1990s to around 2010 was largely based on his research. The NIH's Pubmed database shows Markell authored five works on *Blastocystis*, but only two of those were research studies. The other three works were letters where Markell praised researchers who duplicated his findings, or attacked those who contradicted them. Despite this, both of those studies earned a place in the US Center of Disease Control's factsheet on *Blastocystis*, and one of those studies remain among the most cited *Blastocystis* papers of all time (Table 7.5).

Those five studies were the only ones cited on the US CDC Fact Sheet, which appeared on that organization's web site with few changes from February 2000 to March 2008, according to data archived from that organization's web site (CDC 2000). The agency added four additional references in late 2008, one of which questioned *Blastocystis* pathogenicity, two of which were performed in Zambia and China, and one which described treatment of children in Mexico with the drug nitazoxanide (CDC 2008). The agency did not include any study suggesting *Blastocystis* could cause illness in immunocompetent adults in developed Western countries until 2010, when an entirely revised fact sheet was released. That fact sheet excluded all five of the original studies cited in 2000. The new revision represented the first time when clinical studies of immunocompetent adults performed in the twenty-first century appeared in the fact sheet (CDC 2011). Copies of the original 2000 version and the 2010 revision are included in the supplementary material for this chapter.

In developing decision-making methodologies, there can be value in understanding if the methodology used in selecting research studies may be producing a bias in favor of a particular viewpoint. This can be done by calculating metrics for the research studies selected for an analysis, and determining if those studies are more likely to exhibit a property which could skew the conclusion. In the case of the five studies in Table 7.4, there are several metrics we can calculate, which might help identify a more neutral process for study selection. For example, in 2010 when the CDC data sheet was updated, the average of five studies identified by the sheet was over 18 years.

7.7 Assessing Confidence and Overconfidence in Medical Findings

Collecting statistics about researcher methods can help prevent cultural biases from influencing study conclusion, and later public policy. This process can also be performed in a way which is "conclusion-neutral" in any specific scientific

Table 7.5 The ten most frequently cited *Blastocystis* papers, based on a search for the word "*Blastocystis*" in the title of all journal articles indexed in the scientific collections of Google Scholar, performed on May 29, 2012

N	Number of times cited	Year of publication	First author	Title	Journal
1	288	1996	Dunn	Blastocystis hominis revisited.	Clinical Microbiology Reviews
2	222	1991	Zierdt	Blastocystis hominis—past and future	Clinical Microbiology Reviews
3	127	1986	Sheehan	Association of Blastocystis hominis with signs and symptoms of human disease	Journal of Clinical Microbiology
4	135	1993	Doyle	Epidemiology and pathogenicity of Blastocystis hominis	Journal of Clinical Microbiology
6	134	1993	Boreham	Blastocystis in humans and animals: morphology, biology, and epizootiology	Advances in Parasitology
7	111	1986	Markell	Blastocystis hominis: pathogen or fellow traveler?.	American Journal of Tropical Medicine and Hygiene
8	109	1967	Zierdt	Protozoan characteristics of Blastocystis hominis.	American Journal of Clinical Pathology
9	108	2002	Tan	Recent advances in Blastocystis hominis research: hot spots in terra incognita	International Journal for Parasitology
10	104	1997	Clark	Extensive genetic diversity in Blastocystis hominis	Molecular and Biochemical Parasitology

Google Scholar is a publicly accessible database of scientific literature which, in addition to Pubmed, is commonly cited in statistical studies of scientific literature. It represents one of the largest publicly available collection of citations to such literature. Google Scholar differs from the search engine Google in that the Google Scholar does not index web pages, and instead indexes scientific books and papers.

controversy. That is, we can agree to refrain from using certain practices that would be expected to produce an incorrect decision, and we can create those guidelines before the controversy begins. For example, we might agree that a list of references should not over-represent the work of one particular author. We might agree that we should not rely exclusively on very old studies, because that could also misrepresent the current level of understanding concerning a scientific topic. We might even want to favor studies that are more recent, since science is often viewed as an iterative process, and newer studies are likely to be constructed and reviewed by individuals who have more access to information about the subject.

In examining historical mistakes in medical science concerning microbial pathogenesis (Table 7.7), it can be seen that errors frequently originate from overconfidence in research findings from a single author or group, even before that finding has been reproduced by other researchers. Overconfidence in the lack of an association between a microbe and illness has been demonstrated as well (Table 7.8). Dr. Barry

Table 7.6 An account of Markell's life noted that he waged a "virtually one-man campaign against" the idea of *Blastocystis* being pathogenic, and his work was extraordinarily influential on medical thought in the USA (Table 7.4) This table shows items associated with Markell and Blastocystis from the NIH's Pubmed database.

Year	Type	Journal	Title	Details
September 1986	Study	Journal of Clinical Microbiology	*Blastocystis hominis: pathogen or fellow traveler?*	Asserted that another cause, such as IBS or another infection, could be found in all Blastocystis patients
March 1988	Letter	Journal of Clinical Microbiology	*Association of Blastocystis hominis with human disease*	Markell objects to publications by Sheehan, et al. and Pikula, et al. linking *Blastocystis* to illness in humans
May 1990	Letter	Journal of Clinical Microbiology	*Association of Blastocystis hominis with human disease?*	Markell objects to papers by Qadri, et. al. and Sheehan, et al. linking *Blastocystis* to illness in humans
June 1990	Letter	Western Journal of Medicine	*Blastocystis hominis*	Markell objects to publication by Babb, et al. identifying *Blastocystis* in joints of an immunocompromised patient
July 1993	Study	Journal of Clinical Microbiology	*Blastocystis hominis: prevalence in asymptomatic* versus *symptomatic hosts*	Markell identifies *Blastocystis* at a similar prevalence in symptomatic (12.8 %) and asymptomatic (11.5 %) patients, suggests that patients will be harmed by physicians who investigate *Blastocystis*
July 1995	Letter	Clinical Infectious Diseases	*Is There Any Reason to Continue Treating Blastocystis Infections?*	Markell praises study on expatriates living in Nepal that found *Blastocystis* infection at a similar prevalence in healthy and diarrheal patients

Markell only published two papers on *Blastocystis* before he died in 1998. Most (4/6) of Markell's *Blastocystis* citations are complaints he made against other researchers who published papers that conflicted with his findings, or praise for researchers who corroborated his work

