

## MINIREVIEW

# How To Become a Top Model: Impact of Animal Experimentation on Human *Salmonella* Disease Research<sup>∇</sup>

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***Salmonella* serotypes are a major cause of human morbidity and mortality worldwide. Over the past decades, a series of animal models have been developed to advance vaccine development, provide insights into immunity to infection, and study the pathogenesis of human *Salmonella* disease. The successive introduction of new animal models, each suited to interrogate previously neglected aspects of *Salmonella* disease, has ushered in important conceptual advances that continue to have a strong and sustained influence on the ideas driving research on *Salmonella* serotypes. This article reviews important milestones in the use of animal models to study human *Salmonella* disease and identify research needs to guide future work.**

### THE GLOBAL BURDEN OF *SALMONELLA* INFECTIONS

Most nontyphoidal *Salmonella* (NTS) serotypes are associated with gastroenteritis in immunocompetent individuals, in which the infection remains localized to the terminal ileum, colon, and mesenteric lymph node. Nontyphoidal *Salmonella* gastroenteritis is characterized by a short incubation period, averaging less than 1 day (26), followed by the development of diarrhea, fever, and intestinal inflammatory infiltrates that are dominated by neutrophils (102). The zoonotic pathogens *Salmonella enterica* serotype Typhimurium (*S. Typhimurium*) and *S. enterica* serotype Enteritidis (*S. Enteritidis*) are most frequently associated with this diarrheal disease (63). Although viral agents are generally a more common cause of diarrhea than bacterial agents, nontyphoidal *Salmonella* serotypes stand out by producing a more severe infection, which is associated with considerable mortality rates. As a result, nontyphoidal *Salmonella* serotypes are the leading cause of death from foodborne illness in the United States (48, 73). A recent estimate puts the global burden of NTS gastroenteritis at 93.8 million cases, resulting in 155,000 deaths annually (46).

In immunocompromised individuals, nontyphoidal *Salmonella* serotypes are associated with invasive disease, manifesting as a bloodstream infection known as NTS bacteremia. Individuals with NTS bacteremia present with fever, but symptoms of gastroenteritis are commonly absent (12, 18, 28). Thus, mortality from NTS bacteremia has to be considered separately from deaths attributed to NTS gastroenteritis. NTS bacteremia is a leading cause of hospital admission and death in sub-Saharan Africa (27). The serotypes most commonly associated with this bloodstream infection are *S. Typhimurium* and *S. Enteritidis*. A meta-analysis of 22 reports revealed that non-

typhoidal *Salmonella* serotypes (18.7% of isolates) are second only to *Streptococcus pneumoniae* (23.3% of isolates) in causing bloodstream infections in Africa (67). NTS bacteremia is associated with different risk factors in adults and children. Risk factors for NTS bacteremia in African children include malnutrition, anemia, severe malaria, and human immunodeficiency virus (HIV) infection (10). These risk factors produce an estimated incidence of 175 to 388 child NTS bacteremia cases per 100,000 persons per year (5, 20, 75). In contrast, HIV infection is the predominant risk factor for NTS bacteremia in African adults (29) and is responsible for approximately 2,000 to 8,500 NTS bacteremia cases per 100,000 persons per year in HIV-positive cohorts (25, 89, 91). NTS bloodstream infections are associated with mortality rates in excess of 20% in pediatric and adult populations despite antibiotic therapy (2, 5, 10, 29, 30).

*Salmonella enterica* serotype Typhi (*S. Typhi*) is a strictly human-adapted pathogen associated with an invasive infection in immunocompetent individuals known as typhoid fever. Typhoid fever has an average incubation period of 2 weeks (59), which is followed by the development of nonspecific symptoms, commonly including fever and a slowed heart rate (bradycardia) (54). Unlike gastroenteritis, typhoid fever is not considered a diarrheal disease, because this symptom develops only in approximately one-third of patients. Pathological changes in the intestine are characterized by inflammatory infiltrates that are dominated by mononuclear cells, while neutrophils are scarce (38, 53, 55, 79). The pathogen persists in histiocytic granulomas, known as typhoid nodules, in internal organs, most frequently the bone marrow, the liver, and the spleen (54). Persistence in the gallbladder converts a small fraction (approximately 4%) of patients into chronic carriers, termed “typhoid Marys,” who can transmit the disease for the remainder of their lives. There are an estimated 16 million to 21.6 million cases of typhoid fever each year, with the highest incidence being reported from Southeast Asia. Typhoid fever causes considerable mortality worldwide, with estimates ranging from 216,510 to 600,000 deaths annually (6, 50).

