Intraspecies Variability in the Dose-Response Relationship for \textit{Salmonella} Enteritidis Associated with Genetic Differences in Cellular Immune Response

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ABSTRACT

To evaluate the effects of differences in host cellular immunity, we studied the dose-response relationship for infection with \textit{Salmonella enterica} serovar Enteritidis (SE) in two different rat strains, skewed towards T helper 1 (Th1, Lewis rats) or T helper 2 (Th2, Brown Norway rats) immunoregulation. Rats were exposed orally to different doses of SE after overnight starvation and neutralization of gastric acid. Animals were observed for clinical signs of disease, fecal excretion and SE load in spleen and cecum, histopathology of the cecum, hematology, and cellular and humoral immune responses. Exponential dose-response models were used for binary or continuous outcomes to analyze the experimental data. Cytokine patterns, antibody isotypes, and contact hypersensitivity tests confirmed that Lewis rats are Th1 prone, whereas Brown Norway rats are Th2 prone. The probability of infection per single SE cell was approximately 100 times higher in Brown Norway rats than in Lewis rats. Cellular immune responses were more pronounced in Lewis rats but antibody responses were higher in Brown Norway rats. When infected, colonization levels and inflammation are highest in the intestinal tract of Th2 skewed rats, but systemic infection is more intense in Th1 skewed rats. Successful colonization by only one or two SE clones resulted in a marked increase of neutrophil counts by a factor of two to three in both rat strains.

Hazard characterization is one of the building blocks of microbiological risk assessment, as defined by the Codex Alimentarius Commission (1). Hazard characterization provides a description of the adverse effects that may result from ingestion of a pathogenic microorganism. When data are available, the hazard characterization should present quantitative information in terms of a dose-response relationship and the probability of adverse outcomes. Guidelines for hazard characterization have been published by the World Health Organization and the Food and Agricultural Organization of the United Nations (2). To construct dose-response models, the infectious disease process is usually considered a stepwise process involving a conditional series of events. Exposure to food- or waterborne pathogens may result in infection, defined as a condition in which the organism is able to establish itself in the host and actively multiply there. Methods to detect infection include but are not restricted to fecal excretion and seroconversion. Infection may be asymptomatic but may also lead to acute illness, typically gastroenteritis. Most patients recover eventually, but some may develop chronic sequelae or may die. Here, we concentrate mainly on the first stage of the infectious disease process, the relationship between the probability of infection and the ingested dose of pathogens in food or water.

Dose-infection models as recommended by the World Health Organization and the Food and Agricultural Organization are based on several assumptions: (i) the single hit hypothesis, i.e., each inoculated organism has a (possibly very small but nonzero) probability of causing infection no matter how low the dose, and each surviving organism grows to produce a clone of cells; (ii) the hypothesis of independent action, i.e., the mean probability per inoculated pathogen to cause a (symptomatic or fatal) infection is independent of the number of pathogen cells; (iii) microorganisms behave as discrete particles and cannot be divided into units smaller than one; and (iv) microorganisms are randomly distributed in the inoculum (this assumption is a mathematical convenience but is not necessary; models for nonrandom distributions have been described (6, 16)).

These assumptions lead to the family of single-hit models that are routinely used to describe microbial dose-response relationships. The best known models are the exponential model, the beta-Poisson model, and the hypergeometric model (16).