Marshall, who shared the 2005 Nobel Prize for discovering the role of *H. pylori* in stomach ulcers, devoted a book to the subject of the number of times the relationship had been discovered before and lost to history due to researcher harassment by the medical community (Marshall 2002). The three-century delay between the discovery of *G. intestinalis* and its designation as a pathogen is examined in more detail in the next Chapter. A more recent example of the failure to recognize a pathogen would be

Table 7.7 Type 1 errors (incorrect attribution of a cause and effect) in medical studies

N	Journal	PI, Finding	Retracted/explained
Oct 2001	Science	Mikovits, XMRV found at significantly increased rate in CFS patients	Oct 2011 (Silverman et al. 2011)
Feb 1998	Lancet	Wakefield, MMR virus from vaccinations is found in colonic biopsies from autistic children, and causes autism	Mar 2004 Feb 2010 (full) (Dyer 2010)
Dec 1986	Infection & Immunity	Mirleman, *E. dispar* turns into *E. histolytica*	Dec 1993 (Clark and Diamond 1993)
1926	(Nobel Prize in Medicine)	Fibiger, Cancer can be induced in rats by infecting them with *Spiroptera neoplastica*	1930–1935 (Stolt et al. 2004)

In many cases, studies appeared to be revolutionary, and were embraced by portions of the medical community, but were subsequently found to result from experimental error. Applying simple criteria, such as requiring the finding to be independently produced by three different laboratories in three different countries, can help avoid false alarms

the decision in 2000 by the leadership of South Africa to promote the view that the HIV virus is only incidental in the development of AIDS.

A great deal of the controversy surrounding *Blastocystis* appears to have originated from the extraordinary level of confidence possessed by a few early researchers. In medical research, self-confidence may play a greater role in influencing opinion than it does in scientific research, where repeatability is more valued. As one interesting illustration of this phenomenon, Markell attacked a study where a researcher had identified *Blastocystis* cells in the joint fluid from a patient being treated with immunosuppressive drugs, insisting that the researcher had fabricated the data, or mis-identified the *Blastocystis* cells (Lee et al. 1990). But Markell had never performed a study on an immunocompromised patient. How could he have such confidence that the researcher was in error, since Dr. Markell's studies were exclusively performed on immunocompetent patients?

7.8 Decision Making Theory and Management Errors

Tables 7.7 and 7.8 suggest that there are a number of cases where a great deal of morbidity and mortality could have been avoided by following up on researcher findings in a timely manner. In reviewing this history, it may be tempting to assume that the decision makers of the time were corrupt or diabolical. But the difficulty in developing policies to address this problem lies in the fact that post-mortem investigations show little if any malfeasance on the part of the decision markers.

There is a body of research known as "Behavioral Decision Theory" which examines why this is true, especially as it relates to economic decisions and recent financial disasters (Bazerman and Moore 2009). This field of research is finding its way into medical science: the most frequently accessed paper in the Public Library of Science's journal PLOS-One is entitled, "Why Most Published Research

Table 7.8 Type 2 errors in medical decision making (failure to recognize a cause of a disease).

Organism	Duration of investigatory/indeterminate period (discovery in symptomatic humans to acceptance as pathogen)	Organization or individual credited with transitioning organism to pathogenic status
Vibrio cholerae	36 years (1849–1884)	Robert Koch
Giardia intestinalis	298 years (1681–1978)	US EPA Symposium on *Giardia* (Smith and Wolfe 1980)
Cryptosporidium sp.	10 years (1976–1997)	Various, including US Congress (Matukaitis 1997; Ortega et al. 1993)
Helicobacter pylori	91 years (1892–1984)	Marshall & Warren University of Western Australia (Marshall and Warren 1984)
Escherichia coli	36 years (1947–1983)	Riley US Center for Disease Control (Wells et al. 1983)
Norovirus	2 years (1971–1972)	NIH lab (Wyatt et al. 1974)
Blastocystis	100 years (1911–present)	Various

Errors of judgment are common in major infectious diseases, and disputes generate as many angry comments today asthey did in the 1850s, when experts opposed research identifying *Vibrio choleae* as the causative agent in cholera (Boorom 2009). The field of microbiology has not developed institutionally recognized methodologies for ascertaining pathogenicity. As a result, investigations into enteric pathogens can often span decades. In many cases, the issue of pathogenicity is not decided within the medical or scientific community, but rather by government legislation, as was the case for *G. intestinalis* and *Cryptosporidium* spp.

Findings Are False." An examination of this literature is of value, because it can tell us how we would need to approach decision making differently in medical science in order to avoid situations like those in Tables 7.7 and 7.8.

Harvard Law Professor Max Bazerman proposed the concept of "bounded ethicality" to explain this type of behavior in his book, "Judgment in Managerial Decision Making" (Bazerman and Moore 2009). As noted, in most egregious failures of management decision making, there is little evidence that the people making decisions were consciously harming others to promote their own interests. The committees of physicians that dismissed *Vibrio cholerae* as a cause of cholera in the 1840s had little to gain from the decision. Physicians who intimidated *H. pylori* researchers saw no gain from their behavior (Marshall 2002). Of the researchers listed in Table 7.7, Dr. Wakefield eventually lost his license to practice medicine in the UK, and a warrant was issued for the arrest of Dr. Mikovits, following allegations of the theft of research notebooks. As such, it is difficult to fit such researchers into a paradigm of individuals following well-designed plans to hoodwink the public.

Rather, the decision-making process told leaders that their actions were correct. Experiments on human subjects who are also experts in their field suggests that certain biases are so strong, that experts will consistently make the wrong decisions in certain situations, and that behavior can be repeatably demonstrated in human experiments. (Bazerman and Moore 2009). These biases are strongest in the following situations (Montier 2010):

1. The problem is ill structured and complex.
2. The information is incomplete, ambiguous, and changing.
3. Goals are ill-defined, shifting, or changing.
4. When stress is high due to time constraints or a high stakes.
5. When decisions rely on interaction with others.

In reviewing these factors, it is not difficult to see why physicians make mistakes about pathogenicity, as these factors exist in abundance in medical research. When we understand the idea of "bounded ethicality," the mistakes in Tables 7.7 and 7.8 can be seen in a different light. That is, researchers like Wakefield and Mikovits probably did not intentionally skew the findings of a study in order to produce a specific conclusion. In fact, the factors that lead them to publish and continually defend those findings may have operated at the sub-conscious level.