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<sup>∇</sup> Published ahead of print on 22 February 2011.

TABLE 1. Animal models of human *Salmonella* disease

Model	Advantages	Limitations	Reference(s)
Mouse typhoid	Natural infection; availability of host genetics and immunological reagents; model for fecal-oral transmission; low cost	Not suited to study gastroenteritis; <i>S. Typhimurium</i> does not cause typhoid fever in humans	40, 87
Mouse colitis	Availability of host genetics and immunological reagents; suited to study of intestinal inflammation; low cost	Requires disruption of the microbiota by pretreatment with antibiotics; colitis is accompanied by systemic infection	4
Humanized mouse Guinea pig	Provides a model to study <i>S. Typhi</i> Suited to study intestinal inflammation	Requires extensive manipulation Requires preconditioning through starvation and opium treatment; limited availability of host genetics and immunological reagents	42, 78 84
Calf gastroenteritis	Natural infection that closely resembles human gastroenteritis	Limited availability of genetics and immunological reagents; requires specialized animal facilities	86
Rhesus macaque	Natural infection that closely resembles human gastroenteritis	Limited availability of genetics; requires specialized animal facilities; high cost	36

In summary, the cumulative global death toll from NTS gastroenteritis, NTS bacteremia, and typhoid fever is considerable and highlights the need for research on vaccine development, immunity, and the pathogenesis of these diseases. The increasing prevalence of multidrug-resistant *S. Typhi* isolates that are fully fluoroquinolone resistant and the emergence of multidrug-resistant NTS serotypes with resistance to ciprofloxacin and expanded-spectrum cephalosporins threaten to limit treatment options (60). These data illustrate the need to develop new intervention strategies, which will require the use of adequate animal models. This article reviews how the use of animal models of infection (Table 1) has ushered in important conceptual advances that have improved our understanding of diseases caused by this important group of human pathogens.

**THE OPIUM-TREATED GUINEA PIG MODEL**

Intestinal lesions during gastroenteritis have historically been attributed to toxin production by bacteria. In the sixties, Samuel Formal and coworkers challenged this dogma by demonstrating that bacterial invasion of epithelial cells is a major factor in determining the pathogenicity of *Shigella flexneri* in guinea pigs preconditioned by treatment with opium (39). This work motivated Akio Takeuchi to perform electron microscopic studies of epithelial invasion by *S. Typhimurium* in guinea pigs preconditioned by starvation and treatment with opium (84). In this model, invasion of epithelial cells by *S. Typhimurium* was accompanied by a local degeneration of microvilli and the formation of membrane extrusions (Fig. 1). It took more than two decades for a genetic locus required for epithelial invasion by *S. Typhimurium* to be identified by Jorge Galán and Roy Curtiss III (24). Epithelial invasion by *S. Typhimurium* is mediated by a type III secretion system (T3SS-1) encoded by *Salmonella* pathogenicity island 1 (SPI-1) (51). The main function of T3SS-1 is to translocate proteins, termed effectors, into the host cell cytosol, where they trigger rearrangements in the actin cytoskeleton (23, 104). Stanley Falkow and coworkers demonstrated that these actin cytoskeletal rearrangements result in bacterial uptake through macropinocytosis, a process characterized by the formation of membrane

extrusions, termed ruffles (22), which resemble those originally described by Akio Takeuchi.

**THE MOUSE TYPHOID MODELS**

In 1892, Friedrich Loeffler isolated a pathogen associated with an epidemic, typhoid fever-like disease in mice, which was termed *Bacillus typhimurium* (now *S. Typhimurium*) (44). Mice infected with *S. Typhimurium* do not develop gastroenteritis but instead contract a systemic disease characterized by bacterial multiplication in the liver and spleen, which results in hepatomegaly and splenomegaly (87). The small intestinal pa-

