Parasites and the Food Supply

This Scientific Status Summary, prepared for the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, discusses the sources and incidence of human infection by foodborne parasites and the new technologies that are being developed for their prevention, detection, and inactivation.

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Patterns of travel, trade in foods and food consumption have changed, exposing consumers to pathogens—including parasitic animals— not previously encountered. The globalization of America's food supply increased substantially during the 1990s (Fig. 1). Correspondingly, so did the risk to American consumers of acquiring a foodborne parasite. In 1990, about 13 species of parasitic animals were of concern to food scientists in the United States (Jackson, 1990). Today, that figure has multiplied by more than a factor of 8.

In the past, the risk of human infection with parasites was considered to be limited to distinct geographic regions because of parasites' adaptations to specific definitive hosts, select intermediate hosts and particular environmental conditions. These barriers are slowly being breeched—first by international travel developing into a major industry, and then by rapid, refrigerated food transport which became available to an unprecedented degree at the end of the 20th century.

Figs. 2A and B trace the numbers of international travelers to and from the U.S. over the past decade and provide further projections for international travel through 2003. International travel and population migration are the primary mechanisms by which immunologically naive (previously unexposed) consumers may come into contact with emerging parasites.

Another factor to consider is the rapid transport of foreign food products to the U.S., which has further enhanced the chances that parasites come into contact with consumers. Now, produce picked and seafood harvested or caught early in the week, thousands of miles from our borders, can be consumed fresh in America's heartlands before the weekend. Moreover, cultural habits have shifted toward the consumption of fresh, i.e., raw and undercooked, foods that bypass important preparatory measures in-

tended to reduce or prevent infections by pathogens—especially the long-surviving encysted forms of foodborne parasites.

Food parasitology is an emerging discipline. Although its beginnings coincided with the beginnings of microscopy more than 300 years ago (Dobell, 1920), few of its testing methods are as standardized as those of food bacteriology. Reasons for the underdeveloped state of this science include our inability to readily culture most parasites (Smyth, 1990; Taylor and Baker, 1978) and, for forms encysted on or within plant or animal tissues, our inability to design seeding experiments that are equivalent to working with natural samples.

Frequently, parasitology in its entirety is relegated to the status of a sub-specialty in between microbiology and zoology. Moreover, pathogenic parasites of humans are sometimes considered only in the context of tropical medicine, despite mounting evidence of their prevalence in temperate and arctic climates.

Domestically, for a multitude of reasons, parasitic infections are often not routinely considered as a source of illness when symptoms similar to bacterial infection present themselves. Therefore, many instances of parasite-related illness go undiagnosed, which may lead to a skewed reporting of the incidences of parasitic illness. As a consequence, those parasites that are now considered emerging parasitic pathogens may in fact have been a continual source of human illness in the absence of any clinical recognition.

In the context of foods and parasitic animals, then, there is a need for increased awareness of the impact of parasites on the food supply. This includes recognition of parasites' potential effect on public health and health care issues as well as sensitivity to economic consequences such as worker productivity and agricultural losses. In comparison to other classes of foodborne pathogens, particularly bacteria, the impact of parasites is difficult to assess primarily due to the lack of a uniform standard for monitoring the incidence of foodborne illness directly attributed to parasitic infection. As such, statistical data relating parasitic foodborne illness and economic sequelae are either approximations or lacking.

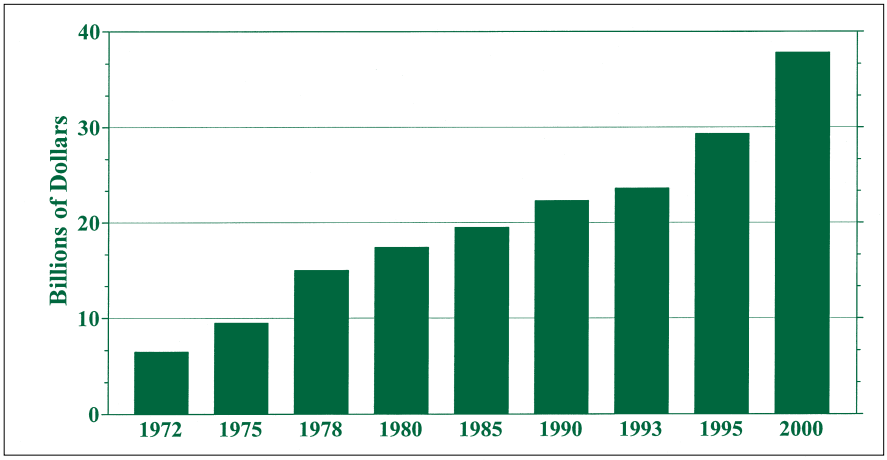

Fig. 1—**Value of agricultural products imported into the United States**. From USDC (2000a).

