CHAPTER 10

Risk Assessment of Parasites in Food

Kristina D. Mena

10.1 PREFACE

Risk is the possibility or probability of an adverse event occurring due to a hazard or hazards. A hazard may be a physical, chemical, or microbial agent, such as a parasite. Determining whether an agent poses a threat—or the extent of that threat—to humans is not always straightforward. With the diversity of hazards present in the environment along with the multiple transmission routes possible, fully understanding the chances of exposure to that hazard as well as the subsequent human health significance are difficult at best.

Risk assessment has emerged as a methodology to address a wide variety of environmental hazards and their associated human health impacts. A risk assessment framework was first developed in the 1970s by the National Research Council (NRC) to systematically evaluate chemical hazards in the environment (NRC, 1983). In the 1980s and 1990s, this framework was applied to address microorganisms, particularly human health risks associated with waterborne pathogens (Haas, 1983; Regli *et al.*, 1991; Rose and Sobsey, 1993; Rose *et al.*, 1991). The application of this paradigm to food-borne microorganisms and food safety issues soon followed in the 1990s (Jaykus, 1996; Lammerding and Paoli, 1997; Rose *et al.*, 1995). This approach is attractive to the food safety arena due to the Sanitary and Phyto-Sanitary (SPS) Agreement of the World Trade Organization (WTO) and the General Agreement on Tariffs and Trade (GATT) (Marks *et al.*, 1998) and many organizations and governmental agencies—such as the US Department of Agriculture (USDA), Health Canada, and the Codex Alimentarius Commission (CAC), have applied this process (CAC, 1999). In addition, results of such an assessment can enhance the food industry's already prevalent HACCP (hazard analysis critical control point) programs, as they can aid in the identification of critical control points during food production and processing.

A goal of a microbial risk assessment is to provide an objective, science-based evaluation of a microbial hazard to risk managers for the subsequent development of strategies to minimize risk. Risk assessment, therefore, is the first component of the risk analysis process, followed by risk management and risk communication (NRC, 1994). The risk assessment component as developed by the NRC (1983) includes four steps: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization. Although the framework is an iterative process, in some cases the food safety approach has applied a modified paradigm that addresses exposure prior to dose-response and has replaced the terms "dose-response assessment" with hazard characterization (Fig. 10.1).

The underreporting of food-borne illnesses in the United States makes it challenging to fully understand the public health significance of food-borne disease

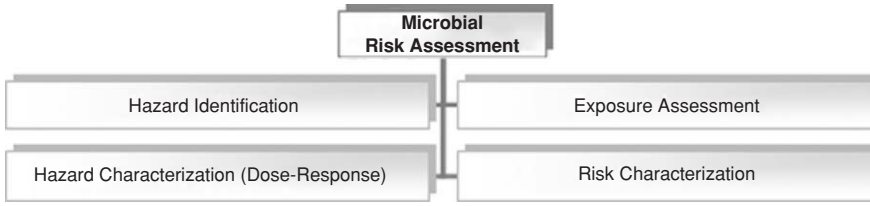

Figure 10.1. The Microbial Risk Assessment Framework.

agents, especially specific food-borne pathogens. Mead *et al.* (1999) estimates that 76 million food-borne illnesses occur in the United States annually leading to 325,000 hospitalizations and 5000 deaths. Protozoan parasites are microorganisms that can be transmitted to humans through either contaminated food or water, resulting in several clinical outcomes such as gastroenteritis. The following sections discuss how this framework can be applied to address parasites in food.

10.2 THE RISK ASSESSMENT FRAMEWORK

10.2.1 Defining the Hazard

This first step of the risk assessment framework provides qualitative information regarding the microorganism itself and its interaction with a host. Available and pertinent information from published epidemiological studies (such as outbreak investigations) as well as laboratory and field data are presented here to determine the extent—if any—of human health impact. Initially, a potential pathogen will be evaluated to determine the range of human illnesses (both acute and chronic) associated with exposure. Once the microorganism is deemed to be pathogenic, other characteristics are identified and reviewed including: (1) endemic and epidemic disease; (2) potential severity of human health consequences, including the determination of morbidity, mortality, and hospitalization ratios; and (3) population characterization, such as the identification of who is likely to be exposed and which subpopulations are more severely impacted.