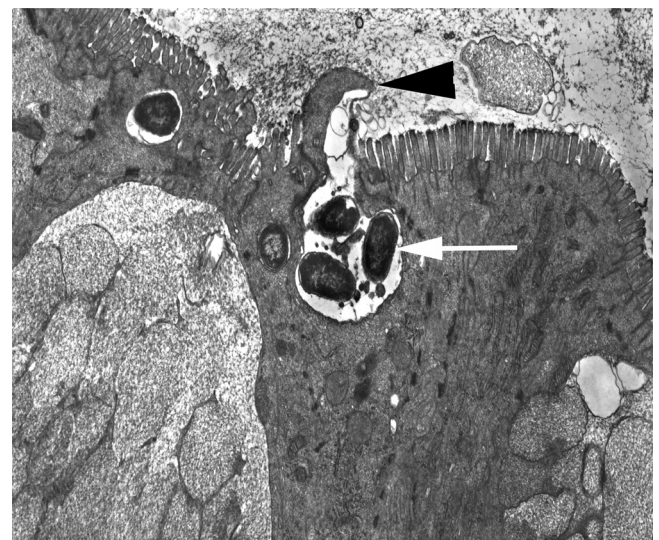


FIG. 1. *S. Typhimurium* invades the intestinal epithelium. A transmission electron micrograph of the ileal mucosa of a calf experimentally infected with *S. Typhimurium* is shown. Local degeneration of microvilli and formation of membrane extrusions at the apical surface (arrowhead) of an enterocyte are associated with internalization of bacteria (arrow). Akio Takeuchi recorded similar images in his pioneering studies of epithelial invasion by *S. Typhimurium* in the guinea pig (84).

thology is characterized by a predominantly mononuclear leukocyte infiltrate, with follicular hyperplasia, capillary thrombosis, hemorrhage, and ulcerations observed at areas of Peyer's patches in moribund animals (72). Some mouse lineages (e.g., C57BL/6 mice and BALB/c mice) are genetically susceptible to lethal *S. Typhimurium* infection due to a point mutation in the *Slc11a1* gene that results in expression of a nonfunctional ferrous iron transporter, termed natural resistance-associated macrophage protein, in the phagosomal membranes of macrophages (7). *S. Typhimurium* also disseminates to the liver and spleen of genetically resistant mouse lineages (e.g., CBA mice and 129sv mice), which possess an intact *Slc11a1* allele. However, genetically resistant mice typically control bacterial multiplication at systemic sites, thereby preventing the development of severe signs of disease.

The use of genetically susceptible animals in the mouse typhoid model was instrumental during the 1980s and early 1990s in establishing a number of new concepts, which had a sustained and powerful impact on the fields of *Salmonella* vaccine development, immunity, and pathogenesis research. For example, the discovery by Bruce Stocker and coworkers that a *S. Typhimurium aroA* mutant is attenuated and immunogenic in mice (35) led to the development of new live-attenuated *S. Typhi* vaccines for humans (82, 83). Pietro Mastroeni and coworkers used the mouse typhoid model to demonstrate that protective immunity to *S. Typhimurium* infection requires both antibodies and CD4<sup>+</sup> T cells (47), a principle that still guides current efforts in vaccine development (1). Paul Gulig and Roy Curtiss III identified the *Salmonella* plasmid virulence (*spv*) operon during an *in vivo* screening for DNA regions that rescue virulence of a plasmid-cured *S. Typhimurium* strain in the mouse typhoid model (31). Finally, Michael Hensel, David Holden, and coworkers identified the second type III secretion system (T3SS-2) of *S. Typhimurium* while using the mouse typhoid model to devise a novel *in vivo* screening method, termed signature-tagged mutagenesis, for the identification of virulence genes (34). Subsequent work by the laboratory of Eduardo Groisman demonstrated that T3SS-2 enables *S. Typhimurium* to survive in host macrophages (58), a property previously shown by Fred Heffron and coworkers to be essential for virulence in the mouse typhoid model (21).

During the 1990s and the first decade of the 21st century, the mouse typhoid model continued to have a marked influence on the methods driving research on bacterial pathogenesis in general. For instance, John Mekalanos and coworkers developed a genetic *in vivo* expression technology (IVET) to identify bacterial genes specifically induced in tissue (77). The laboratories of Dirk Bumann and Brad Cookson used the mouse typhoid model to work out green fluorescent protein (GFP)-based approaches to follow bacterial gene expression during infection using flow cytometry (13, 17, 68). The mouse typhoid model remains an important platform for developing novel screening approaches for the *in vivo* identification of virulence genes, such as the array-based analysis of cistrons under selection (ABACUS) recently described by Helene Andrews-Polymenis, Michael McClelland, and coworkers (70). Finally, the laboratory of Ferric Fang led the way in combining knockout mice and bacterial genetics to investigate how virulence factors overcome host defenses in the mouse typhoid model (45, 90),