7.9 A Metric for Assessing Confidence in Research Findings

How can we develop systems that provide us with reliable and timely answers about microbial infections? One approach is to implement a structured decision-making process, as a substitute for unstructured decision making by experts. A researcher who calculates confidence intervals for relative risk from a contingency table uses a structured process, while a physician who draws a conclusion from a "gut feeling" is using an unstructured process. When a structured process replaces expert guidance, there is sometimes objection that a formula cannot replace the experience and wisdom of an expert. However, in many cases, an evidence-based structured process will out-perform unstructured decision making by experts, because such unstructured decision making is prone to bias (Montier 2010).

In the next chapters, we will discuss specific findings about *Blastocystis*, and we will use a structured decision making system to assess the level of confidence we should have in those findings. The following criteria are used to classify findings:

1. *High confidence findings* are those corroborated by at least three different research groups operating in three different countries, and where no contradictory studies exist, or where contradictory studies number <10 % of studies with the positive finding.
2. *Medium confidence findings* are those reported in at least one study, the conclusions of which are not contradicted by another study. Additionally, the findings must either identify a phenomenon which is known to be true for all similar infectious diseases or which is supported by the presence of circumstantial evidence from several other studies.
3. *Findings which do not meet any of these criteria* occasionally work their way into the medical community, and these are discussed as well.

The following provides two examples of the application of these criteria:

1. "***Is Blastocystis transmitted by contaminated water?"*** In 2000, researchers from the USA and Jordan had independently suggested an association between contaminated or untreated water and *Blastocystis* infection (Nimri and Batchoun 1994; O'Gorman et al. 1993). With just two studies, this could not be a High Confidence Finding. Since all highly prevalent pathogenic protozoa (*Cryptosporidium* spp., *G. intestinalis*, *E. histolytica*) are known to be transmissible via contaminated water, this would be classified as a "Medium Confidence Finding." An additional study appeared in 2004 from Thailand implicating waterborne transmission of *Blastocystis* infection (Leelayoova et al. 2004). Between 2007 and 2012 close to a dozen studies appeared in the literature from multiple countries, including ones which genotyped *Blastocystis* in humans and water samples (Leelayoova et al. 2008). As such, at the current time, transmission of *Blastocystis* by contaminated water would be considered to be a "High Confidence" finding.
2. ***"Can symptomatic and asymptomatic Blastocystis infection in humans be explained primarily by variation in pathogenicity of different Blastocystis subtypes?":*** As a second example, in 2006, some findings could be extended to suggest that symptomatic and asymptomatic infection may be dependent on different subtypes of *Blastocystis* (Tan and Suresh 2006a,b). However, the evidence for this was based on findings from one laboratory, which does not mean it was incorrect, but it may have been related to some other variable or process. Additionally, while parasite genotype has been important in differentiating *E. histolytica*/*dispar* infection, it has not been as significant in explaining symptomatic and asymptomatic in *G. intestinalis* or *Cryptosporidium* spp., so it does not meet the universality criteria described previously. Additionally, there was not a great deal of circumstantial evidence, for example, symptomatic and asymptomatic *Blastocystis* infection did not cluster in certain areas or families. Subsequent studies from multiple sites showed that the same subtypes of *Blastocystis* are present in symptomatic and asymptomatic patients (Dogruman-Al et al. 2009; El-Shazly et al. 2005), suggesting other factors may be more important in determining asymptomatic or symptomatic infection. As such, the proposal that symptomatic and asymptomatic infection is determined by *Blastocystis* subtype does not meet the criteria for either a high-confidence or a medium-confidence finding at this time.

The *Blastocystis* Research Foundation (BRF) began categorizing studies on *Blastocystis* infection in 2006 for our metastudy paper. The initial effort was limited to categorization of studies by researcher finding (pathogenic or nonpathogenic). We continued distributing the list of those two categories of studies, but we found that researchers were also interested in lists of other studies into specific areas of *Blastocystis*. In, we extended the original two-category list to include additional categories, with the complete list as follows (as of March 17, 2012):

Studies Identifying *Blastocystis* as Pathogenic or Correlated with Symptoms (157 studies)
Studies Identifying *Blastocystis* as Nonpathogenic or Uncorrelated with Symptoms (17 studies)
Studies Identifying *Giardia* and *E. histolytica* as Nonpathogenic, Uncorrelated with Symptoms, or Not Requiring Treatment (37 studies)

Studies of *Blastocystis* and Irritable Bowel Syndrome (IBS) (22 studies)
Studies Linking *Blastocystis* to Inflammatory Bowel Disease (12 studies)
Studies Describing the Physiological Effect of *Blastocystis* in Humans and Pathogenesis of Blastocystosis (16 studies)
Studies Describing How to Detect *Blastocystis* in Humans (31 studies)
Animal Models for *Blastocystis* Infection (19 studies)
Antibody Response to *Blastocystis* (19 studies)
Culturing *Blastocystis* (63 studies)
PCR Detection of *Blastocystis* (50 studies)
Blastocystis Treatment Studies and Reviews (46 studies)
Studies Identifying the Proportion of *Blastocystis* Monoinfections that are Symptomatic (3 studies)
Selected Studies on the Role of IL-8 and Nitric Oxide in *Blastocystis* infection, IBS, and *Helicobacter pylori* infection (23 studies)

7.10 High Confidence Findings

In population studies of groups of 10 or more adults from non-Asian countries, between 68 % and 100 % of all individuals with Blastocystis mono-infections report gastrointestinal symptoms: A study published in 2002 by a US researcher surveyed 2,896 stool samples collected in 2000 and found that 69 % (400/581) of the individuals the *Blastocystis* mono-infections reported symptoms (Amin 2002). A 2010 study noted that 63 % (12/19) of zookeepers studies were found to have *Blastocystis* infection and only *Blastocystis* infection (Parkar et al. 2010). All of those cases (100 %, 12/12) were symptomatic. And a study of 108 food handlers in Egypt who were mono-infected with *Blastocystis* found that 68.5 % were symptomatic (Fathy 2011).

Blastocystis can be transmitted by contaminated water: Researchers have identified an increased risk of *Blastocystis* infection in groups consuming untreated water in studies from the USA (O'Gorman et al. 1993), Thailand (Leelayoova et al. 2004), and China (Li et al. 2007b). Genotyping studies have shown the type of *Blastocystis* present in water matches the type in individuals infected with *Blastocystis* (Leelayoova et al. 2004; Li et al. 2007b). Viable *Blastocystis* has been demonstrated in sewage samples (Suresh et al. 2005) and in recreational water samples (Ithoi et al. 2011).