Appropriately selecting pertinent information for the hazard identification step is critical to the overall risk assessment. Information learned through the completion of this step is incorporated throughout the risk assessment. It is important for the risk assessor to be able to critically review available information to determine its justification for inclusion in the assessment as well as be able to appropriately interpret study findings. Moreover, an understanding of the difficulties in determining a microorganism's ability to initiate infection and illness (*causation* as opposed to *association*) is necessary to adequately evaluate an agent as a (potential) hazard, particularly when addressing food-borne agents. Information available in the peer-reviewed literature regarding food-borne microorganisms is often descriptions of food-borne outbreak investigations that lack crucial information including the identification of the specific pathogen, food vehicle, and number of illness cases.

Table 10.1. Food-borne Protozoan Parasites of Human Health Concern (Haas *et al.*, 1999).

Parasite	*Health Outcome*
Cryptosporidium parvum	Gastroenteritis
Cyclospora	Gastroenteritis
Entamoeba histolytica	Gastroenteritis Intestinal tissue abscesses
Giardia lamblia	Gastroenteritis Chronic joint pain Lactose intolerance
Toxoplasma gondii	Congenital malformations Mental retardation Seizures

10.2.1.1 Important Food-borne Parasites

Table 10.1 provides a list of protozoan parasites that may be transmitted through food (and perhaps water and/or person-to-person). Clinical symptoms usually include gastroenteritis but more severe consequences may result, particularly in the immunocompromised. Although most risk assessments of food-borne pathogens focus on bacterial agents, risk assessment approaches have been undertaken for *Cryptosporidium parvum*, *Giardia lamblia*, and *Toxoplasma gondii.* The focus on the former two was on the waterborne route of transmission and the production of microbially safe drinking water (Haas *et al.*, 1996; Lammerding and Paoli, 1997; Rose *et al.*, 1991; Teunis and Havelaar, 2002). Unfortunately, the protozoa more commonly associated with food, such as *T. gondii* and *Cyclospora*, are lacking critical (dose-response) data for risk assessments to be conducted. Components of a risk assessment process have been explored for *T. gondii* and specific data needs were realized and are discussed in the following sections of this chapter.

10.2.2 Exposure Assessment

In the exposure assessment step, the risk assessor determines the intensity and frequency of human exposure to the hazard (parasite). Information is collected or determined regarding the source of exposure, the number of pathogens in the source, the extent (duration) of exposure, the population exposed, and perhaps events leading to exposure. This information may be obtained during the hazard identification step for perhaps a general model development or some of the information may be obtained from an epidemiological food-borne outbreak investigation leading to the development of a model that is situation-specific. Scenario trees have been built and applied to describe specific potential situations or sequences of events that need to occur for a particular outcome to take place (Jaykus, 1996; Marks *et al.*, 1998). Jaykus (1996) describes two types of "trees"—"fault trees" and "event trees"—where one is describing the series of events (probabilities) that would occur to lead to the preidentified "fault" and where the other attempts to predict the events that would

need to occur for a food-borne contamination incident to lead to human disease. These "scenario" approaches can provide useful information that can be incorporated in HACCP programs. In addition, predictive microbiology—which has been applied to food-borne bacteria—develops mathematical models to predict the number of microorganisms present throughout a food production process (McMeekin *et al.*, 1993). The mathematical models incorporate variables related to food composition and food processing that may potentially impact a microbe's growth or die-off.

A probabilistic model has been developed for *T. gondii*, although not specifically addressing food-borne transmission (Cassin *et al.*, 1996). This model explores other factors related to exposure such as maternal exposure during pregnancy, effect of drug therapies, population age and immunity profiles, and cat ownership. The goal is to evaluate the role of various risk factors in the incidence of toxoplasmosis to then develop appropriate reduction strategies.