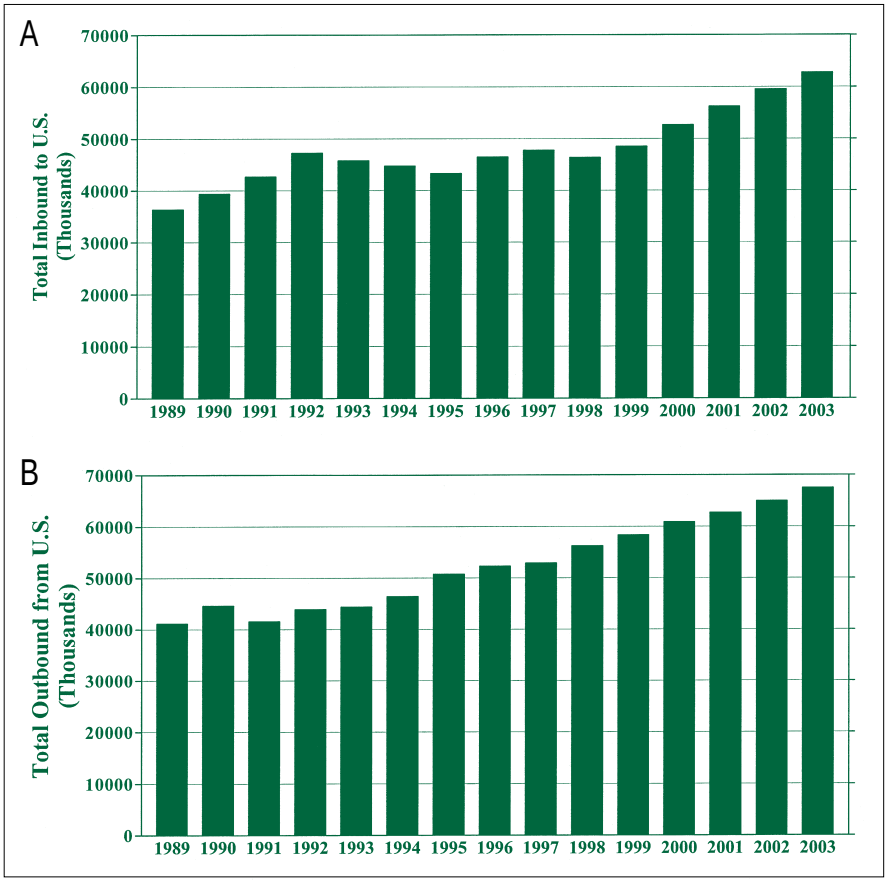

Fig. 2—**International travel (A) to the U.S. and (B) from the U.S.** From USDC (2000b).

Scope of the Problem

There are about 107 known species of parasitic animals that can be foodborne (Table 1). While not all species are reported to infest domestic food sources or infect consumers in the U.S. and its territories, the likelihood of this possibility has significantly increased in recent years with the emergence of a truly global market place. Planetary statistics on foodborne illnesses due to parasitic infections have been difficult to estimate. Norman R. Stoll's classic "This Wormy World" (Stoll, 1947) estimated that in the global population of 2.2 billion people, there were 664 million *Ascaris lumbricoides* infections (30% prevalence) and 355 million infections with *Trichuris trichiura* (16%) compared to the update by Michael et al. (1997) which estimated 1273 million (24%) and 902 million (17%) infections 50 years later when the human population was 5.6 billion. What percentage of these cases is foodborne has not been determined; however, their overall impact as demonstrated by substantial in-

Table 1—Parasites transmitted by food and water

Parasite	Stage	Pathogenicity	Source[a]	Geographic area[b]	Key feature/ food/hosts
PROTISTA					
Amoebae					
Endolimax nana	Cyst	–	W,F,H	U	
Entamoeba chattoni	Cyst	–	W,F,H	U	
Entamoeba coli	Cyst	–	W,F,H	U	
Entamoeba dispar	Cyst	–	W,F,H	U	
Entamoeba invadens	Cyst	–	W,F,H	U	
Entamoeba hartmanni	Cyst	–	W,F,H	U	
Entamoeba histolytica	Cyst	+	W,F,H	U	May invade extraintestinal sites
Entamoeba moshkovskii	Cyst	–	W,F,H	U	Morphologically similar to *E. hystolytica*
Entamoeba polecki	Cyst	±	W,F,H	U	Pigs, monkey reservoirs
Iodamoeba butschlii	Cyst	–	W,F,H	U	Pigs, monkey reservoirs
Flagellates					
Chilomastix mesnili	Cyst	–	W,F,H	U	
Dientamoeba fragilis	Trophozoite	±	H	U	No cyst known
Enteromonas hominis	Cyst	–	W,F,H	U	Rare
Giardia (lamblia) intestinalis	Cyst	+	W,F,H	U	Many alternate hosts
Pentatrichomonas hominis	Trophozoite	±	W,H	U	
Retortamonas intestinalis	Cyst	–	W?	U	Uncommon
Retortamonas sinensis	Cyst	±	W	C	
Ciliates					
Balantidium coli	Cyst	+	W,F,H	U	Pig reservoirs
Sporozoa and Coccidia					
Cryptosporidium parvum	Oocyst	+	W,F,H	U	Many hosts, cider, and bivalve molluscs
Cyclospora cayetanenesis	Oocyst	+	W,F,H?	U	Travel? Seasonal? Fresh produce
Isospora belli	Oocyst	±	W,F,H	U	More common in tropics
Sarcocystis hominis	Cyst	+	F		Beef intermediate host
Sarcocystis spp.	Oocyst	+	W,F	A	Sources unknown
Sarcocystis suihominis	Cyst	+	W,F		Pig intermediate host
Toxoplasma gondii	Oocyst	+	W,F	U	Severe illness in fetus
Encephalocytozoon cuniculi	Spore	+	U	U	Immune-compromised humans
Encephalocytozoon hellem	Spore	+	U	U	Immune-compromised humans
Encephalocytozoon intestiniale	Spore	+	U	U	Immune-compromised humans
Enterocytozoon bieneusi	Spore	+	U	U	Immune-compromised humans
METAZOA					
Trematoda					
Achillurbainia recondita	Metacercaria	+	F	C	
Alaria americana	Metacercaria	+	F	NA	Frogs
Appophalus donicus	Metacercaria	+	F		Fish
Artyfechinostomum mehrai	Metacercaria	+	F	I	Fish
Centrocestus spp.	Metacercaria	+	F	AS	Fish, eggs may enter circulation
Centrocestus formosanus	Metacercaria	+	F	AS	Fish, eggs may enter circulation