Blastocystis infection is found at an elevated rate in IBS patients from all non-Asian countries: The first study that suggested *Blastocystis* was causing IBS was published in 1997 by a University research group in Karachi, Pakistan (Hussain et al. 1997).[3] Six studies from five countries are summarized in Table 7.9. The relationship between *Blastocystis* and IBS does not appear in Thai patients (Surangsrirat et al. 2010; Tungtrongchitr et al. 2004).

[3] This finding was in contrast to Markell's 1986 paper, which indicated that *Blastocystis* patients could be diagnosed with IBS (Markell and Udkow 1986).

Table 7.9 *Blastocystis* is significantly correlated with irritable bowel syndrome (IBS) in all studies from non-Asian countries

No.	Year	Country	*Blastocystis* infection rate in IBS patients	*Blastocystis* in asymptomatic controls or general population
1	2011 (Jimenez-Gonzalez et al. 2011)	Mexico	31.1 % (14/45)	13.3 (6/45)
2	2010 (Yakoob et al. 2010a)	Pakistan	53 % (90/171)	16 % (25/159)
3	2010 (Dogruman-Al et al. 2010)	Turkey	76 % (13/21)	11.6 (5/43)
4	2005 (Windsor 2007)	UK	38 % ($n > 800$)	7 %
5	2004 (Yakoob et al. 2004b)	Pakistan	46 % (44/95)	7 % (4/55)
6	1999 (Giacometti et al. 1999)	Italy	18.5 % (15/81)	7.5 % (23/307)

This relationship does not appear to be present in Thailand, based on data from two studies (Surangsrirat et al. 2010; Tungtrongchitr et al. 2004). Variations in prevalence of infection in IBS patients between studies may be largely due to different detection methods used, which included PCR detection, stool culture, and less-sensitive staining techniques

Experimental infection of laboratory animals with Blastocystis will produce gastrointestinal or physiological symptoms, but only if large numbers of cysts are used: One of the first reports of symptoms in a laboratory animal following experimental infection was published by a research group from Singapore, describing experimental infection of mice in 1997 (Moe et al. 1997). This was followed by additional studies in mice from China in 2005 and 2006 which reported severe symptoms and death (Yao et al. 2005; Zhang et al. 2006). A study followed on experimental infection of rats with 40 million cysts in Egypt in 2008 which reported severe inflammation and precancerous polyps (Hussein et al. 2008). A 2010 study on murine infection noted no changes mice inoculated with 100 or 100,000 cysts, but did note significant changes in mice inoculated with 47 million cysts (Elwakil and Hewedi 2010) Studies describing high levels of oxidative stress in urine samples from experimentally infected rats were reported by a Malaysian group in 2010 (Chandramathi et al. 2010b), and the group noted that urine samples from humans exhibited a similar phenomenon in humans (Chandramathi et al. 2009). A 2009 study from a Japanese group noted changes in cytokine response in experimentally infected rats, but no histological changes in biopsies of rats. The animals were inoculated with 100,000 cysts (Iguchi et al. 2009).

The prevalence of Blastocystis infection varies substantially between different geographies and different groups within a single country: The largest study of *Blastocystis* infection in different groups within the same country was performed in China and published in 2010 (Li et al. 2007a) and noted a prevalence varying from a low of 1.9 % in the Shanghai municipality to a high of 32.6 % in Yunnan province.

This is the only large population study ($n = 2321$) performed using a detection technique (stool culture) considered by most researchers to be reliable. Studies from additional countries showing substantially different prevalences of infection are shown in Table 7.9. A study on *Blastocystis* in zookeepers from Australia and Europe reported a prevalence of 63 % (12/19) in the zookeepers (Parkar et al. 2010), which was higher than the prevalence in the zoo animals (!) All individuals infected with *Blastocystis* in that study reported gastrointestinal symptoms.

It should be noted that researchers sometimes misrepresent the frequency of detection of *Blastocystis* in clinical samples as the prevalence in the population. However, this practice is incorrect, as clinical samples usually come from patients with an illness, and such samples will often contain *Blastocystis* much more often than samples from the general population. Additionally, as clinical detection methods have a sensitivity of <40 % (Dogruman-Al et al. 2010; Leelayoova et al. 2002; Stensvold et al. 2007), it is not reasonable to represent figures from those methods as a prevalence.

The diagnostic techniques commonly used in clinical settings fail to detect most Blastocystis infections: One of the first studies to note this relationship was published by a Thai research group in 2002, noting that the sensitivity of smear and concentration techniques was about 32.3 % compared to in vitro culture (Leelayoova et al. 2002). This finding was repeated in 2004 with a study on UK patients (Suresh and Smith 2004). Additional studies performed in Denmark noted that staining and concentration techniques in common use in Europe would detect about a third of the infections identifiable by polymerase chain reaction (PCR) or stool culture (Stensvold et al. 2006, 2007). A pair of US studies noted the identification of *Blastocystis* infection in a majority of patients with chronic gastrointestinal illness, even though most patients had been found to parasite-negative by clinical laboratories (Jones et al. 2009; Whipps et al. 2010). A study on Turkish patients published in 2010 noted that trichrome staining would detect only about a third of the infections detectable by PCR and stool culture (Dogruman-Al et al. 2010). A 2010 comparison study in which the same stool samples were analyzed by multiple European reference laboratories found that agreement between laboratories for *Blastocystis* detection was the worst for any intestinal parasite examined (Utzinger et al. 2010).

In Blastocystis mono-infections, the following symptoms are among the most common reported by Blastocystis patients: diarrhea, abdominal pain, vomiting, flatulence, and fatigue: This symptomatic distribution was first reported in a study of 239 mono-infected patients from Saudi Arabia in 1989 (Qadri et al. 1989). A similar distribution was found in a study of *Blastocystis* mono-infection in 39 children in the USA in 1993 (O'Gorman et al. 1993), as well as a study of 38 mono-infected schoolchildren in Jordan in 1993 (Nimri 1993). Similar symptoms were noted in a study of 23 mono-infections from Egypt in 2005 (El-Shazly et al. 2005), and in 108 mono-infections reported from Libya in 2007 (Al-Fellani et al. 2007), and a detailed molecular study of *Blastocystis* subtypes, coinfections, and symptoms in 92 patients from Denmark in 2009 (Stensvold et al. 2009).