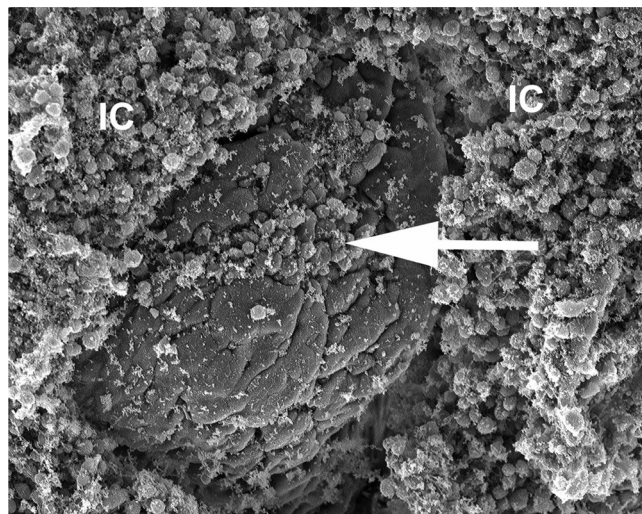


FIG. 2. Neutrophils migrate into the intestinal lumen in response to *S. Typhimurium* infection. A scanning electron micrograph of the ileal mucosa of a calf experimentally infected with *S. Typhimurium* is shown. The image shows epithelial erosion at the tip of an intestinal villus (arrow) and marked accumulation of inflammatory cells (IC) on the luminal surface of the intestinal mucosa.

and this “genetics-squared” approach is now widely used in innate immunity and bacterial pathogenesis research (61).

In addition, the innovative use of the mouse typhoid model continues to provide important insights into the pathogenesis of infections caused by *Salmonella* serotypes. For example, the laboratories of Denise Monack and Corrella Detweiler pioneered the use of genetically resistant mouse lineages to elucidate mechanisms of chronic carriage in tissue (11, 41, 52, 56, 76). Genetically resistant mouse lineages were utilized to study mechanisms of intestinal persistence (19, 37, 93) and to develop an animal model for *S. Typhimurium* transmission (40). Finally, the laboratories of John Gunn and Brett Finlay demonstrated that the mouse typhoid model provides an opportunity to investigate colonization of the gallbladder (16, 49), a reservoir important for transmission of typhoid fever.

One limitation of the mouse typhoid model is that *S. Typhimurium* causes gastroenteritis rather than typhoid fever in humans. Since the mouse typhoid model is not suited to study the pathogenesis of NTS gastroenteritis, this disease manifestation remained largely unexplored until alternative animal models became more widely used in the late 1990s and the first decade of the 21st century.

#### THE CALF GASTROENTERITIS MODEL

*S. Typhimurium* is a natural cause of gastroenteritis in calves, and infection is associated with signs of disease that parallel the symptoms observed in humans. The most severe pathological lesions accompanying this localized infection are restricted to the intestinal mucosa and mesenteric lymph nodes (86, 100). Animals develop intestinal inflammation characterized by a severe diffuse infiltrate composed predominantly of neutrophils (86, 100). Neutrophil recruitment is associated with necrosis of the upper mucosa (57) and migration of neutrophils into the intestinal lumen (Fig. 2). In severe cases,