[a] Source: W = water, F = food, H = food handler

[b] Geographic area: U = Ubiquitous, A = Africa, AS = Asia, C = China, E =Egypt, I = India, , IN = Indonesia, H = Hawaii, HA = Holartic, J = Japan, NA = North America, SA = South America, SEA = South East Asia, O = Orient, P = Phillipines, R = Russia, US = United States.

cidences of foodborne trematode infections (*Opistorchis*, *Clonorchis*) in Southeast Asia and the Pacific region (WHO, 1995) underscores the need for increased awareness of this class of human pathogens. Particularly with the consumption of raw or undercooked seafood and the advent of a global market place, the need for precautions in processing methods and consumption habits is essential to prevent the spread of foodborne parasitic infections. This awareness also applies to food-related illnesses caused by gastrointestinal protozoa, although statistics comparable to those for worms are not available.

In the U.S., the latest survey of foodborne illnesses by the Centers for Disease Control and Prevention estimates that there are 2.5 million cases annually due to food- and beverage-borne parasites (Mead et al., 1999). This is approximately 7% of the annual food- and beverage-borne disease incidence caused by known infectious agents—fewer than the 13% caused by bacteria and the 80% caused by viruses. However, the parasite *Toxoplasma gondii*, a coccidian protozoa, is responsible for 20.7% of foodborne deaths due to known infectious agents. The flagellated protozoa, *Giardia* (*lamblia*) *intestinalis*, causes the greatest number of parasite-related disease cases, with an estimated 2,000,000 illnesses annually, equaling 1.4% of the food- and beverage-borne total for known pathogenic agents. *Cryptosporidium parvum* is reported to cause 30,000 cases (0.2%) and the recently recognized *Cyclospora cayetanensis* caused 14,638 cases (0.1%), due primarily to imported fresh produce. Although it is difficult to distinguish foodborne from

