Applied Biosafety, 8(4) pp. 160-165 © ABSA 2003

OSHA Infectious Dose White Paper

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During the past summer, OSHA requested ABSA's technical support to develop a white paper regarding the concept of infectious dose. ABSA was asked to consider whether infectious doses for organisms could be defined in such a way to potentially develop permissible exposure levels to those infectious agents. The following paper was researched and developed by ABSA's Technical Review Committee and Council in support of the ABSA/OSHA Alliance.

Executive Summary

A pathogen's infectious dose (ID) is one of many factors that are considered when a biological hazard analysis is performed. The NIH Recombinant DNA Guidelines and the CDC/NIH Guidelines for Biosafety in Microbiological and Biomedical Laboratories recognize many factors interact and contribute to an organism's ability to infect the host. Since the ID varies based on a number of factors, it is often prudent to conduct specific job hazard analysis or risk assessments to determine the appropriate precautions used in a microbiological laboratory. Factors to be considered in determining the level of containment include agent factors such as virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. The infectious dose of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The laboratory worker's immune status is directly related to his/her susceptibility to disease when working with an infectious agent (NIH Guidelines, 2002; CDC-NIH, 1999). By analogy with the LD_{50} metric that is used to communicate chemical toxicity, OSHA has asked ABSA to evaluate whether infectious dose would provide a meaningful parameter for communicating the relative and absolute risks of infectious agents.

In this paper, ABSA examines the current opportunities associated with the development or extrapolation of infectious dose values, and the subsequent application of infectious dose in a regulatory setting. ABSA has identified the challenges in defining endpoints for the term "infection" (and consequently, for "infectious dose"), and the challenges associated with the interpretation of data from disparate studies.

ABSA believes that the current internationally recognized system for assigning pathogens to one of four Risk Groups based on numerous factors is prudent and has worked well over time, and should continue to do so in the future. ABSA has concluded that the vast amount of resources needed to develop scientifically valid, quantitative values for infectious dose, the potential assignment of permissible exposure limits, and the subsequent sampling and laboratory analyses that would then be required would not improve worker protection. ABSA believes that OSHA would see a better return in terms of worker health and safety if these valuable and limited, human and financial resources were used for promoting other safety initiatives.