Other exposure-related factors—such as food composition, parasite survivability in the environment, and parasite resistance to treatment—also need to be addressed as they can impact the magnitude and duration of exposure. Food production, processing, and consumption also influence exposure and involve a variety of players including growers, manufacturers, distributors, and consumers. Moreover, consumers are the final point of contact with the potentially contaminated food and associated characteristics such as demographics and sociocultural factors influence food preparation practices and consumption patterns, which will also impact an exposure assessment. In addition, the (in many cases) inevitable role of secondary transmission needs to be considered during this step of the risk assessment.

10.2.2.1 Data Gaps and Challenges

Having quantitative data on the occurrence of specific pathogens (parasites) in food is of utmost importance and perhaps presents the biggest challenge in conducting risk assessments for any type of microbial hazard. Exposure data derived from detection methodologies that are both sensitive and specific, address pathogenicity, and are quantitative (rather than presence/absence tests), are critical to conduct meaningful risk assessments. Such occurrence data for microbial risk assessments are usually obtained from published surveillance studies of water, for example, but are more difficult to obtain for the food-borne route. Current methods for the detection of *Cryptosporidium parvum* and *Giardia lamblia* in water, for example, are time-consuming and labor-intensive with several opportunities for error (Teunis and Havelaar, 2002), which obviously impacts the integrity of risk assessment results if such data are incorporated. Some information is available as a result of food-borne outbreak investigations, but to a limited extent (Rose *et al.*, 1995) due to the inherent complexities of the investigations. The challenges associated with adequately defining factors related to exposure introduce the greatest amount of variability and uncertainty in the microbial risk assessment process. This will be discussed further in the Risk Characterization section.

10.2.3 Hazard Characterization (Dose-response Assessment)

The hazard characterization describes the relationship between the dose of the microorganism (parasite) and the extent of the adverse human health effect (infection,

illness, and death). In essence, it is predicting the ability of a pathogen to overcome a host's defenses to initiate infection and perhaps disease. Determining the likelihood of infection or disease in an exposed population is determined from a dose-response curve developed from experimental data. Unlike with chemical hazards, most of the dose-response data regarding microbial hazards—including parasites—were obtained from human studies (rather than animal experiments) (Dupont *et al.*, 1995; Rendtorff, 1954; Rendtorff and Holt, 1954; Teunis *et al.*, 2002). In human studies, participants are given a range of doses (through ingestion, inhalation, or direct contact) and a human health endpoint of interest (infection or disease or both) is determined. Infection may be determined through direct microscopic count of cysts or oocysts in stool samples of participants, for example, and disease would be determined through the observation of appropriate clinical symptoms. Limitations of such human studies include the fact that healthy adults were used and a relatively high amount of (low virulent) dose was administered in order to be able to observe the desired health endpoint of interest using as few participants as possible. In a typical food contamination situation, a person may be exposed to a (more virulent) lower dose of microorganisms. (However, dose-response datasets for protozoan parasites do include low doses.) A dose-response assessment will attempt to predict what the human health outcomes would be in such a situation using the information obtained from dose-response studies.

Although it is the exposure assessment that is the greatest source of variability and uncertainty in a microbial risk assessment (as explained above), most of the controversy surrounding the application of microbial risk assessment results to "real-world" situations targets the dose-response assessment. Haas (1983) was the first to evaluate the ability of dose-response models to adequately represent the microorganism-host interaction and concluded that the models representing the best fit (using maximum-likelihood methods) were the following nonthreshold models: the exponential and the beta-Poisson. More recently, other models such as the Weibull-Gamma, log-probit, and Gompertz, have been evaluated although primarily for bacteria (Holcomb *et al.*, 1999; Teunis *et al.*, 1999). An ideal model not only fits the available data but is also flexible, conservative (to be protective of subpopulations), and simple (Holcomb *et al.*, 1999). Currently, the exponential and beta-Poisson models are recommended for food-borne and waterborne pathogens (Haas, 1983; Haas *et al.*, 1999; Teunis and Havelaar, 2000).