Table 1—Parasites transmitted by food and water, *continued*

Parasite	Stage	Pathogenicity	Source[a]	Geographic area[b]	Key feature/ food/hosts
Clonorchis sinensis	Metacercaria	+	F	CJ	Fish
Cryptocotyle lingua	Metacercaria	+	F		Fish
Dicrocoelium dendriticum	Metacercaria	+	F	U	Vegetables, ant transport host
Diorchitrema formosenum	Metacercaria	+	F		Fish, eggs may enter circulation
Diorchitrema pseudocirratum	Metacercaria	+	F		Fish, eggs may enter circulation
Diorchitrema spp.	Metacercaria	+	F		Fish, eggs may enter circulation
Echinochasmus spp.	Metacercaria	+	F		Fish
Echinoparyphium recurvatum	Metacercaria	+	F	U	Fish
Echinostoma iliocenum	Metacercaria	+	F	P	Snails, clams
Echinostoma lindoensi	Metacercaria	+	F	I	Dog reservoirs, molluscs
Echinostoma revoltum	Metacercaria	+	F	U	Snails, clams
Echinostoma spp.	Metacercaria	+	F	A	Molluscs
Episthmium caninum	Metacercaria	+	F		Fish
Euparyphium melis	Metacercaria	+	F		Fish
Eurytrema pancreaticum	Metacercaria	+	F	O	Fish, can occlude pancreas
Fasciola gigantica	Metacercaria	+	F	ME	Vegetables
Fasciola hepatica	Metacercaria	+	F	U	Sheep reservoir, vegetables
Fasciolopsis buski	Metacercaria	+	F	AS	Pig reservoir, vegetables
Fischoederius elongatus	Metacercaria	+	F		Vegetables
Gastrodiscoides hominis	Metacercaria	+	F	AS	Vegetables
Gymnophalloides seoi	Metacercaria	+	F	K	Fish?
Haplorchis spp.	Metacercaria	+	F		Fish, eggs may enter circulation
Heterophyes heterophyes	Metacercaria	+	F	U	Mullet primary vector
Heterophyes nocens	Metacercaria	+	F	AS	Fish, eggs may enter circulation
Heterophyopsis continua	Metacercaria	+	F	U	Fish, eggs may enter circulation
Himasthla muehlensi	Metacercaria	+	F	US	Clams
Hypoderaeum conoideum	Metacercaria	+	F		Fish
Isoparorchis hypselobagri	Metacercaria	+	F		Fish, man accidental host
Metagonimus minutus	Metacercaria	+	F		Fish, eggs may enter circulation
Metagonimus yokogawai	Metacercaria	+	F	AS,E	Fish
Nanophyetus salmincola	Metacercaria	+	F	NA	Fish (California to British Columbia)
Nanophyetus schikhobalowi	Metacercaria	+	F	R	Fish (Siberia)
Neodiplostomum seoulensis	Metacercaria	+	F	K	Snakes
Opisthorchis viverrini	Metacercaria	+	F	SEA	Fish
Opisthorchis felineus	Metacercaria	+	F		Fish
Paragonimus spp.	Metacercaria	+	F	U	Fish
Paryphostomum surfrartyfex	Metacercaria	+	F	I	Unknown intermediate hosts
Phagicola spp.	Metacercaria	+	F		Fish
Phaneropsolus bonnei	Metacerceria	+	F	SEA	Fish
Plagiorchis spp.	Metacercaria	+	F	U	Snail intermediate
Poikilorchis congolensis	Metacercaria	+	F	A	Fish?
Procerovum spp.	Metacercaria	+	F		Fish
Prohemistomum vivax	Metacercaria	+	F	E	
Prosthodendrium molenkampi	Metacercaria	+	F		Fish
Pygidiopsis summa	Metacercaria	+	F	SEA	Fish
Stellantchasmus spp.	Metacercaria	+	F	AS	Fish
Stellantchasmus falcatus	Metacercaria	+	F	O,H	Fish
Stictodora fuscata	Metacercaria	+	F		Fish
Watsonius watsoni	Metacercaria	+	F	A	Vegetables, monkey alternate host ▶

waterborne illnesses attributed to these species, their impact on food safety and public health both nationally and internationally appears to be significant (Käferstein, 2000).

The relationship between enteric parasitic protozoa, the environment, contamination of food, and human illness is extremely complex. Environmental factors play a significant role in the transmission of most foodborne parasitic diseases. This impact is particularly apparent with protozoa, which are readily transported to food by contaminated water (Slifko et al., 2000a). Fecal contamination of water sources used in crop irrigation, food processing and meal preparation are important sources of human infection. In this regard, contamination of fresh fruits and vegetables is causing the greatest concern. These commodities are intimately influenced by the environment and agricultural practices, and often receive no processing that is lethal to protozoa.

The deployment of more efficient and rapid means of transporting perishable goods worldwide enables fresh produce to be available in the U.S. nearly year-round. Together, these factors have influenced the emergence and recognition of some parasites as new human pathogens.

Cryptosporidium parvum and *Cyclospora cayetanensis*, for example, rapidly became important human pathogens in the U.S. during the 1980s and 1990s, infecting immunocompromised and immunocompetent individuals alike. While not generally associated with human disease earlier in the 20th century, *C. parvum* and *C. cayetanensis* are now frequently identified as causative agents in human illness. Additionally, each is linked epidemiologically to