The outcome of exposure to pathogens is dependent on the ingested dose and is modulated strongly by the complex interactions among the pathogen, the host, and the food or water matrix. These interactions must be taken into account when analyzing available data sets or when extrapolating from available data to other exposure situations. In experi-
imental situations, such as volunteer experiments, the conditions are well controlled and are typically chosen to reflect a homogeneous situation. The statistical uncertainty in the resulting dose-response relationship is only descriptive of that single combination of pathogen, host, and food matrix and may not be reflective of real-world exposure. Observational data sets, such as those generated by outbreak investigations, typically reflect a heterogeneous situation with regard to host characteristics and pathogen strains, and food matrices vary among outbreaks. The challenge is to pool different data sets together. Various questions should be addressed. Are all exposed individuals in a particular outbreak similar with respect to susceptibility to infection and illness? Are different strains of one pathogenic species in different outbreaks similar with respect to virulence and pathogenicity? Was the effect of the food or water matrix similar in different outbreaks? Some information can be derived from statistical analysis, but arbitrary decisions are difficult to avoid. To overcome these difficulties, a broad set of experimental data reflecting different combinations of pathogen, host, and matrix would be desirable. Ethical, economic, and logistic considerations restrict extensive experimentation involving humans, making laboratory animals an interesting alternative.

Previously published dose-response studies in the Wistar Uniliver (WU) rat indicated that the severity of *Salmonella enterica* serovar Enteritidis (SE) intestinal and systemic infection and the specific immune responses (such as increased neutrophil counts in peripheral blood) and specific T-cell responses were highly dose dependent (7, 15). There is significant evidence that T cells, especially the Th helper (Th) cells, can be subdivided into at least two subtypes based on different functions and cytokine profiles, Th1 and Th2 cells (10, 11). The subsets of Th cells do not derive from distinct lineages but rather develop from the same Th cell precursor under the influence of environmental and genetic factors acting at the level of antigen presentation. Among these factors, the early presence of interleukin (IL)–4 is the most potent stimulus for Th2 differentiation, whereas IL-12 and interferon-gamma (IFN-γ) favor Th1 development (4). Th cells recognize antigens using their specific antigen receptor (T-cell receptor) in the context of major histocompatibility complex class II molecules on antigen-presenting cells such as macrophages. Th1 cells release especially IL-2, IFN-γ, and tumor necrosis factor α but not IL-4, IL-5, and IL-13, whereas Th2 cells release in majority IL-4, IL-5, IL-6, IL-10, and IL-13 but no IL-2 and IFN-γ. Another probably better endpoint readout parameter for Th1- versus Th2-mediated immune responses might be the detection of Th1- and Th2-associated immunoglobulin (Ig) isotopes, although animal species differ with respect to this readout system. For the rat, IgG2b and IgG2c are Th1-related isotopes, whereas IgG1 and IgG2a are Th2 related (3).

The processes such as phagocytosis and cytotoxicity that are crucial for effective resistance to intracellular pathogens such as mycobacteria, *Listeria*, and *Salmonella* species are highly dependent upon IFN-γ, which is produced especially by Th1 cells and natural killer cells. Th1 cells also seem to be crucial for contact-type hypersensitivity and delayed-type hypersensitivity and some forms of autoimmune diseases. In contrast, Th2 cells are especially involved in classical types of IgE-associated allergies and asthma and seem to be involved in the immune response against intercellular pathogens such as parasites and some fungi. The ultimate immunological effect depends partly on the balance between the different subsets of Th cells (Th1 versus Th2). Under physiological conditions, cytokines probably only transiently shift the balance along the Th1-Th2 axis, without permanently fixing the Th phenotype (13). The extreme situation of a fixed Th1 or Th2 phenotype is seen only in severe pathological conditions. However, the ratio of these Th cell subsets determines whether the immune system is able to respond appropriately to specific stimuli from pathogens such as SE.

Different studies in animals and humans have confirmed the crucial role for Th1-mediated immune responses in resistance to *Salmonella* infection. Pashine et al. (12) indicated that Th1 cells are a crucial part of the immune response to live *Salmonella* Typhimurium in mice. For this process, bacterial invasiveness but not persistence was required. De Jong et al. (5) demonstrated that IL-12–deficient patients suffer from very severe *Salmonella* infections. IL-12 is a crucial cytokine produced by antigen-presenting cells and macrophages leading to the stimulation of IFN-γ production and Th1-dominated immune responses.