The following is the exponential model:

$$P_i = 1 - \exp(-rN)$$

where P_i = the probability of infection from a single-dose exposure, r = a constant that represents the number of microorganisms that survive and are capable of initiating an infection (i.e., microorganism-specific), and N = the number of microorganisms ingested or inhaled. This model assumes a random distribution of pathogenic microbes and a constant microorganism-host interaction. The parameter r is further defined as:

$$-r = \ln(0.5)/N_{50}$$

Table 10.2. Dose-Response Models for Food-borne Protozoa.

Microorganism	*Model*	*Animal*	*Reference*
Cryptosporidium parvum	Exponential $r = 0.0042$	Humans	Haas *et al.*, 1996
Giardia lamblia	Exponential $r = 0.01982$	Humans	Rose *et al.*, 1991

where N_{50} equals the median infectious dose. The beta-Poisson distribution assumes a heterogeneity between the microorganism-host interaction resulting in two parameters, α and β. The following is the beta-Poisson model:

$$P_i = 1 - (1 + N/\beta)^{-\alpha}$$

where P_i = the probability of infection from a single-dose exposure, N = the number of microorganisms ingested or inhaled, and α and β represent the dose-response curve (microorganism-specific). Ninety-five percent confidence limits to the dose-response parameters can be computed. β can be further defined as:

$$\beta = N_{50}/(2^{1/\alpha} - 1),$$

resulting in the following equation for the beta-Poisson model:

$$P_i = 1 - [1 + N/N_{50}(2^{1/\alpha} - 1]^{-\alpha}.$$

Risks of illness and death can be calculated by multiplying the risk of infection (P_i) by the appropriate morbidity ratio (risk of illness) and then by multiplying the risk of illness by the appropriate mortality ratio (risk of death). Morbidity ratios for *Giardia* and *Cryptosporidium* are reportedly not dose-dependent and are approximately 50% (Rose *et al.*, 1991) and 39% (Haas *et al.*, 1996), respectively. Mortality ratios for both protozoa are about 0.1% (Haas *et al.*, 1999).

More data are needed to fully address the dose-response relationship of food-borne parasites, particularly for *T. gondii* and *Cyclospora*. Factors associated with the food vehicle (e.g., composition), the host (e.g., health status and immune status), as well as the protozoan itself (e.g., virulence factors and strain variation) need to be considered. A cell culture approach has recently been applied to address dose-response issues regarding *Cryptosporidium* (Slifko *et al.*, 2002). Dose-response and model selection information are available for two food-borne protozoa (Haas *et al.*, 1996; Rose *et al.*, 1991) (Table 10.2).

10.2.4 Risk Characterization

The objective of the risk characterization step is to integrate all of the information from the first three steps and provide both a qualitative assessment of the potential human health impact from exposure to the parasitic hazard and (ideally) a quantitative estimate (e.g., 1:10, 1:10,000, 3:100) of the probability of certain human health outcomes actually occurring due to that exposure. Therefore, the probability risk estimate reflects both the likelihood that a microorganism will cause adverse health outcomes within a population as well as the severity of those outcomes.

Risk estimates may be computed as a point-estimate that represents perhaps a "worst-case scenario" where a conservative approach was taken during the risk

assessment to be protective of subpopulations, such as the immunocompromised. A more realistic, useful approach is to compute a distribution of risk that represents a range of exposure scenarios. Nevertheless, the goal is to provide science-based direction for risk managers in the mitigation of environmental hazards and risks.

A common approach in the regulatory arena is to define an action level—either in the food or water industry—above which predicted risks are unacceptable. When considering risks associated with protozoa and consumable products, Teunis and Havelaar (2002) propose an action level be based on the following factors: exposure (maximum amount of pathogen consumed), effect (maximum incidence of human health consequences), and associated costs (regarding both human health effects and product maintenance). Although an action level would reflect a maximum level of acceptable risk, this risk limit should be determined from a range (distribution) of exposures. An inevitable reality when resources to mitigate risks are limited, is the challenge of risk-risk comparisons and subsequent decision-making, particularly when such comparisons aren't compatible.