Table 1—Parasites transmitted by food and water, *continued*

Parasite	Stage	Pathogenicity	Source[a]	Geographic area[b]	Key feature/ food/hosts
Cestoda					
Bertiella studeri	Plerocercoid	–	F?W?	AS	Mite intermediate host
Braunia jasseyensis	Plerocercoid	+	F	AS	Probably transmitted by fish
Diphyllobothrium latum	Plerocercoid	+	F	U	Fish, pernicious anemia
Diphyllobothrium spp	Plerocercoid	+	F	U	Fish
Diplydium caninum	Plerocercoid	+	F?,W?	U	Fleas, mainly infants
Diplogonoporus grandis	Plerocercoid	+	F?W?	U	Crustacea, normal host whales
Digramma brauni	Plerocercoid	+	F		Fish
Echinococcus granulosus	Egg	+	F,W,H	U	Picnics
Echinococcus multilocularis	Egg	+	F,W,H	U	Picnics
Echinococcus vogeli	Egg	+	F,W,H	U	Picnics
Hymenolepis dimunita	Egg	+	F,W,H	U	Fleas
Hynenelepis nana	Egg	+	F,W,H	U	Fleas
Inermicapsifer madagascariensis	Egg?	+	F,W,H	A	Unknown life cycle
Ligula intestinalis	Plerocercoid	–	F		Unknown life cycle
Mesocestoides spp.	Plerocercoid	+	F		Unknown flesh
Multiceps multiceps	Egg	+	F,W	U	
Raillietina demerariensis	Plerocercoid	+	F		
Spirometra sp.	Procercoid?	+	F		Fish
Spirometra erinacei	Plerocercoid	+	F		Fish
Taenia serialis	Egg	+	F,W,H		
Taenia multiceps	Egg	+	F,W	U	
Taenia saginatta	Cysticercoid	+	F	U	Cows
Taenia solium	Cysticercoid/Egg	+	F	U	Pigs, autochthonous
Taenia spp.	Cysticercoid	+	F	U	
Acanthocephala					
Bulbosoma spp.	Juvenile	+	F	R	Fish
Corynosoma strumosum	Juvenile	+	F	R	Fish
Nematoda					
Ancylostoma duodenale	Larva	+	F	U	Vegetables
Anisakis spp.	Larva	+	F	U	Fish
Angiostrongylus cantonensis	Larva	+	F,W	U	Vegetables
Angiostrongylus costaricensis	Larva	+	F,W	NA,SA	Vegetables
Ascaris lumbricoides	Egg	+	F,W	U	Vegetables
Capillaria hepatica	Larva	+	F	U	Vegetables
Capillaria philippinensis	Larva	+	F	P	Fish
Capillaria spp.	Larva	+	F	NA,SA	Fish?
Contracaecum sp.	Larva	+	F	U	Fish
Dioctophyme renale	Larva	+	F	U	Fish
Dracucculus medinensis	Larva	+	W	A	Near eradication
Echinocehpalus sp.	Larva	+	F	U	Shellfish
Enterobius vermicularis	Egg	±	F,W,H	U	Mainly children
Gnathostoma spp.	Larva	+	F	U	Molluscs?
Gnathostoma spinigerum	Larva	+	F	AS	Reptiles, birds
Mammomonogamus spp.	Egg	+	F,W	T	Vegetables?
Porrocaecum spp.	Larva	+	F	U	Fish
Pseudoterranova spp.	Larva	+	F	U	Fish
Rhabditis sp.	Larva	–	F,W	U	Vegetables
Syphacia obvelata	Egg	±	F,W	U	Vegetables
Toxocara cati	Egg	+	F,W	U	Vegetables
Toxocara canis	Egg	+	F,W	U	Vegetables
Trichinella nativa	Larva	+	F	HA	Wild animals
Trichinella pseudospiralis	Larva	+	F	U	Wild animals
Trichinella spiralis	Larva	+	F	U	Domestic animals
Trichinella spp.	Larva	+	F	U	Wild animals
Trichuris trichiura	Egg	+	F,W	U	Vegetables
Trichostrongylus spp.	Larva	+	F	U	Vegetables

the consumption of fresh produce in both sporadic and clustered outbreaks (Table 2). Although both these protozoa can be foodborne pathogens, it is highly likely that they are more often waterborne organisms and transmitted to humans by environmental factors and agricultural practices. The microsporidia, another rapidly emerging group of human pathogens, may also belong in this category.

Although the contribution of these protozoan parasites to overall foodborne illness, as reflected in recent statistics (Mead et al., 1999), appears to be small, it is highly likely that the numbers are underestimates.

Analytical Methods

Methods for the detection of food- and water-borne parasites have expanded from traditional microscopic techniques to include such molecular tools as the polymerase chain reaction (PCR) and species-specific immunologically-based assays. Whereas morphological identification of worms (helminths) and protozoan pathogens remains a vital aspect of analysis, molecular and sometimes immunologic diagnoses are rapidly being incorporated as more sensitive standard practices.

Detection and identification of tissue-encysted or encapsulated helminths rely heavily on morphological characterization by visual inspection and use methods ranging from direct tissue examination to mechanical and enzymatic tissue disruption. Candling is still the standard practice for detecting and recovering anisakid nematodes in fish flesh (Bier et al., 1995). This method can be applied to fresh or frozen white-fleshed fish that are processed as fillets, steaks, or minced fish. Using a "cool white" light source,

the appearance of parasitic worms may vary from reddish to a chalky white. A similar procedure using reflected long-wave ultraviolet light (366 nm) in which the worm's larval stages fluoresce blue or green may be used for fish with dark flesh (Brattey, 1988). Compression candling is applied to such translucent foods as shellfish. Alternatively, tissue disruption is highly effective in liberating parasites for easier identification and establishing accurate counts. The simple technique of homogenizing fish flesh in a food processor and inspecting the diluted debris under shortwave UV light is an efficient means of detecting larval anisakid nematodes of the genera *Anisakis* and *Phocanema* (Brattey, 1988). Alone or in combination with saline elution or pepsin-HCl (enzyme acid) digestion of the homogenate, this approach offers the investigator a greater degree of parasite recovery. Using these types of disruptive methods, the whitish plerocercoids of *Dyphyllobothrium* spp. (larval tapeworms) can also be more easily recovered and identified (Arambulo, 1982). Metacercaria and mesocercaria (larval forms of trematodes) are also more easily detected and identified with the aid of these methods.