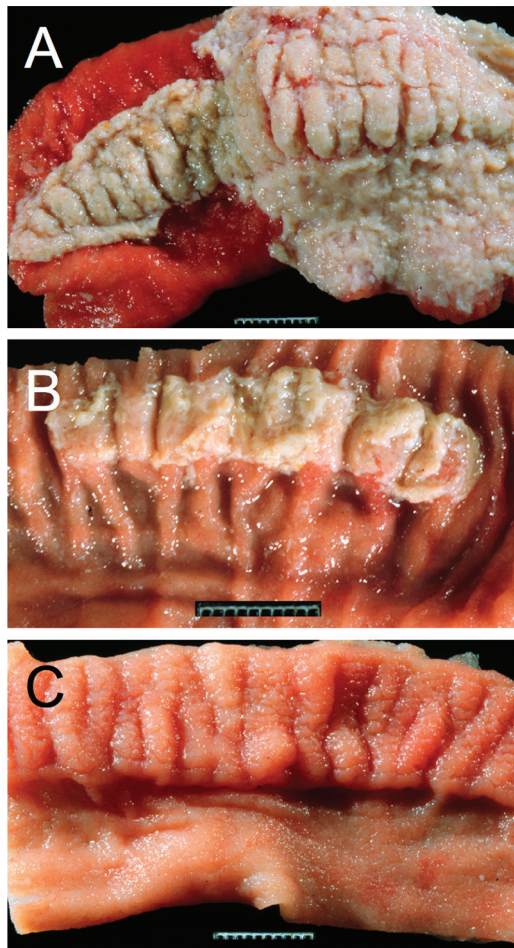


FIG. 3. T3SS-1 and T3SS-2 contribute to intestinal inflammation in the calf gastroenteritis model. (A to C) Gross pathological appearance of the luminal surface of the terminal ileum collected 2 days after oral infection of calves with the *S. Typhimurium* wild-type (A), a T3SS-2-deficient mutant (B), or a T3SS-1-deficient mutant (C). Compared to the severe lesions caused by infection with the wild type (severe acute fibrinopurulent necrotizing enteritis with segmental or continuous pseudomembrane formation [A]), intestinal inflammation is reduced in calves infected with a T3SS-2-deficient mutant (moderate to marked subacute fibrinopurulent enteritis often confined to Peyer's patches [B]) and absent in calves infected with a T3SS-1-deficient mutant (normal Peyer's patch and ileal mucosa [C]). (Reprinted from reference 86.)

necrosis of the upper mucosa leads to formation of a pseudomembrane, a gross pathological change observed in the terminal ileum and the cranial portions of the colon (Fig. 3) (86).

Analysis of *S. Typhimurium* mutants in the calf model in the late 1990s provided important first insights into the role virulence factors play during gastroenteritis, which exposed some parallels to, but also some differences from, their respective roles in the mouse typhoid model. Oral-challenge studies revealed that T3SS-1 is essential for the ability of *S. Typhimurium* to cause intestinal inflammation and diarrhea (86, 92, 103), while inactivation of T3SS-2 reduces the severity of intestinal lesions in calves (86) (Fig. 3). In contrast to its important role during systemic infection of mice, the *spv* operon

plays only a small role during the localized infection caused by *S. Typhimurium* in the bovine host (86).

The development of a bovine ligated-ileal loop model provided the opportunity to study the development of intestinal inflammation at defined early time points after *S. Typhimurium* infection (71, 74, 101, 103). The use of this model revealed that flagella contribute to neutrophil recruitment in the intestinal mucosa during *S. Typhimurium* infection (74). The role of flagella in eliciting inflammatory responses is in part indirect, by promoting bacterial invasion, and in part direct, by serving as a pathogen-associated molecular pattern (PAMP) that stimulates innate pathways of inflammation (97). Importantly, a role for flagella in virulence had not been apparent from work in the mouse typhoid model (43, 74).

Limitations of the calf model include the scarcity of reagents available to manipulate the host and limited availability of animal facilities to perform the research. Research on *S. Typhimurium*-induced gastroenteritis therefore experienced a second boost when a mouse colitis model gained popularity in the first decade of the 21st century.

### THE MOUSE COLITIS MODEL

Work performed by Marjorie Bohnhoff and coworkers in the 1950s and 1960s established that mice preconditioned by treatment with streptomycin exhibit a greatly increased susceptibility to oral *S. Enteritidis* and *S. Typhimurium* infections (8, 9). Subsequently, streptomycin pretreatment was shown to be associated with enhanced growth of *S. Typhimurium* in the murine cecum (62). In 2003, these initial observations were followed up by Wolf-Dietrich Hardt and coworkers, who demonstrated that *S. Typhimurium* infection of streptomycin-pretreated mice results in the development of intestinal inflammatory infiltrates that are dominated by neutrophils (4). The most severe pathological lesions in the intestines of streptomycin-pretreated mice are restricted to the cecum (Fig. 4). Streptomycin-pretreated mice can thus be used to model the orchestration and the consequences of intestinal neutrophil recruitment during *S. Typhimurium*-induced gastroenteritis (mouse colitis model).

The initial use of the mouse colitis model confirmed observations from the calf model that flagella, T3SS-1, and T3SS-2 contribute to intestinal inflammation during *S. Typhimurium* infection (4, 15, 80). A subsequent important conceptual advance was the finding that intestinal inflammation enables *S. Typhimurium* to outgrow the resident intestinal microbiota in the intestinal lumen (3, 81). Recently, the mouse colitis model was used to demonstrate that inflammation promotes a luminal outgrowth of *S. Typhimurium*, because the respiratory burst of neutrophils, which transmigrate into the intestinal lumen (Fig. 2), oxidizes an endogenous sulfur compound, thio-sulfate, to generate tetrathionate (98). Tetrathionate serves as a respiratory electron acceptor for *S. Typhimurium* (33), thereby empowering the pathogen to use respiration to outgrow the resident microbiota, which rely on fermentation for energy production during growth in the anaerobic environment of the gut (98). Finally, luminal outgrowth of *S. Typhimurium* was shown by Denise Monack and coworkers to enhance transmission of the pathogen (40).