Risk assessments of parasites in food have been minimally explored although this approach has been used to address *Giardia lamblia* and *Cryptosporidium parvum* in water (Aboytes *et al.*, 2004; Haas *et al.*, 1996; Regli *et al.*, 1991). The USEPA recommends that yearly risks of microbial infection not exceed 1:10,000 for potable waters so risk assessments addressing waterborne pathogens can use this risk level when interpreting risk outputs. Approaches have involved either determining the water treatment level required to meet USEPA's recommendation (Rose *et al.*, 1991) or determining the dose associated with a specified risk level (Haas *et al.*, 1996). More recently, risk estimates were computed addressing the occurrence of *Cryptosporidium parvum* in water where not only quantitative data were available, but the detection method was able to identify *infectious* oocysts (Aboytes *et al.*, 2004).

Research regarding exposure factors and dose-response relationships of foodborne parasites—such as *Cyclospora* and *T. gondii*—are needed to fully conduct comprehensive risk assessments. Such assessments could provide a tool to identify risk factors or "critical control points" along with a food production chain as well as during food distribution and processing. Specific food matrix factors and characteristics/parameters associated with the host and the specific microbe(s) of interest would all need to be considered (Buchanan *et al.*, 2000).

10.2.5 Assumptions, Assumptions, Assumptions

Variability and uncertainty are inherent to all risk assessments. Variability can result due to heterogeneous parameters such as those factors related to exposure. Uncertainty can have a role when certain parameters are unknown or specific data are lacking. Assumptions may be made in order to forward the risk assessment process and it is critical that all assumptions are stated as such for proper risk assessment interpretation to occur. There are several places within the risk assessment process where factors are introduced that either underestimate or overestimate the calculated human health risks. Issues related to exposure such as detection method inefficiencies, human consumption patterns, secondary/tertiary transmission, and immunity and multiple exposures may all contribute to inaccurate estimations of risk. In addition, microbial-related factors such as the assumption that all detected microbes

are infectious to humans, for example, may also inappropriately impact a computed risk estimate.

Computer software programs are available that address variability and uncertainty. A Monte Carlo simulation can be performed to develop a risk distribution from point estimates (Burmaster and Anderson, 1994). Random variables (representing a defined probability distribution) are entered literally thousands of times resulting in a final probability distribution. This final probability distribution reflects many distributions and input combinations providing perhaps a more realistic evaluation of the risks associated with a particular hazard or hazards.

10.2.6 Emerging Applications of Microbial Risk Assessment

The quantitative microbial risk assessment framework has been used by both the water and food industries as a means to provide and incorporate science-based information during regulatory decision-making and is increasingly becoming part of microbial water monitoring studies (Aboytes *et al.*, 2004). Microbial risk assessment gives public health meaning to laboratory data and can provide direction for addressing microbial-contaminated media, particularly where information gaps are apparent. Although HACCP programs are actually a risk management tool, the risk assessment approach can greatly enhance such program development, particularly during the hazard identification and exposure assessment steps. In addition, with the increasing global food market, risk assessments can provide a means of standardizing the food production process and/or the evaluation of such processes. Recently, the risk assessment framework has been applied in a water/food combined approach to assess the role of microbial—(such as parasites) laden waters (irrigation water, produce wash water, etc.) in contaminating fresh fruits and vegetables. This distinctive application incorporates issues related to both water and food routes of transmission, provides crucial information to enhance (or develop) effective HAACP programs for fruit and vegetable production, and has the potential to have global implications—both for the produce industry as well as human health.

Quantitative microbial risk assessment provides an adaptable, flexible framework for evaluating the public health impacts associated with exposure to a variety of pathogens in different settings. The limited data on food-borne parasites—especially data related to exposure and dose-response—currently restrict the applicability of the framework to adequately address human health risks associated with parasites in food; however, it has also had a role in identifying emerging threats, such as *Cyclospora* (Jaykus, 1996). Quantitative microbial risk assessment—particularly for protozoa—in the food safety arena has yet to be fully realized.

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