Visual examination is the predominant method of inspection for the presence of the cysticerci (larval forms) of *Taenia solium* (pork tapeworm) in pig carcasses and *Taenia saginata* (beef tapeworm) in bovine carcasses. Post-mortem macroscopic inspection of selective muscle tissue is the primary means of detecting these parasites, although enzyme-acid digestion of tissue similar to that used to examine fish is also employed (Arambulo, 1982). Ante-mortem detection methods using serological tests are currently available. Although hemagluttination tests show some promise, these and other procedures have demonstrated variable specificity and sensitivity and may not be practical due to high cost. Additionally, a host's weak immunological response to light infections may complicate definitive diagnoses.

In the past, detection and identification of pathogenic protozoa from water sources and foods were equally dependent on traditional microscopy. However, success with microscopy has proven to be much more difficult for protozoa than for the helminths. Definitive microscopic identification of coccidian parasites such as *C. cayetanensis* and *C. parvum* is linked to such criteria as oocyst size, shape, and sporulation characteristics (Goodgame, 1996). Staining and autofluorescent properties (such as those of *C. cayetanensis*) further distinguish these parasites, although, these means of identification are complicated by variability in staining patterns (Negm, 1998; Visvesvera et al.,1997). Inefficient isolation and concentration techniques—used to counteract small size, low numbers, and the large sample sizes required—create additional problems for analyses that already are laborious and time-consuming. Additionally, as in all microscopic diagnoses, identification is highly dependent on the skill, experience and expertise of the microscopist (Goodgame, 1996).

While microscopy remains an important diagnostic component for definitive identification of protozoan parasites, newer techniques are more sensitive, specific, and time-efficient. PCR protocols for *Cyclospora* and *Cryptosporidium* detection have been used successfully over the last several years for clinical, environmental and, more recently, food surveys (Rose and Slifko, 1999). These protocols include methods to differentiate among species or closely related genera. To distinguish between *C. cayetanensis* and *Eimeria* spp., PCR products also must be examined either by restriction fragment length polymorphism analysis (RFLP) (Jinneman et al., 1998) or an oligonucleotide ligation assay (OLA) (Jinneman et al., 1999).

With the recent description of several *Cyclospora*-like species isolated from non-human primates (Eberhard et al., 1999; Lopez et al., 1999; Smith et al., 1996), a new nested PCR protocol, capable of differentiating *C. cayetanenesis* from related non-human parasites (Orlandi, 2001) is currently under development at the U.S. Food and Drug Administration (FDA).

Molecular techniques provide considerable advances in detection sensitivity, specificity and ease of analysis. These methods, however, still depend on our ability to isolate and concentrate the parasite from the sample matrix and prepare a suitable DNA template free of matrix-derived substances that may inhibit PCR. An extraction-free, filter-based method of preparing DNA templates now exists for the PCR detection of such protozoa as *C. cayetanensis*, *C. parvum*, and the various microsporidia genera and species in a va-

Table 2—Foodborne outbreaks associated with parasites

Infection	Parasite	Food item(s) implicated/associated	Reference
Amebiasis	*Entamoeba histolytica*	Ice cream, fruit	de Lalla et al. (1992)
Anisakiasis	*Anisakis*	Raw saltwater fish Raw fish	Machi et al. (1997) Muraoka et al. (1996)
Ascariasis	*Ascaris lumbricoides*	Imported vegetables	Raisanen et al. (1985)
Cryptosporidiosis	*Cryptosporidium parvum*	Chicken salad	CDC (1996b)
	Cryptosporidium	Green onions	CDC (1998)
Cyclosporiasis	*Cyclospora cayetanensis*	Basil Raspberries Dessert	Lopez et al. (2001) Caceres et al. (1998) Fleming et al. (1998)
Diphyllobothriasis	*Diphyllobothrium*	Salmon	Ruttenber et al. (1984)
Fascioliasis	*Fasciola hepatica*	Lettuce	Espino et al. (1998)
Giardiasis	*Giardia lamblia*	Raw sliced vegetables Fruit salad	Mintz et al. (1993) Porter et al. (1990)
Nanophyetiasis	*Nanophyetus salmincola*	Salmonid fish	Eastburn et al. (1987)
Trichinellosis	*Trichinella*	Horse meat Cougar jerky	Ancelle et al. (1998) CDC (1996b)

riety of complex matrices (foods, environmental samples, and clinical specimens) (Orlandi and Lampel, 2000). This protocol increased the sensitivity of PCR detection and alleviated the need for time-consuming purification schemes that can contribute to significant sample losses and affect detection sensitivity. The protocol was successfully used to identify *C. cayetanensis* as a contaminant in a chicken-basil-pasta salad implicated in a 1999 outbreak of cyclosporiasis in Missouri (Lopez et al., 2001).

In addition to PCR, several immunologically-based assays were developed for *C. parvum*. Monoclonal antibodies to oocyst surface antigens are currently available for use in immunofluorescence microscopy or as a component in commercial (enzyme-linked-immune-sorbent assay) kits. Immunomagnetic separation techniques are also being refined and applied as a means of isolating, concentrating, and purifying *C. parvum* oocysts from complex matrices. No commercially available immunological reagents currently exist for *C. cayetanensis*.