Blastocystis subtype 3 is the most common type of Blastocystis infection in most Western countries: The first subtyping of isolates from a Western country was performed in Denmark in 2006 and identified subtype 3 as the most prevalent variant (Stensvold et al. 2006). This was followed by similar findings from a Greek study in 2008 (Menounos et al. 2008), a US study in 2009 (Jones et al. 2009), a French study in 2009 (Souppart et al. 2009), a Mexican study in 2011 (Jimenez-Gonzalez et al. 2011), and a Swedish study in 2012 (Forsell et al. 2012). Subtype 3 is also the most common *Blastocystis* type found in Egypt (Hussein et al. 2008; Souppart et al. 2010) and China (Li et al. 2007a). Different subtype distributions have been found in a 2012 Australian study (Nagel et al. 2012), where Subtype 1 was the most common (45 %) and subtype 3 was as common as subtype 4 (36 %). In Nepal, 86.4 % of *Blastocystis* infections were found to be made up of subtype 4. Additionally, in Denmark, subtype 4 was found to be the most common subtype among patients with acute (rather than chronic) diarrhea (Stensvold et al. 2011).

In Western countries, age is a risk factor for the development of long-term illness with Blastocystis infection. That population consists primarily of individuals over 30: The average age of individuals with long-term illness associated with *Blastocystis* mono-infection is usually significantly greater than the average age of the population studied. This relationship holds true in studies conducted in Turkey (Dogruman-Al et al. 2010), Mexico (Jimenez-Gonzalez et al. 2011) and Pakistan (Yakoob et al. 2010b), with a typical average age of 41 years reported in such studies.

Blastocystis upregulates production of the inflammatory cytokine IL-8: This was first reported in 2001 in a German study of *Blastocystis* cells co-cultured with human colonic cells (Long et al. 2001), and then again in two studies in 2008 from Singapore labs which noted differences between subtypes in IL-8 upregulation behavior (Mirza and Tan 2009; Puthia et al. 2008). Similar findings were reported in 2010 from a co-culture study performed in Malaysia (Chandramathi et al. 2010a). An interesting finding noted in the next section is that individuals with a polymorphism which upregulates production of the cytokine IL-8 are much more likely to show symptoms when infected with *Blastocystis* (Olivio-Diaz, 2012).

7.11 Medium Confidence Findings

In adult Blastocystis patients, the duration of infection approaches or exceeds 1 year and may last indefinitely in some individuals: A Japanese study was the first to report multiyear *Blastocystis* infection, which was accompanied by a steadily increasing immune response to the infection (Kaneda et al. 2000). Studies from the USA and other countries have related *Blastocystis* infection to long-term illness in patients, which has been found to be of a duration of 5 years or more (Jones et al. 2009). A Thai study followed adult caregivers at an orphanage and found that children cleared *Blastocystis* in an average of 2.6 months (median age 10 months),

while adults cleared *Blastocystis* in an average of 10 months (median age 37 years) (Pipatsatitpong et al. 2012).

Expression of symptoms in Blastocystis infection may depend significantly on the host's genetic characteristics: The first study to note a specific host genetic effect in *Blastocystis* infection was performed on 45 patients and 45 controls in Mexico and found that two mutations that up-regulate IL-8 and IL-10 production were significantly associated with symptomatic expression in hosts, with the IL-8 characteristic potentially accounting for 45 % of the etiological fraction of illness (Olivo-Diaz et al. 2012). Relationships between host genetic traits and symptomatic expression in microbial illness can be identified for most major pathogens. The most common example may be that heterozygous carriers of the sickle cell trait exhibit resistance to extreme symptoms in malaria. Blood type A has been found to confer protection against infection with *E. histolytica* (Haque et al. 2003). Hosts with blood types A and AB may be more likely to experience symptomatic infection with enterotoxic *E. coli* (ETEC) (Qadri et al. 2007).

The antimicrobial therapies commonly used to treat Blastocystis infection may fail to eradicate the infection in most or all cases, especially in Western countries: The development of resistance to metronidazole was first reported in the mid-1990s (Zaman and Zaki 1996), and isolates from "IBS" patients were tested for antimicrobial resistance for the first time in a study from Pakistan in 2004 (Yakoob et al. 2004a). A poster presented in 2007 at the 15th United European Gastroenterology Week in Paris by Borody and Wettstein noted that most Australian *Blastocystis* patients appeared to be receiving no benefit from metronidazole treatment, and described a combination of drugs which provided better outcomes. A 2010 review paper, aptly named, "Antimicrobial therapy in *Blastocystis*: Reality or Delusion," critically examined the evidence surrounding the use of antimicrobials in treating *Blastocystis* patients (Stensvold et al. 2010). Surprisingly, there are very few studies in which patients who are treated for *Blastocystis* infection are subsequently tested for the infection to determine if the therapy can be said to eradicate the microbe. One of the first such studies was performed on 11 Australian patients in 2011, and it reported that every (11/11) patient with long-term *Blastocystis* symptoms who was treated with 400 mg of metronidazole for 11 days was still positive for *Blastocystis* infection at the end of treatment, with *Blastocystis* detected by PCR (Nagel et al. 2012).

7.12 Findings Which Do Not Meet any of These Criteria

Blastocystis only produces symptoms in immunocompromised patients: It is not clear where this concept originates. Overall, when we reviewed *Blastocystis* literature in 2008, we identified 86 studies pointing to symptomatic *Blastocystis* infection in immunocompetent patients, but only about half that number in immunocompromised patients. That trend has continued post-2008, with about 80 % of the current studies addressing immunocompetent patients, or the general population, and only a few studies focusing on HIV, cancer, and organ transplant patients. A 1990 study of

130 Canadian *Blastocystis* patients noted that only a few were immunocompromised (Doyle et al. 1990).

The origin of this idea may be related to the fact that some other microbes only cause symptoms in HIV and patients undergoing chemotherapy. The physician thus extrapolates that finding to assume that variability in symptomatic expression for all microbial infections is due to HIV infection and cancer treatment. Alternately, it may be the case that HIV and cancer patients visit physicians more often, and have more tests performed, and the discovery of *Blastocystis* in those patients establishes a mental correlation between *Blastocystis* infection and host immune status.

Blastocystis infection is easily treatable in all patients with metronidazole: In the few studies that have followed up with patients treated with metronidazole using sensitive detection techniques, that drug has been shown to have a high failure rate, particularly in patients in developed countries (Nagel et al. 2012; Stein 2007).

7.13 Is Giardia Good For You? Understanding Population Studies with Analytical Methods

A 2006 paper on *Blastocystis* from the London School of Hygiene and Tropical Medicine (Scicluna et al. 2006) noted that:

> Most population-based studies find no difference between rates of infection in symptomatic and asymptomatic individuals. In contrast, in individual infections a strong case can be made for the organism being the cause of disease.