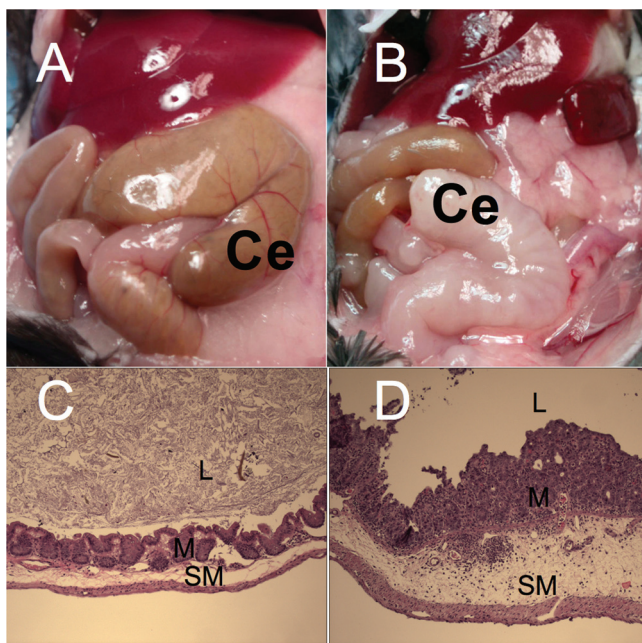


FIG. 4. Inflammation of the cecum in the mouse colitis model. (A) Normal appearance of the cecum (Ce) in a mock-infected control animal. (B) Shrunken and edematous cecum (Ce) from a *S. Typhimurium*-infected mouse. (C and D) Histopathological appearance of the murine cecum of a mock-infected mouse (C) or a mouse infected with the *S. Typhimurium* wild type (D) 72 h after infection. Note that *S. Typhimurium* infection (D) is associated with severe diffuse neutrophil infiltrate in the mucosa (M) and submucosa (SM) and severe edema in the submucosa. L, lumen. (The images in panels C and D were reprinted from reference 32.)

Collectively, these new insights connect previous observations to provide a coherent picture of *S. Typhimurium*-induced gastroenteritis: *S. Typhimurium* uses its virulence factors to trigger inflammation by using flagella and T3SS-1 to invade the intestinal epithelium, followed by T3SS-2-mediated survival in tissue macrophages. The ensuing inflammatory response generates a new respiratory electron acceptor that supports outgrowth of *S. Typhimurium* in the gut lumen, thereby promoting its transmission by the fecal-oral route.

One caveat of the mouse colitis model is that development of acute cecal inflammation requires the use of an antibiotic to disrupt the microbiota. Furthermore, *S. Typhimurium* disseminates systemically in both genetically resistant and genetically susceptible mouse lineages (Fig. 5), thus making it difficult to study mucosal barrier functions in the mouse colitis model.

#### COINFECTION MODELS

While recent years have seen significant advances in our understanding of NTS gastroenteritis, the pathogenesis of NTS bacteremia remains understudied. The relative paucity of studies on this topic is compounded by the emergence of NTS bacteremia as a leading cause of death in sub-Saharan Africa (27, 67). Risk factors for NTS bacteremia include an underlying malaria infection or HIV disease. Initial studies suggest that animal models are well suited to interrogate the pathogenesis of these coinfections.

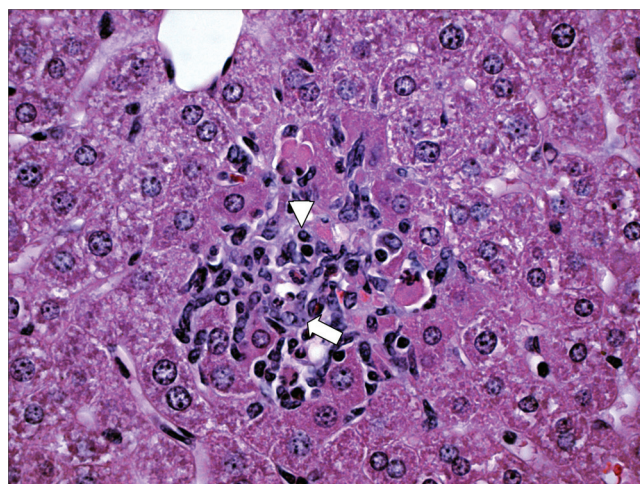


FIG. 5. *S. Typhimurium* disseminates systemically in the mouse colitis model. A microgranuloma in the liver of a streptomycin-pre-treated mouse infected with *S. Typhimurium* is shown. The image shows focal accumulation of lymphocytes (arrowhead) and epithelioid macrophages (arrow) in a section of the liver.