A series of experiments were conducted in which rats with a different immunological genotype regarding Th1 and or Th2 skewing were evaluated for microbiological, hematological, immunological, and pathological changes following oral exposure to different doses of SE. Sensitivity analyses for many different kinds of immunomodulating agents revealed that Lewis (Le) rats were Th1 prone and Brown Norway (BN) rats were Th2 prone (14). We hypothesized that the Th1 skewed animals are less susceptible to SE infection. We also attempted to quantify the shift in the dose-response relationship.

**MATERIALS AND METHODS**

**Experimental design.** The experimental design was similar to that used in our previously published work (7). Specific-pathogen-free male Le and BN rats were obtained from the breeding colony at Harlan CPB (Zeist, The Netherlands). WU rats were obtained from the breeding colony of the National Institute of Public Health and the Environment (Bilthoven, The Netherlands). The breeding colonies were prescreened and monitored for endogenous pathogenic viruses and bacteria and were found negative. Animals 6 to 9 weeks of age were housed individually in macron cages. Drinking water and conventional diet (RMH-B, Hope Farms BV, Woerden, The Netherlands) were provided ad libitum. After 1 to 2 weeks of rest, the animals were starved overnight (water ad libitum). SE 97-198, a patient isolate (origin RIVM) and *Escherichia coli* W5G, a nalidixic acid–resistant derivative of *E. coli* C (8), were used to prepare inoculum cultures suspended in 3% (wt/vol) NaHCO₃ as previously described (7). After 16 h of starvation, 1 ml of a bacterial suspension was orally administered to the animals by gavage. Directly after gavage (day 0), food and water were provided ad libitum. Each dose group consisted of four animals of each rat strain, and the average dose...
per animal was $3.0 \times 10^2$, $5.2 \times 10^4$, and $4.6 \times 10^6$ for SE 97-198 and $6.4 \times 10^5$ for E. coli WG5.

Blood samples were taken via orbita plexus puncture using a capillary tube under light ether anesthesia 10 to 14 days before and 5 days after oral inoculation just before administration of the delayed type hypersensitivity (DTH) challenge.

Daily clinical observations were made to monitor the general health of the animals. Feces were macroscopically evaluated, and samples were tested for bacterial counts the same day. The animals were sacrificed on day 6 after oral inoculation by exsanguination from the abdominal aorta under anesthesia. The cecum and spleen were removed aseptically. The cecum was divided into two parts for microbiology and pathology, respectively.

**Contact type hypersensitivity and DTH.** Picryl chloride (Chemotronix, Swannanoa, N.C.), used as contact sensitizer, was recrystallized three times from methanol-H$_2$O before use and protected from light during storage at 4°C. Animals were sensitized by application of 250 $\mu$L of 5% picryl chloride solution in ethanol-acetone (3:1) to the shaved abdomen and four feet. Control rats were sham-sensitized by topical application of 250 $\mu$L of ethanol-acetone (3:1). Four days after sensitization, both ears of the rats were challenged by topical application of one drop (27-ga needle) of 0.8% picryl chloride in olive oil. Prior to and 24 h after challenge, duplicate measurements of ear thickness were made using an engineer’s micrometer (Mitutoyo 193-10, Veenendaal, The Netherlands). The rats were anesthetized by intramuscular injection of 0.8% ketalar (Parke Davis, Madrid, Spain), 3 ml of rompun (20 mg/ml; OPG, Utrecht, The Netherlands). The negative control of each ELISA contained samples of serum from a pool of noninfected animals and serum samples obtained prior to the infection from each individual animal. The 96-well plates were coated with LPS SE (Sigma, Zwijndrecht, The Netherlands) overnight at room temperature. The coated plates were blocked with blocking solution of 0.01 M phosphate-buffered saline (PBS) containing 0.5% coffee creamer (Koninklijke Buisman B.V., Zwartsluis, The Netherlands) on a shaker for 1 h at room temperature. Serum sample dilutions including dilutions of the hyperimmune serum batch and negative controls were incubated for 1 h at room temperature. After standard washing procedures, the plates were incubated with antibodies against IgG2a, IgG2b, IgG1, and IgM (Sigma; 1:1,000 diluted in 0.01 M PBS) overnight at room temperature. The coated plates were blocked with blocking solution of 0.01 M phosphate-buffered saline (PBS) containing 0.5% coffee creamer (Koninklijke Buisman B.V., Zwartsluis, The Netherlands) on a shaker for 1 h at room temperature. Serum sample dilutions including dilutions of the hyperimmune serum batch and negative controls were incubated for 1 h at room temperature. After standard washing procedures, the plates were incubated with antibodies against IgG2a, IgG2b, IgG1, and IgM (Sigma; 1:1,000 diluted in 0.01 M PBS + 0.05% Tween). Incubation was performed at room temperature for 1 h with continuous shaking. Plates were then incubated with rat anti-mouse IgG PO (1:1,000) for 1 h at room temperature with TMB (3,3′,5,5′-tetra methyl benzidine) as substrate. After 10 min, the reaction was stopped with 1 M H$_2$SO$_4$. The absorbance was read at 450 nm.