Why is this? And is the phenomenon unique to *Blastocystis*? A short time spent reviewing papers on established pathogenic gastrointestinal protozoa will show that many researchers have performed population studies and found that these organisms can, in some populations, show no correlation or little correlation with illness (Janoff et al. 1990; Markell et al. 1983, 1984). Patients with *G. intestinalis* infection have even been reported to be healthier than those without such infection (Veenemans et al. 2011). Does this mean that protozoal infections are good for you?

Surprisingly, there are few papers in which a researcher has begun with known properties of existing pathogens, and determined if it should be possible to consistently demonstrate pathogenicity in all epidemiological situations. That is, can the known properties of established gastrointestinal protozoa account for researcher findings that show no correlation between *G. intestinalis* infection and symptoms?

Consider the Relative Risk and statistical significance (Fisher's Exact Test or Chi square) of a 2×2 contingency table representing a population of 1,000, all of whom are infected with *Giardia*, but only 50 % of whom show symptoms. We can assume that the asymptomatic individuals have acquired or innate immunity, for example.

	Giardia+	*Giardia*−	Total
Diarrhea	500	0	500
No diarrhea	500	0	500
Total	1,000	0	1,000

We can now show that in this population, the relative risk of having diarrhea is the same, whether the patient is infected with *G. intestinalis* or not. So since the RR = 1, the contingency table shows no relationship between *G. intestinalis* and diarrhea.

However, lack of correlation does not prove lack of causation, and in this case, we can see that if symptomatic expression is dependent on a host genetic or immunological factor, correlation studies may not demonstrate pathogenicity for a particular microbe. As a further examination, consider the effect of adding 250 individuals, all of whom are infected with *Cryptosporidium* sp., and all of whom have diarrhea.

	Giardia+	*Giardia*−	Total
Diarrhea	500	250	750
No diarrhea	500	0	500
Total	1,000	250	1,250

We now see that 67 % (500/750) of the patients with diarrhea test positive for *G. intestinalis*, but 100 % of the patients who are healthy have *G. intestinalis*. Thus, people with *G. intestinalis* are healthier than those without *G. intestinalis*! The example can be repeated with larger numbers of patients to create a "landmark" study. Such studies which might involve thousands of patients are just as susceptible to confounding variables as smaller studies.

The results do not tell us anything about whether we should treat patients, or whether *G. intestinalis* is causing illness. If we refuse to treat *G. intestinalis* patients based on this study, we leave up to half of the population with a treatable type of diarrhea. It is worth noting that once the correlation calculation is performed, many researchers will stop analyzing the results, and conclude that *G. intestinalis* (or *Blastocystis*) is either harmless or even healthful:

> Healthy day care children with asymptomatic *Giardia* infection show no disadvantage and perhaps even an advantage in nutritional status and freedom from other illnesses. (Ish-Horowicz et al. 1989)
>
> An additional study examining Tanzanian children noted that children who tested positive for *Giardia intestinalis* were less likely to have diarrhea, but that relationship was lost when the children were treated with a nutritional supplement. (Veenemans et al. 2011)

To focus on the question of pathogenicity, it is necessary to remove confounding variables:

1. The inclusion patients with other infectious diseases that produce the same symptoms as the microbe under study will weaken statistical correlation, or even reverse it.

2. If adaptive or innate immunity can exist for the microbe under study, including patients who are immune to the infection will weaken the correlation. Limiting the study group to patients who are older or have specific genetic traits can improve correlation figures for *Blastocystis* (Olivo-Diaz et al. 2012).

7.14 Acquired Immunity and Peak Shifting

Researchers have noted seemingly enigmatic behavior in both *E. histolytica* and *G. intestinalis*, as they discover groups with a high infection rate, and few symptoms. This effect has largely been ignored in public health efforts, which generally focus on reducing exposure, rather than building immunity through vaccination. It is necessary to understand this effect in order to properly assess the significance of potential new enteric microbes and also to understand potential effects of reducing exposure to these microbes. One remarkable study followed 82 schoolchildren for months and showed they exhibited no symptoms from *G. intestinalis* infection:

> We prospectively evaluated excretion of *Giardia lamblia* in children in day care centers in Houston by conducting two prevalence studies of 600 children enrolled in 30 day care centers, and an 18-month longitudinal study in 82 children in one center. In the two prevalence surveys, *Giardia* cysts were identified in 72 (21 %) and 67 (26 %) children, respectively, who provided stool specimens. Trophozoites were found in 15 (4 %) and 8 (3 %), respectively. There was no correlation between the frequency of recent diarrheal episodes and the finding of *Giardia*. In the longitudinal study, cysts were detected in stool specimens from 27 (33 %) of the 82 children at least once during the survey. Twelve children had *Giardia* cysts in weekly stool specimens for a mean of 6.2 $\pm$ 1.2 months and trophozoites for 3.3 $\pm$ 1.2 months. The number of enteric symptoms observed in children and the classification of nutritional status based on monthly height and weekly weight measurements did not differ significantly when infected and noninfected children were compared.
>
> –Occurrence of *Giardia lamblia* in children in day care centers, 1984 (Pickering et al. 1984)

> *Giardia lamblia* infection was identified in 33 of 89 (37 %) 3-month-old to 3-year-old children who were followed with monthly stool examinations for up to 12 months in a day care center. The infection was mainly asymptomatic and usually associated with prolonged carriage of the parasite. There were no significant differences for height and weight achievements and mean hemoglobin values between *Giardia*-positive and *Giardia*-negative children. However, *Giardia*-positive children tended to achieve higher weight and height for age than *Giardia*-negative children; weight for age was above the 50th percentile in 69 % of *Giardia*-positive vs. 40 % of *Giardia*-negative children (alpha = 0.01). *Giardia*-positive children tended to have fewer symptoms related to the gastrointestinal and respiratory tracts as recorded by a weekly questionnaire. Lactase deficiency was detected by breath hydrogen testing in 8 of 26 *Giardia*-positive vs. only 1 of 21 *Giardia*-negative children (*P* less than 0.02). Healthy day care children with asymptomatic *Giardia* infection show no disadvantage and perhaps even an advantage in nutritional status and freedom from other illnesses.
>
> –Asymptomatic giardiasis in children, 1989 (Ish-Horowicz et al. 1989)