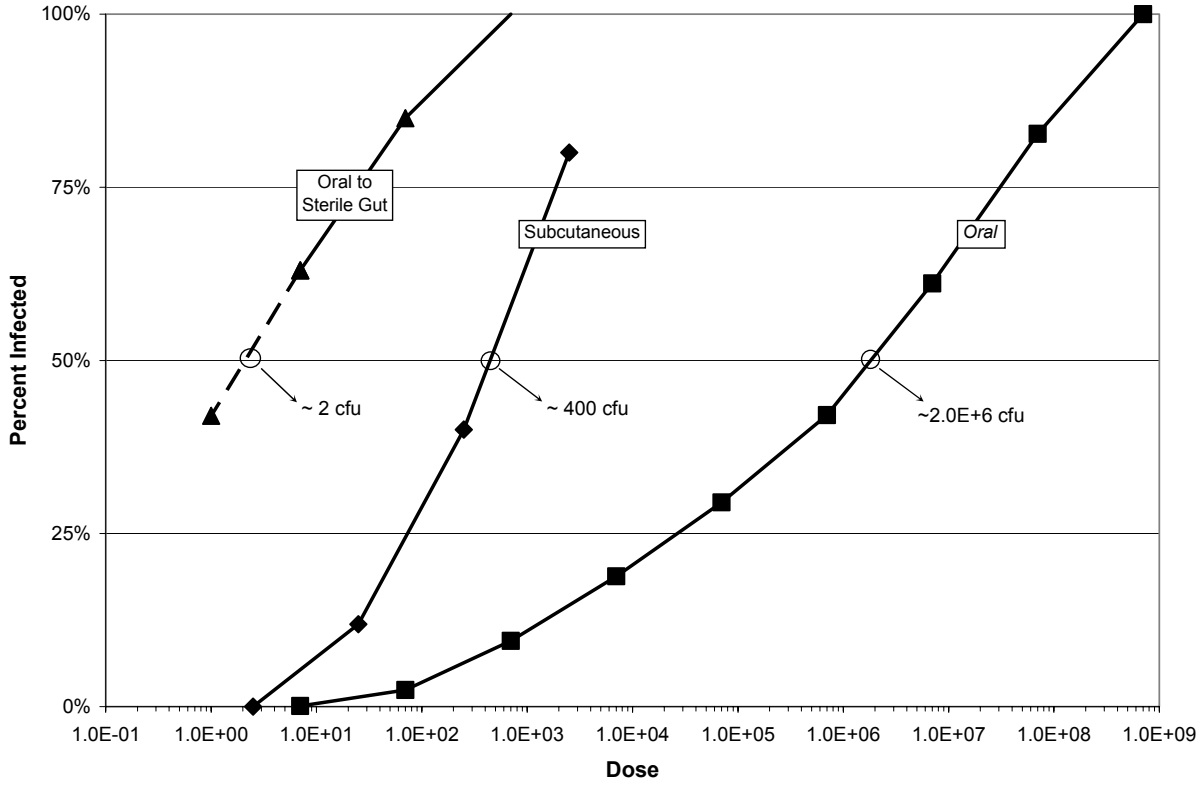
Defining Infectious Dose

The term "infectious" is defined in Stedman's Medical Dictionary (22nd ed.) as "capable of being transmitted by infection," and "denoting a disease due to the action of a microorganism." However, the term "infectious dose" is not found in medical texts. The likely reason for this is that the host response to infection is highly variable, and is dependant on the interrelationship of many host, agent, and environmental factors, and ranges from nonapparent infection to overt disease (Mandell et al., 1990). In day-to-day layman's terms, it is typically described as the number of organisms necessary to cause disease. Under this definition, the concept of infectious dose becomes linked to the virulence or pathogenicity of the organism and is an oversimplified definition. For example, by this definition, it is impossible to define an infectious dose for an avirulent organism. In addition, this definition does not take into account asymptomatic or subclinical infections, or colonization. Those individuals who are infected but not symptomatic may shed virulent organisms into the general population but may not be recognized as source of infectious organisms.

There are clear examples of infections which do not result in disease. Live vaccines are an obvious example. Oral polio vaccine is efficacious precisely because of a transient, asymptomatic intestinal infection. Vaccinia immunization requires viral growth to induce a robust immune response and, with the exception of rare side effects, causes primarily local lesions.

A working definition of infectious dose will vary as a function of the endpoint used in measuring infection. To encompass all possible degrees of infection, a less rigorous definition of infectious dose may be necessary. For practical purposes it might be defined as "a dose at which an organism can reproduce in the host and produce a measurable effect." This effect may not be limited to the display of symptoms,

Figure 1



but may also include postconvalescence antibody titers, development of cellular immunity, and the presence of nucleic acid incorporation. While these endpoints are measurable, it is with varying degrees of difficulty, requiring preinfection samples for comparison to identify the time of infection, and in some cases may not be obtained until postmortem examination (i.e., identification of prion protein in the brain).

The Host

Infectious Dose Measurement in Animals

Despite the seeming simplicity of infectious dose measurements, the pitfalls are many and complex. A good example of the difficulties involved in determining a unique ID_{50} can be found in the results of a study by Miller and Bohnhoff (1962). Various doses of Salmonella enteritidis were administered to outbred mice orally, subcutaneously, and intraperitoneally. Infection was defined as the presence of bacteria in the feces 3 weeks after administration. Some of the results are summarized in the figure below (Bohnhoff & Miller, 162).

These data summarize many of the difficulties with the infectious dose concept.