**Cytokine analyses.** For cytokine analyses, spleen cell cultures were prepared. The culture medium used was Iscove’s modified Dulbecco’s medium (Gibco, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (PAA, Linz, Austria), 100 $\mu$g/ml streptomycin, and 100 IU/ml penicillin. Cell suspensions were made by pressing the spleens through a stainless steel screen. Connective tissue was removed from the suspensions in a fiberglass column. Cells were counted using a Coulter counter (Coulter Electronics, Luton, UK). The suspensions were cultured at 10$^7$ cells per ml of culture medium with concanavalin A for T-cell activation (3.3 $\mu$g/ml) or heat-killed SE (10$^8$ bacterial cells per ml) for approximately 24 h. After culture, the cells were pel-
cessed for SE and WG5, as previously described (7).

Microbiology. Fecal samples and internal organs where assessed for SE and WG5, as previously described (7).

Pathology. Immediately after exsanguination, the abdomen and thorax were inspected and the cecum was removed. Half of the cecum was fixed as a Swiss roll (9) in 3.8% (wt/vol) phosphate-buffered formaldehyde, trimmed, and embedded in paraplast. Sections (4 to 5 μm thick) were prepared (three sections per animal) and routinely stained with hematoxylin and eosin. Sections were scored on a scale of 0 to 5 for mucosal infiltration and erosion without knowledge of the treatment: 0 = negative, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, and 5 = severe. For a final score per animal, the average of two sections was taken.

Dose-response modeling. We employed the exponential dose-response model for the analyses of the infection data as previously described (7). The frequency at which SE was isolated from spleen and cecum samples is related to the average dose D by

\[ P_{\text{inf}} = 1 - e^{-rD} \]

where \( r \) is the probability of infection per inoculated SE cell.

For continuous responses such as neutrophil counts, we used the extended exponential models as described by Takumi et al. (15). The neutrophil count \( X \) is related to the average dose \( D \) by

\[ X = P_{\text{inf}}YZ \cdot \tanh(rD/c) + (1 - P_{\text{inf}})Y \]

\[ Y = \text{lognormal}(\mu, \sigma) \]

where \( Y \) (mean \( \mu \), SD \( \sigma \), both on an ln scale) describes the baseline neutrophil counts and \( Z \) (mean \( \rho \), SD \( \sigma \), both on ln scale) describes the proportion by which the neutrophil count increases from the baseline due to multiplication of a clone, resulting from a single surviving SE cell. The function \( \tanh(rD/c) \) implements the neutrophil response to saturate with increasing dose. We interpret the parameter \( c/r \) as the critical dose at which the immune response saturates to the maximum level and the parameter \( c \) to be the number of surviving SE cells that initiated infection at this critical dose. The parameters of the dose-response models were estimated by the maximum likelihood method.