Whereas molecular methods have provided a means for rapid and sensitive detection of parasites in foods, the question of whether those organisms detected in complex matrices are viable and therefore infective still remains. Animal infectivity studies, where possible (i.e., *C. parvum, G. intestinalis*), provide the most definitive answer to the question of viability in comparison to sporulation, excystation, and tissue culture models (*in vitro* studies). However, animal infectivity studies are laborious, time-consuming, and expensive (Neumann et al., 2000).

Deng et al. (1997) proposed an alternative method using immunomagnetic capture PCR to distinguish viable and dead *C. parvum*. In addition, viability stain assessment and fluorescent *in situ* hybridization studies have also proved useful (Deere et al., 1998; Neumann et al., 2000; Vesey et al., 1998). For other parasitic protozoa such as *Cyclospora* and the microsporidia, where genetic and surface antigen information is lacking, assessment of viability remains difficult. Sporulation, excystation (e.g., *Cyclospora*) and spore germination in conjunction with tissue culture infectivity (e.g., microsporidia) are currently the only method for gauging viability (Ortega et al., 1998; Wittner and Weiss, 1999).

Control Measures

Contamination of food products by parasites may occur at several points along the path from growing and harvesting food at the farm or fishing grounds to consumption by the consumer. Possible contamination sources include the use of parasite contaminated irrigation or spraying water, contamination of surfaces during harvesting or processing, and contamination of food products during final preparation and packaging. Furthermore, certain genotypes of *C. parvum, T. gondii* and *G. intestinalis* have reservoir hosts that increase their frequency in the environment and thereby their threat to public health.

A number of control measures are used to protect food products from parasites. Foremost among the traditional measures are the cleaning and cooking of food items prior to consumption. Most parasites' heat resistance is not impressive, and temperatures as low as 56–60°C for several minutes will, in many instances, eliminate the infectivity of helminths (Fayer et al., 2001). Heating to > 72°C for 1 min or 45°C for 10–20 min inactivates *C. parvum* oocysts (Steiner et al., 1997). However, the heat must uniformly penetrate the entire food matrix because parasites such as *Trichinella* or *Anisakis* may be encysted deep inside the tissues.

Protozoa . . . are now among the dominant public health concerns in several nations and can cause disease outbreaks that encompass entire communities.

Other processes, used primarily for the preparation and preservation of fish are effective in inactivating helminths. These include hot smoking, fermentation in brine, and drying (WHO, 1995); cold smoking and salting, however, may not be effective against fishborne anisakid nematodes. Cleaning methods include such actions as peeling, washing or scrubbing fresh produce that is usually contaminated at or near the surface. Mixtures of chemicals such as the in-situ generation of mixed oxidants, may be more effective than a single chemical, such as chlorine, which does not inactivate protozoan cysts and oocysts (Venczel et al., 1997).

Consumer preferences for the consumption of fresh fruits and vegetables preclude the use of heat as a control measure against parasitic contamination. Application of cold temperatures may serve as a useful alternative: freezing *C. parvum* to –20°C and –70°C rendered oocysts noninfectious and nonviable after 24 and 1 hr, respectively (Fayer and Nerad, 1996). FDA's "Fish and Fisheries Products Hazards and Controls Guide" recommends that raw fish served for consumption should be frozen at either (1) –35°C (or below) until solid and stored at –35°C or below for 15 hr, (2) –35°C (or below) until solid and stored at –20°C (or below) for at least 24 hr, or (3) –20°C or below for 7 days (total time) prior to being sold (FDA, 2001). Proper freezing of fish products destroys helminths capable of causing disease after consumption of such raw fish dishes as sushi, sashimi, or ceviche. Freezing, particularly in the short term, however is unpredictable—freezing can inactivate parasites but under certain conditions also may preserve them.

Water serves as an important vehicle for transmission of foodborne parasites. In developed nations, treatment of public water sources with halogenated compounds, predominantly chlorine, significantly reduces the public health threat of bacterial pathogens such as *Vibrio cholerae*. Protozoa such as *G. intestinalis* and the coccidia that are resistant to antibacterial levels of chlorine are now among the dominant public health concerns in several nations and can cause disease outbreaks that encompass entire communities.

Furthermore, the Environmental Protection Agency standard for water quality is based on a total bacterial coliform count (EPA, 1990). This criterion does not appear to be a reliable indicator for parasite contamination of water. This was apparent during the 1993 *C. parvum* outbreak in Milwaukee, in which the implicated water source met all federal quality standards (Mac Kenzie et al., 1994). Filtration of water also has improved water quality, although the small size of some parasites may allow their passage through certain

types of filters. An absolute 1-µ filter is required to exclude *Cryptosporidium*.