- First, the route of administration has a major effect on the ID_{50}. The ID_{50} of subcutaneous administration and oral administration differ by nearly four orders of magnitude. Similarly, S. enteritidis virulence is affected by the route of administration. In the experiments displayed above no more than 3% of the mice given oral S. enteritidis died. For experiments not shown in the graph, the authors report that intraperitoneal administration of 10^7 S. enteritidis led to 90% mortality.
- Second, there is huge animal-to-animal variability. In the right-hand curve, a small fraction of outbred mice became infected at $1x10^2$ organisms, while some animals were not infected at doses greater than $1x10^8$ cfu.
- Third, the slope for subcutaneous administration is steeper than for oral administration. This reflects a lower animal-to-animal variability by this route than among orally treated mice.
- Fourth, mice pretreated with streptomycin to remove the existing intestinal flora were five orders of magnitude more sensitive to oral challenge by S. enteritidis (labeled "Oral to Sterile Gut") than animals with populated intestines.
- Fifth, extrapolation to humans fails. In an analysis of an S. enteriditis outbreak involving contaminated ice cream, Vought and Tatini (1998) estimated the oral pathogenic dose to be no more than 28 organisms. The Health Canada MSDS for Salmonella species gives a human oral infectious dose of 10^2 - 10^3 cfu, three orders of magnitude below the ID_{50} for or mice (Health Canada, 2003). It appears humans are more sensitive to S. enteriditis than mice.

Table 1

***B. anthracis* Infectious Doses, Variability Among Host Species**		
	Parenteral LD_{50}	Inhalation LD_{50}
Guinea pig	<10 spores	$5 x10^4$ - $8.6x10^5$ spores (depending on particle size)
Cynomolgus monkey	Not given	$4.1x10^3$ spores
Rhesus monkey	$3x10^3$ spores	$5.3x10^4$ - $7.6x10^5$ spores (depending on particle size)
Mouse	5 spores	$1.4x10^4$ spores
Rat	10^6 spores	$2.6x10^4$ spores
Pig	10^9 spores	$2.7x10^7$ spores
Dog	$5x10^{10}$ spores	$1.8x10^7$ spores
Human	Not given	**NOT LD_{50}:** $6.0x10^2$ - $2.2x10^3$ spores.

Host Variability

Unlike the case where infection and immunity studies can be conducted with inbred strains of age- and sex-matched animals, such research can not be conducted in human populations due to ethical morays, as well as the logistical inability to find a genetically homogeneous population of subjects, small sample size, research expense, patient tracking, and complex secondary interactions (Salem & Gardner, 1994). Variables in the human population likely to alter infectious dose include sex, age, nutritional status, pregnancy, metabolic disorders, gastric acidity, gastric contents, gastric flora, immune competence, previous exposure to the agent, use of medications, immunization, health status (secondary infection), histocompatability markers, and their genetic makeup.

Bacillus anthracis infection data provide a good example of host variability. A large literature on LD_{50} for this pathogen has been summarized by Watson and Keir (1994). The LD_{50} for B. anthracis spores varies widely with species and route of exposure. Some data from the Watson and Keir compilation are given below.

Some of the variability in the inhalation B. anthracis LD_{50} value reflects variations in the particle size, number of spores in each particle, viability of the spores in the particle, the bacterial strains used, and the condition of the animals involved. There have been no systematic species-to-species comparisons.

There is wide variability in infectious doses among inbred strains of a single animal species. For instance, Scott, Williams, and Stephenson (1987) tested 42 mouse strains for their response to Coxiella burnetii (Q fever). They found the mice could be separated into three groups according to their sensitivity to this pathogen. For instance, an intraperitoneal dose of $5x10^9$ cfu killed 30% of C57BL/6J mice while 100% of A/J mice were killed by a dose two orders of magnitude less. The infectious dose for C. burnetii via the inhalation route in humans is said to be 10 organisms (Eitzen et al., 1998).

Results in occasional human studies relate poorly to results in other species. The S. enteriditis experience described earlier suggests infectious dose differences of more than three orders of magnitude between laboratory animals and humans. Human infectious dose experiments involving Crytosporidium parvum have been published (DuPont et al., 1995; Messner, Chappell, & Okhusen, 2001). In these and several other experiments these investigators found wide variation in the human infectious dose among three C. parvum strains; from ~2,000 oocytes for the UCP isolate, ~130 for IOWA to ~10 for TAMU. Even so, this was in stark contrast to the complete resistance of normal laboratory mice (O'Donoghue, 1995). Some mutant strains of mice are sensitive. Disease can be induced at doses of 10^6 to 10^7 in SCID (Severe Combined Immune Deficiency) mice (Mead et al., 1995). Knockout mice lacking γ-interferon are susceptible to as few as 10 oocytes (Griffiths et al., 1998).