> We conducted a point prevalence survey for enteric protozoa in 205 institutionalized orphans 1-61 months of age in Bangkok, Thailand. *Cryptosporidium* was identified in 17 children (8%), *Giardia lamblia* in 42 (20%), and 3 children (1%) had both parasites. At the

> time of diagnosis, diarrheal symptoms were present in a minority of subjects: 36% of children with *Cryptosporidium* alone, 10% with G. lamblia alone, and in 20% of those with neither parasite. . .Although neither infection with *Cryptosporidium* nor G. lamblia was consistently associated with acute diarrheal symptoms, *Cryptosporidium* was more often associated with depressed acute nutritional status than *G. lamblia*.
>
> –Endemic *Cryptosporidium* and *Giardia lamblia* infections in a Thai orphanage (Janoff et al. 1990)

The most interesting example of asymptomatic *E. histolytica* infection may come from a 1925 paper from Harvard Medical School professor Dr. SF Chiang, who infected rats with *E. histolytica* from his own stool samples:

> Material for the second series of experimental infections was furnished by Dr. S., he himself being a healthy carrier of *E. histolytica*, *E. coli* and *Endolimax nana*.
>
> –The Rat as a possible carrier for *E. histolytica* (Chiang 1925)

The rats did, in fact, develop illness. Can these studies help us understand some of the mystery behind why some people show symptoms in *Blastocystis* and others do not? Why does the long-term morbidity keep showing up in Europe but not in Africa or Asia (so far)?

One commonality of all these studies is that they focus on individuals in hyper-endemic environments, where exposure is occurring. We can see a similar phenomenon in a modern study of *E. histolytica* in Bangladeshi children:

> The contribution of amebiasis to the burden of diarrheal disease in children and the degree to which immunity is acquired from natural infection were assessed in a 4-year prospective observational study of 289 preschool children in an urban slum in Dhaka, Bangladesh. *Entamoeba histolytica* infection was detected at least once in 80%, and repeat infection in 53%, of the children who completed 4 years of observation. Annually there were 0.09 episodes/child of *E. histolytica*-associated diarrhea and 0.03 episodes/child of *E. histolytica*-associated dysentery.
>
> –*Entamoeba histolytica* infection in children and protection from subsequent amebiasis (Haque et al. 2006)

In some pathogens, groups with the greatest exposure also experience the greatest morbidity. This is clearly true for exposure to *Mycobacterium tuberculosis* and the HIV virus. But a pattern can be seen for gastrointestinal protozoa in which individuals who are exposed frequently or continuously do now show severe manifestations of the illness. This is one reason researchers have focused on the development of a vaccination for *E. histolytica* (Haque 2006).

Studies showing that enteric protozoa as uncorrelated with symptoms are often conducted on populations from day cares, orphanages, prisons, or on gay men, where exposure is common (Table 7.10). On the other hand, studies of individuals with infrequent exposure show illness, which is often severe. Cases like this include vacationing travelers from developed countries, or community exposures in areas that usually have uncontaminated water supplies (de Lalla et al. 1992).

Individuals in a Thai community are found to maintain high levels of immunity to *G. intestinalis* throughout their lifetime, while individuals in the USA were found to exhibit declining levels of immunity after age 19 (Fig. 7.6). One interpretation of

Table 7.10 Many studies suggest that patients with *G. intestinalis* or *E. histolytica* are just as healthy as those without such infections

Year	Study group	Region	Organism	Finding
1954	Prisoners	Southern USA	*G. intestinalis*	Experimental infection of prisoners with *G. intestinalis* produces no clinical manifestations of giardiasis (Rendtorff 1954)
1954	Prisoners	Southern USA	*E. histolytica*	Experimental infection of prisoners with *E. histolytica* produces no clinical symptoms of amoebiasis (Rendtorff and Holt 1954)
1986	Gay Men	UK	*E. histolytica*	*E. histolytica* appears to be a "commensal" in gay men (Allason-Jones et al. 1986)
1984	Children in daycare	Houston	*G. intestinalis*	No correlation between symptoms and infection (Pickering et al. 1984)
1989	Children in day care	Israel	*G. intestinalis*	Children with *G. intestinalis* appear healthier (Ish-Horowicz et al. 1989)
1990	Children in Thai orphanage	Thailand	*G. intestinalis* *Cryptosporidium* spp.	No correlation between diarrhea and *G. intestinalis* (Janoff et al. 1990)
2011	Children	Tanzania	*G. intestinalis*	*G. intestinalis* provides protection against diarrhea unless a food supplement used (Veenemans et al. 2011)

These studies are often performed on population where the infection is endemic, suggesting the possibility that acquired immunity may play a significant role in asymptomatic infection

the data would suggest that public health efforts do not prevent all exposures to gastrointestinal protozoa, but rather have the effect of delaying the first exposure and creating more time between exposures. This produces an epidemiological characteristic known as peak shifting, where the peak in prevalence vs. age occurs at a later time. For some microbial infections which are more severe when first exposure occurs later in life, the improved efforts at sanitation may have the paradoxical effect of increasing morbidity in the population by reducing the frequency of exposure, but increasing the severity of illness following an exposure.

7.15 How Much is *Blastocystis* Costing Communities?

A number of papers have provided cost estimates for outbreaks of acute illness due to food-borne bacterial and *Cryptosporidium* spp. (Corso et al. 2003, Batz et al. 2012). Comparable studies for *Blastocystis* would be complicated by the clinical use of low-sensitivity diagnostic tests, and a lack of reporting mechanisms for infections.

A more feasible approach would be to use existing cost estimates for IBS and adjust those by the percentage of cases which are due to *Blastocystis* infection. The latter factor might be calculated by identifying the percentage of IBS patients who