A number of parasite control measures are being considered and evaluated for food processing (Rose and Slifko, 1999). Ozone is a powerful oxidizing agent and has been suggested as a possible disinfectant for some parasites. Treatment of *Cryptosporidium parvum* in ozone-demand-free buffered H_2O with 1 ppm ozone for 5 min decreased both oocyst excystation and infectivity to mice by greater than 90% (Korich et al., 1990).

Ozone inactivation, however, is dependent on a number of parameters including temperature, medium pH, and the amount of extracellular organic matter residing around the parasite; therefore, penetration of ozone into food crevices where parasites may reside may not occur (Kim et al., 1999).

Irradiation serves as another possible measure for parasite control. UV irradiation, as a method for inactivating *Cryptosporidium* in apple cider, is currently being investigated. Preliminary results demonstrate a 5-$\log_{10}$ reduction in oocyst viability (Hanes, 2001). Whereas UV irradiation has the potential to serve as an efficient method for inactivation of *Cryptosprodium* for even small producers, its economic feasibility has yet to be determined.

Ionizing irradiation is effective in controlling helminths such as *Opisthorchis viverrini, Anisakis simplex, Clonorchis sinensis,* and *Paragonimus westermani* (Venugopal et al., 1999). In the control of *Trichinella spiralis*-infected pork, FDA approved the use of irradiation at an absorbed dose of 0.3 kGy-1.0 kGy (FDA, 1985). While ionizing irradiation is useful for inactivation of a number of parasites, it does not uniformly inactivate all parasites to the same degree; a considerable range in the log reduction is reported (Enigk et al., 1975).

Also, inactivation of parasites may require a range of doses because the effectiveness of the process is dependent on the parasite, the stage of the parasite that has contaminated the food matrix, and the types and characteristics of the food matrix itself (Farkas, 1998). Hence, many parameters must be examined before irradiation will become practical for a particular product. Another factor is some consumers' concern about ingesting irradiated food. This has mitigated against the use of irradiation on food products.

Hydrostatic pressure is a recently proposed means by which we may be able to decontaminate foods. Studies by Slifko et al. (2000a) demonstrated that more than 99% of *C. parvum* oocysts in apple and orange juice were inactivated following > 60 sec high hydrostatic pressure treatment. The use of hydrostatic pressure on fish for inactivation of helminths is currently being investigated. While some of the control measures discussed exhibit efficacy against certain parasites, none of these procedures has been demonstrated to be as uniformly reliable as heat.

Control measures used to decontaminate food products once contaminated by parasites are only one intervention to prevent human illness caused by the ingestion of foods harboring parasitic pathogens. Surveillance programs are another means to control and limit the impact of foodborne parasites on public health. Such programs can serve as effective indicators of potential contamination problems through periodic testing. Though somewhat costly, surveillance of those foods, such as fresh produce, previously identified as "at-risk" for parasitic contamination acts to protect both the producer and the consumer and minimize the risk of large foodborne outbreaks. A step towards such a surveillance program is the development and implementation of policies, similar to seafood HACCP, designed to limit, control, and monitor sources of parasite contact with food.

In an effort to preserve the raspberry industry in Guatemala in 1999, the Model Plan of Excellence (MPE) was developed through the cooperative efforts of the U.S. Food and Drug Administration, the Guatemalan government, and the Guatemalan Berry Commission to guard berries intended for export against contamination with *C. cayetanensis*. The MPE is a strict version of Good Agricultural Practices and is intended to decrease the risk of contaminating crops with *Cyclospora*. As a system designed to monitor many food safety aspects of farming, it provides for oversight and surveillance of sanitation practices, water source development, irrigation methods, food handling, and employee hygiene. Though originally designed and implemented to stem the growing problem of cyclosporiasis attributed to contaminated berries epidemiologically linked to the spring crop of Guatemalan raspberries, the MPE has the potential to serve as a model policy document for other produce-trading partners to limit the risk of contaminating crops with other pathogens (Calvin et al., 2000).

Conclusions

In the past, in the U.S., consideration of parasitic animals as foodborne pathogens waned with the incorporation of better food handling and sanitation practices, and inspection procedures. However, events during the latter part of the 20th century forced us to focus again on the potential health risks posed by parasitic protozoa and helminths. Globalization of food trade, preferences for raw and undercooked dishes, ease of international travel, and increasing numbers of immunocompromised individuals are factors that have contributed to the increase in foodborne parasitic infections. This is further complicated by the emergence of parasites not previously associated with pathogenesis in humans.

Educational outreach and research programs developed through the U.S. government's Food Safety Initiative have been relatively successful in increasing public awareness of food safety issues and potential health risks (FSI, 2001). Nevertheless, it is probable that the incidence of illness attributed to parasitic contamination of food and water is underreported. The reasons for the underreporting are: the lack of recognition of potential effects of parasite contamination on public health and the absence of routine screening protocols for these pathogens.

With the emphasis on food safety continuing and food security being a major concern since September 11, 2001, the development of new technologies for the prevention, detection and inactivation of foodborne parasites is being accelerated. In conjunction with an increased awareness of the health risks associated with parasitic contamination and illness, the control of foodborne parasites is expected to become more effective.

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