The vastly different outcome of infection by Cercopithecine Herpes Virus 1 (Herpesvirus simiae) on macaques and humans is a dramatic example of species-to-species difference (Eberle & Hilliard, 1995). In macaques this virus is a chronic, nearly asymptomatic, infection, while in humans untreated infections are invariably fatal.

The Pathogen

Microorganism Variability

Variations in the organism affect its infectivity as well. These may include variation in gene expression, variation in bacterial cell surface due to preinfection environmental conditions, mutations affecting virulence, pH sensitivity, interactions with other organisms, its viability, and for airborne agents, droplet size and resistance to drying.

B. anthracis is a well-studied example of pathogen factors involved in infectious dose. Its virulence is primarily dependent on the products of two autonomous plasmids, pX01 and pX02. Coker et al. (2003) measured vaccinated guinea pig survival following intramuscular administration of 10,000 B. anthracis spores from 36 different natural isolates in which plasmid numbers per cell varied from 24 to 243 (pX01) and 1 to 32 (pX02). Survival at 14 days ranged from 0% to 94%. Survival was thought to be inversely related to the number of plasmids per cell. It appears that no single B. anthracis isolate can be thought of as having a characteristic ID for the species.

Extrapolation

For all but the most benign pathogens, direct tests in humans are ethically impossible. In fact, any human experiments involving pathogens require long and careful consideration by the investigator and the institutional ethics review board, as well as lengthy patient tracking and complex compliance with patient record keeping and disposition.

Unfortunately, reliable extrapolation of animal studies to humans has not been demonstrated in determining repeatable and unequivocal human infectious dose estimates. Animal studies are useful in qualitative studies that, for example, illustrate pathology caused by infection, or approximate protection factors afforded by vaccines in studies administered under strict challenge controls. Even in these instances care must be used in interpreting the results, as animal models are not an absolute physiologic replacement for humans. In many cases where a comparison can be made, infectious doses often differ by several orders of magnitude.

Sources of General Data on Infectious Dose

There is no comprehensive and critical listing of experimental infectious dose data. At present, two of the best sources for human infectious dose estimates are entries in the Health Canada MSDSs (Health Canada, 2003) for infectious agents and Medical Management of Biological Casualties (Eitzen et al., 1998). Unfortunately, these excellent references do not give direct source citations. Without knowing how this information was obtained, experimental conditions and sensitivity of assay, and whether it was extrapolated from animal studies, ABSA cannot verify or validate these values for use in regulatory guidelines. An additional source is the FDA publication, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, commonly called the "Bad Bug Book" that lists rough infectious dose estimates for 14 enteric disease organisms based on epidemiological investigations (Food and Drug Administration, 2003).

Conclusion

In summary, the studies described above support ABSA's position that attempts to develop quantitative values for human infectious dose are not currently feasible. Infectious dose values developed using past studies would not accurately characterize the relative hazard of pathogenic organisms in humans. The reasons for this conclusion are:

- Lack of a clear and universally acceptable definition of the term "infectious dose."
- There is no single standardized protocol for testing infectious dose in animals, making legitimate controlled comparisons of study results very difficult.
- Extrapolation of infection and toxicity data among animal species and from animals to humans has proven to be unreliable for most biological (and chemical) agents.
- Inbred animal strains are a poor surrogate for predicting human response, as humans are a highly variable outbred population.
- Infectious dose is affected by numerous, complex secondary interactions to include condition of the host, its genetics, and previous exposure to the biological agent or vaccine. Risk estimates must take these and many other factors into consideration.
- Bacteria of a single species can vary widely in virulence and infectious dose. It is not possible to make a broad or generalized statement about the infectious dose of a species of bacteria.
- Infectious dose in part depends on the route of exposure. A complete picture of a single pathogen's infectious dose profile requires inhalation, percutaneous, oral, im, ip, iv, etc. data. These data are currently unavailable.

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