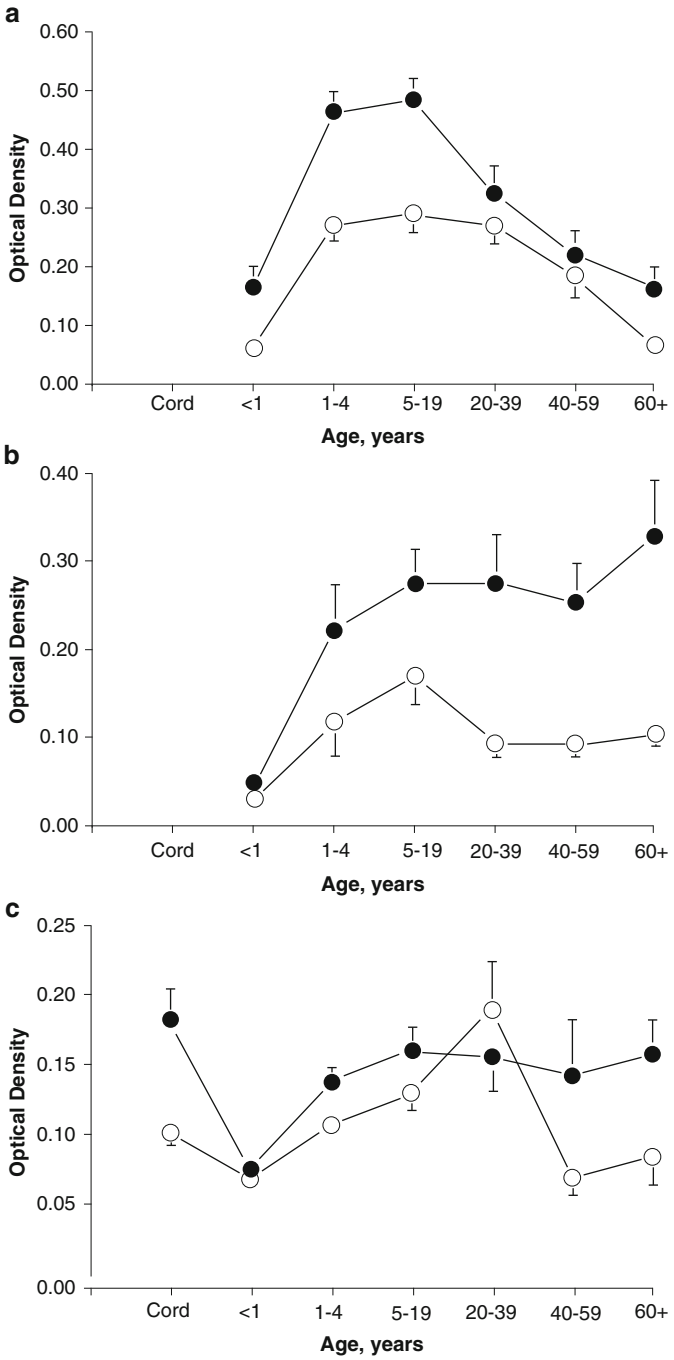

Fig. 7.6 Average serum antibody to *G. intestinalis* antigens response for 210 residents of Denver, Colorado versus 207 residents of Soongern, Thailand. In the USA, antibody response declines substantially after age 19, which is not seen in Thailand. If illness is more severe or long lasting in immunologically naïve adults over 30, a counterintuitive trend may develop, where the population with a lower prevalence of an infection sees more incidences of severe illness

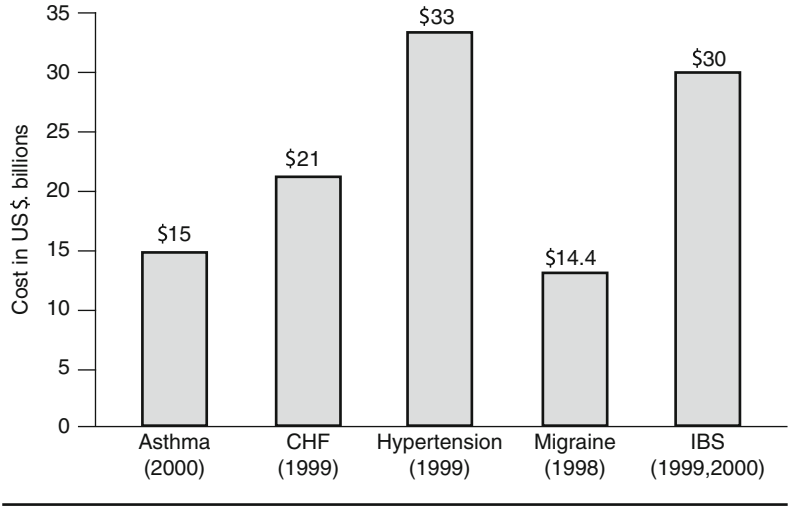

Fig. 7.7 Comparison of the total costs for various long-term illnesses in the USA (Cash et al. 2005). Existing studies suggest that 24.4–46 % of IBS morbidity may be attributable to *Blastocystis*, which would place the cost of *Blastocystis* infection at between $7.3 and $13.8 billion in the USA

are infected with *Blastocystis* by the percentage of those infections which are expected to be symptomatic:

$$\text{Cost of Blastocystis} = P_{\text{IBS}} \times P_{\text{symp}} \times \text{cost of IBS}$$

Where P_{IBS} is the percentage of IBS patients who are infected with *Blastocystis* and P_{symp} is the percentage of *Blastocystis* infections that are symptomatic.

This formula accounts for the argument that some patients who are infected with *Blastocystis* do not show symptoms, so a portion of the *Blastocystis*-positive IBS patients could represent asymptomatic *Blastocystis* carriers who have another cause for the illness.

When studies using stool culture or PCR detection are considered, P_{IBS} has been reported as 31, 53, 76, 38, and 46 % in existing studies (Table 7.9), giving 48.8 % as an average.

Numbers for P_{symp} for non-Asian countries have been reported to be in the range of 69–100 % (see High confidence findings). One of those studies used a small sample, and two of those studies could have arguably been influenced by patient self-selection (Amin 2002; Yoshikawa et al. 2004), so an argument could be made that P_{symp} could be lower. However, in studies of other enteric protozoa in healthcare-seeking individuals, resolution of symptoms occurs in over 95 % of healthcare-seeking patients following eradication of the infection, suggesting that P_{symp} for healthcare-seeking patients may be higher than P_{symp} for the general population.

For example, P_{symp} for *E. histolytica* is estimated to be only 10 %, but in treatment study of patients with symptoms and *E. histolytica* infection, 80 to 90 % of patients report resolution of symptoms (Latonio, 1988).

Using a value of P_{symp} ranging from 50 to 95 %, combining the two coefficient gives:

$$\text{Cost of } \textit{Blastocystis} \text{ infection} = (0.244 \textit{ to } 0.46) \textit{ cost of IBS}$$

Figure 7.7 illustrates how this cost-multiplier can be applied to existing cost estimates of IBS to estimate the cost of *Blastocystis* infection alone.

It may be worth noting that most existing studies have assumed the rate of IBS is relatively static, and that the illness is due largely to psychosomatic causes. If a substantial portion of IBS cases are caused by an infectious disease, this would suggest that the illness is contagious, and if cases are untreated, the prevalence of the illness will increase over time.

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