RESULTS

Le rats are Th1 prone and BN rats are Th2 prone. As an in vivo model for cellular immune responses in which Th1-mediated immune responses play a crucial role, contact hypersensitivity to picryl chloride was tested. Le rats were better responders to picryl chloride–induced con-
The effects in BN rats were more pronounced (Table 1). Inflammation of the cecum was present in all SE dose groups in BN rats but only in the highest dose group in Le rats. The severity of the lesions in BN rats was highly dose dependent (Fig. 3).

Microbiology: BN rats excrete higher numbers of SE than do Le rats. Exposure to SE resulted in a sharp increase in fecal SE counts, followed by a transient decrease and subsequent increase (Fig. 4). Doses of $<10^4$ CFU did not result in detectable fecal excretion in Le rats. In contrast, all inoculation doses did lead to fecal excretion in BN rats. The fecal excretion in BN rats was significantly higher than that in Le rats at all doses tested.

The results of microbiological examination of the cecum indicated much higher SE loads in BN rats than in Le rats (data not shown). Even the lowest dose tested did lead to cecum colonization in some BN rats. Systemic infection (i.e., colonization of the spleen) occurred at lower doses in BN rats than in Le rats, but when systemic infection did occur, the SE load in Le rats was higher than that in BN rats (Fig. 5A).

Probability of infection: Lewis rats are more resistant to SE invasion. We fitted the exponential dose-response model to the spleen data (Fig. 5B). Estimates for the parameter $r$ were much less than 1, indicating that only a small fraction of the inoculum successfully initiated infection. We estimate that on average 5 in 1,000 SE cells initiated systemic infection in BN rats (Table 2). The success ratio was even lower in Le rats; on average only 4 in 100,000 SE cells initiated systemic infection in this rat strain. Thus, Le rats are roughly 100 times more resistant to SE infection than are BN rats. In fecal and cecum samples, interfering natural flora was present, and we cannot eliminate the possibility that low SE counts were not detected. For this reason, we did not perform further mathematical analysis of the cecum data.

DTH reaction to SE antigen in the skin is dose dependent in both rat strains. Injection of heat-killed SE antigens induced ear swelling in all animals (Fig. 6). Animals previously exposed orally to SE had significantly more swelling than did control rats exposed to E. coli WG5. The degree of swelling clearly increased with dose. The background response in the Th1-prone Le rats was much higher than that in the Th2-prone BN rats. For this reason, further mathematical analysis was not performed (Fig. 6).

Serum antibodies: production of IgG is dose dependent and the dominant isotype differs between rat strains. Specific IgG2a and IgG2b concentrations increased in a dose-dependent manner in both strains of rats (Fig. 7).

### TABLE 2. Dose-response parameter $r$ for SE colonization of the spleen in three rat strains

<table>
<thead>
<tr>
<th></th>
<th>Brown Norway</th>
<th>Wistar Unilever</th>
<th>Lewis</th>
</tr>
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<tbody>
<tr>
<td>$r$ (CFU$^{-1}$)</td>
<td>$4.6 \times 10^{-3}$</td>
<td>$1.2 \times 10^{-3}$</td>
<td>$3.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>90% CI</td>
<td>$1.7 \times 10^{-3}, 1.3 \times 10^{-3}$</td>
<td>$4.1 \times 10^{-4}, 2.6 \times 10^{-3}$</td>
<td>$1.5 \times 10^{-5}, 9.3 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

* From (14).
In Le rats, Th1-associated IgG2b concentrations were higher than Th2-associated IgG2a concentrations, and in BN rats the reverse was true (IgG2a concentrations higher than IgG2b concentrations). Specific IgA, IgG1, and IgM concentrations were apparently below detection levels in both rat strains tested.