To study the underlying susceptibility of children with severe malaria to NTS bacteremia, a mouse coinfection model was developed in resistant CBA mice, using the rodent malaria parasite *Plasmodium yoelii* subsp. *nigeriensis* and *S. Typhimurium* (69). Using this model, the investigators demonstrated that increased systemic loads of *S. Typhimurium* during coinfection were caused by both hemolytic anemia and malaria parasite-mediated immunosuppression. A reduction in circulating interleukin-12 (IL-12) found in the coinfection model suggests that impaired inflammatory responses in patients with severe malaria may be one factor that predisposes them to NTS bacteremia.

Ligated ileal loops in rhesus macaques infected with simian immunodeficiency virus (SIV) and *S. Typhimurium* have recently been used to model NTS bacteremia in HIV-infected individuals (66) (Fig. 6). Initial characterization of this model revealed that SIV-mediated depletion in the ileal mucosa of IL-17-producing CD4<sup>+</sup> T cells (Th17 cells) selectively blunted expression of IL-17, IL-22, IL-26, CCL-20, and lipocalin-2 in response to *S. Typhimurium* infection and was associated with increased bacterial dissemination to the mesenteric lymph nodes. Furthermore, IL-17 deficiency resulted in increased systemic dissemination of *S. Typhimurium* in the mouse colitis model. These data suggest that Th17 depletion is one factor that predisposes HIV-infected individuals to NTS bacteremia.

#### ADAPTATION OF MODELS TO STUDY VIRULENCE OF HUMAN-ADAPTED *S. TYPHI*

Although the mouse typhoid model has been used extensively to study *S. Typhimurium* infection, the pathogenesis of *S. Typhi* infections remains poorly understood. This is in part due to a limitation of the mouse typhoid model, namely, the fact that *S. Typhimurium* causes gastroenteritis rather than typhoid fever in humans. In other words, some of the genes that enable *S. Typhi* to cause typhoid fever in humans are not present in *S. Typhimurium*. The second reason *S. Typhi* pathogenesis is

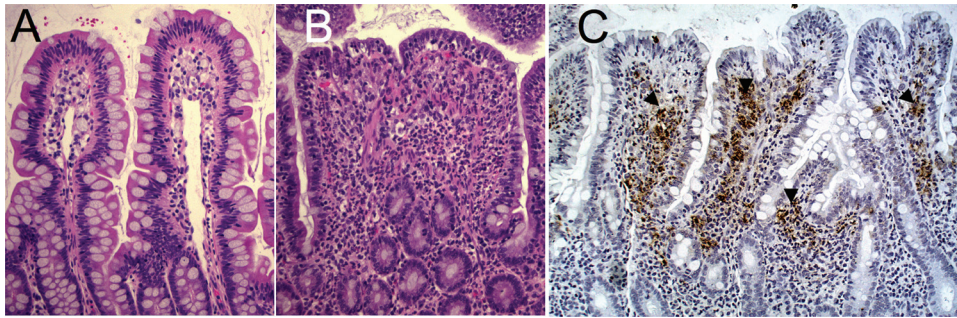


FIG. 6. The rhesus macaque (*Macaca mulatta*) model. (A) Image of the uninfected ileal mucosa. (B) Section of the intestinal mucosa 8 h after infection of a ligated ileal loop with *S. Typhimurium*. Note the blunting of intestinal villi and the marked infiltration of inflammatory cells, predominately neutrophils. (C) Immunohistochemical labeling (brown precipitate) of *S. Typhimurium* in the intestinal mucosa of a rhesus macaque (*Macaca mulatta*) 8 h after inoculation of a ligated ileal loop with *S. Typhimurium*. Note the marked immunolabeling (arrowheads) of *S. Typhimurium* in the lamina propria, which illustrates the invasive nature of the infection.

understudied is the fact that this pathogen is strictly human adapted, which has long prevented the use of animal models to study its virulence *in vivo*. The recent utilization of innovative new approaches to overcome these limitations holds promise for improving our understanding of *S. Typhi* pathogenesis.

One approach to study *S. Typhi*-specific virulence factors *in vivo* is their introduction into the mouse-pathogenic *S. Typhimurium*. This approach revealed that introduction of the *S. Typhi*-specific *viaB* locus into *S. Typhimurium* attenuates inflammation and neutrophil recruitment in bovine ligated ileal loops (65) and the mouse colitis model (32) by preventing complement receptor 3 (CR3)-mediated clearance (95) and reducing Toll-like receptor 4 (TLR4) stimulation (94). These stealth properties of the virulence (Vi) capsule-encoding *viaB* locus might help to explain the long incubation period of typhoid fever and the scarcity of neutrophils in intestinal infiltrates in typhoid fever patients (64, 88). Separate studies showed that the TviA regulatory protein encoded within the *viaB* locus rapidly represses flagellum expression and induces Vi capsule expression when the pathogen enters intestinal tissue (85, 99). The TviA-mediated repression of flagellum expression during the transition through the intestinal epithelium might help *S. Typhi* to evade detection of flagellin by the innate immune system through TLR5- or IL-1 $\beta$ -converting enzyme-protease activating factor (IPAF) (96, 97). Collectively, these data suggest that repression of PAMPs during entry into tissue enables *S. Typhi* to evade detection by the innate immune surveillance system through a stealth strategy, which might promote bacterial dissemination (64, 88). Consistent with this idea, introduction of *tviA* into the *S. Typhimurium* genome increases bacterial dissemination to internal organs in a chick model of infection (99). Since *S. Typhimurium* infection causes little clinical disease in chickens, this species is not discussed here as a model for human disease and the interested reader is referred to a recent review article on this subject (14). In summary, studies of *S. Typhi*-specific virulence factors in animal models are starting to reveal mechanistic differences in host-pathogen interactions that occur during a localized infection (*S. Typhimurium*-induced gastroenteritis) and a systemic infection (typhoid fever).

A second approach for studying the strictly human-adapted *S. Typhi* is the development of humanized *in vivo* models.

Ferric Fang and coworkers recently pioneered the use of a humanized mouse model to study *S. Typhi* pathogenesis (42). Nonobese diabetic immunodeficient (*scid*) mice that lack the  $\gamma$  chain of the IL-2 receptor were engrafted with human hematopoietic stem cells (hu-SRC-SCID mice) and were shown to support growth of *S. Typhi* after intraperitoneal infection. Similar results were reported by Jorge Galán's group, who used immunodeficient (*Rag2*) mice lacking the IL-2 receptor  $\gamma$  chain engrafted with human fetal-liver hematopoietic stem and progenitor cells (78). The initial characterization of these models demonstrated that humanized mice can be used for the *in vivo* characterization of *S. Typhi* mutants (42, 78). Further use of humanized mice promises to provide rare direct insights into *S. Typhi* pathogenesis *in vivo*.

## CONCLUSIONS

Animal models have been instrumental in establishing a number of conceptual advances in our understanding of human *Salmonella* disease. These advances were made possible by approaches that exploited the unique strengths of each animal model. However, each animal model also has shortcomings that limit its usefulness for studying certain disease manifestations associated with *Salmonella* serotypes. Current limitations of animal models have contributed to the relative paucity of knowledge about NTS bacteremia, a leading cause of death in sub-Saharan Africa. Furthermore, the pathogenesis of the strictly human-adapted pathogen *S. Typhi* remains understudied. Innovative new approaches and models to help fill these key gaps in knowledge are promising developments that should guide future research efforts. Before these new animal models can become widely used, a number of additional limitations, such as high cost, the unavailability of adequate animal facilities, and difficulty in manipulating the host, need to be overcome. Due to these restrictions, the development of future top models will likely continue to rely predominantly on mice.

## ACKNOWLEDGMENTS

We thank Melita Gordon, Jennie Musto, and John Crump for insightful discussions on the global epidemiology of *Salmonella* serotypes and Sebastian E. Winter for helpful comments to improve the manuscript.

Work in A.J.B.'s laboratory is supported by Public Health Service grants AI040124, AI044170, AI073120, AI076246, and AI088122.

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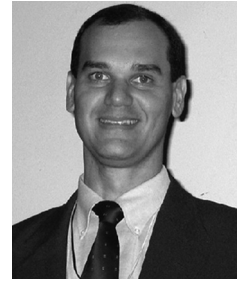
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