**Hematology:** Le rats are less susceptible than BN rats, as indicated by neutrophil counts. In both Le and BN rats, oral exposure to SE resulted in significant, reproducible, and dose-dependent changes in several hematological parameters, as previously reported for WU rats (7). The most pronounced effect was the increase in absolute and relative numbers of neutrophils (Fig. 8). The lowest SE dose tested already induced a neutrophil increment in the blood in some animals. One Le rat given a dose of 300 CFU had a high neutrophil count, but the spleen data indicated that this rat was not systemically infected. Higher SE doses did lead to higher neutrophil counts in a dose-dependent fashion.

We fitted the extended dose-response model to the neutrophil data of both rat strains and estimated its parameters (Table 3). Le rats have higher baseline neutrophil counts than do BN rats. Based on the estimates for the parameter \( r \), we estimate that on average 2 of 1,000 SE cells initiated a neutrophil response in BN rats, whereas only 3 of 100,000 SE cells initiated a neutrophil response in Le rats. Thus, consistent with the spleen (SE load) data, BN rats are roughly 100 times more susceptible to SE infection than are Le rats. In both strains, the estimates for the parameter \( c \) are very low, which indicates that the first few SE cells

![Figure 6](http://meridian.allenpress.com/jfp/article-pdf/67/9/2008/1675258/0362-028x-67_9_2008.pdf)

**FIGURE 6.** DTH (percentage increase in ear thickness) to SE antigen 24 h after ear challenge. Triangles (top) represent BN rats, and circles (bottom) represent Le rats. Ear thickness before challenge was 0.43 mm for BN rats and 0.45 mm for Le rats.

![Figure 7](http://meridian.allenpress.com/jfp/article-pdf/67/9/2008/1675258/0362-028x-67_9_2008.pdf)

**FIGURE 7.** IgG isotypes in serum of Le and BN rats in relation to the oral dose of SE. Triangles represent BN rats, and circles represent Le rats. Closed symbols represent IgG2b, and open symbols represent IgG2a. Values are mean (\( n = 5 \)) and SD. AU, absorbance units (490 nm).

![Figure 8](http://meridian.allenpress.com/jfp/article-pdf/67/9/2008/1675258/0362-028x-67_9_2008.pdf)

**FIGURE 8.** Neutrophil counts in blood (open symbols, preinfection; closed symbols, 5 days postinfection). Triangles represent BN rats, and circles represent Le rats. The solid line is the best-fitting dose-response model, and the dashed lines represent the 95% confidence intervals.
TABLE 3. Dose-response parameters for neutrophil response in three different rat strains

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Brown Norway</th>
<th>Wistar Unilever</th>
<th>Lewis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$ (CFU$^{-1}$)</td>
<td>$2.0 \times 10^{-3}$ ($6.4 \times 10^{-4}$–$6.0 \times 10^{-3}$)</td>
<td>$3.4 \times 10^{-4}$ ($1.0 \times 10^{-4}$–$6.8 \times 10^{-4}$)</td>
<td>$2.9 \times 10^{-5}$ ($1.3 \times 10^{-5}$–$6.4 \times 10^{-5}$)</td>
</tr>
<tr>
<td>$\mu$ (cell/µl)</td>
<td>$5.6 \times 10^{2}$ ($4.8 \times 10^{2}$–$6.4 \times 10^{2}$)</td>
<td>$6.1 \times 10^{2}$–$7.3 \times 10^{2}$</td>
<td>$9.9 \times 10^{2}$ ($8.6 \times 10^{2}$–$1.2 \times 10^{3}$)</td>
</tr>
<tr>
<td>$\nu$</td>
<td>1.6 (1.4–1.8)</td>
<td>1.7 (1.5–1.9)</td>
<td>1.5 (1.3–1.7)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>3.8 (1.2–45)</td>
<td>1.4 (1.1–6.8)</td>
<td>3.5 (1.5–13)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>1.0 (0.79–1.4)</td>
<td>1.0 (1.0–2.0)</td>
<td>1.0 (0.74–1.5)</td>
</tr>
<tr>
<td>$c$ (CFU)</td>
<td>1.2 (0.36–4.3)</td>
<td>6 (1–16)</td>
<td>1.1 (0.51–2.7)</td>
</tr>
</tbody>
</table>

$^{a}$ Values are means with standard deviations (SD) in parentheses.  
$^{b}$ Adopted from (14).  
$^{c}$ Relative counts in the original publication converted to absolute counts.  
$^{d}$ SD in the original publication converted to geometric SD.

initiating infection were sufficient to trigger the majority of the neutrophil response.

**DISCUSSION**

The dose-response relationship for exposure to SE by intragastric gavage of adult male Le and BN rats seemed to be reproducible and in part comparable to the previously reported response in WU rats (7, 15). Overall, the BN rats were very sensitive to infection, even more sensitive than WU rats, and the Le rats were considerably less susceptible. WU rats seemed to be intermediate, both in Th expression and in sensitivity to infection. This difference in sensitivity could be attributed to the fact that Le rats are Th1 prone and BN rats Th2 prone. However, host factors other than Th1 and or Th2 skewing might be involved in the sensitivity to SE infection in the different rats strains tested. More detailed studies with isogenic mutants are necessary to further characterize the effect of differential Th expression on the dose-response relationship.

In vivo experiments in laboratory animals offer a powerful tool for evaluating the effects of variability in the pathogen-host-matrix triangle on the dose-response relationship for food- and waterborne pathogens. However, these experiments also pose a new challenge: how to extrapolate results from animals to humans. There are several possible approaches.

**Direct extrapolation.** This approach assumes that the dose-response relationship in animals is the same as that in humans. This approach is the same as that used in toxicology experiments, where safety factors are used to account for intra- and interspecies variability. However, the results of the present study indicate an intraspecies variability of at least a factor of 100 for SE in rats, which is considerably more than the factor of 10 typically assumed for chemicals. Furthermore, the interaction between pathogens and host tissues is usually more specific than that for chemicals, which warns against the use of universal safety factors. For example, the (exponential) dose-response parameter of Shiga-toxin producing *E. coli* in rabbits is $6 \times 10^{-8}$ but was $9 \times 10^{-3}$ for children in an outbreak in Japan involving a school lunch (17).

**Scaling factors.** The exponential model is scalable, i.e., a change in the dose-response parameter $r$ can be compensated by a proportional change in the dose. This property can be used for extrapolation in the pathogen-host-matrix triangle, assuming that relative changes between different situations are the same for animals and humans. For example, one could argue that the difference between Le and BN rats observed in this study predicts that the probability of infection by SE in allergic humans is 100 times higher than that in nonallergic persons. Although the beta-Poisson model is also scalable (the parameter $\beta$ is a scale parameter), the situation is more complex because heterogeneity in the host-pathogen interaction must be taken into account. The hypergeometric model is not scalable.

**Parallelogram.** The parallelogram approach is used in immunotoxicology to improve extrapolations from animals to humans by incorporating the results of an in vitro test that is considered relevant for the disease process being evaluated (18). For example, in our animal model we suspended the inoculum in a sodium bicarbonate solution to prevent inactivation by stomach acid. If inactivation in the human stomach were known or could be modeled for some exposure situation, this information could be used to modify the ingested dose.

**Dynamic models.** The interaction between pathogens and their host is complex, nonlinear, and dynamic. Any extrapolation using simple factors is likely to be of limited value. Dynamic models explicitly include some of these complexities and may offer a less biased approach to extrapolating results from animals to humans. Such models will be complex and have not yet been published for intestinal pathogens. A recent description of a dynamic model for tuberculosis demonstrates that this approach is feasible (19).

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