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A rabbit model of non-typhoidal *Salmonella* bacteremia

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ABSTRACT

Bacteremia is an important cause of morbidity and mortality in humans. In this study, we focused on the development of an animal model of bacteremia induced by non-typhoidal *Salmonella*. New Zealand White rabbits were inoculated with a human isolate of non-typhoidal *Salmonella* strain CVD J73 via the intra-peritoneal route. Blood samples were collected at specific time points and at euthanasia from infected rabbits. Additionally, tissue samples from the heart, lungs, spleen, gastrointestinal tract, liver and kidneys were obtained at euthanasia. All experimentally infected rabbits displayed clinical signs of disease (fever, dehydration, weight loss and lethargy). Tissues collected at necropsy from the animals exhibited histopathological changes indicative of bacteremia. Non-typhoidal *Salmonella* bacteria were detected in the blood and tissue samples of infected rabbits by microbiological culture and real-time PCR assays. The development of this animal model of bacteremia could prove to be a useful tool for studying how non-typhoidal *Salmonella* infections disseminate and spread in humans.

1. Introduction

Bacteremia and sepsis constitute important sources of morbidity and mortality in humans. Several bacteria including non-typhoidal *Salmonellae* (NTS) are important causes of infections and have shown to result in disseminated infections, even in modestly compromised hosts. Such infections cause significant mortality in humans. Non-typhoidal strains of *Salmonella enterica* (*S. enterica*) serotypes Enteritidis or Typhimurium have been reported to be major causes of such illnesses [1–7]. In a study from Spain of 172 cases of *Salmonella* bacteremia (70% Enteritidis and 17% Typhimurium), 16% of patients developed septic metastases, and 16% died [8]. In the 1990s, in

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Massachusetts General Hospital, 18% mortality was observed in 45 patients affected with bacteremia induced by NTS [9].

S. enterica serotype Enteritidis is a facultative intracellular pathogen that is a frequently isolated serotype in the United States and accounts for nearly 15% of human cases of salmonellosis. An infection with *S. enterica* generally causes a localized intestinal infection. However, *S. enterica* serotypes can spread systemically in the elderly, in young children and in immunocompromised individuals causing serious conditions such as septicemia and septic shock [10,11]. *S. enterica* serotypes Typhimurium and Enteritidis have been reported to account for 79–95% of all bacteremic NTS infections in sub-Saharan Africa [12–14]. Reports from the United Kingdom suggest that infection with drug resistant serotype Typhimurium may result in greater morbidity and mortality than infection with other serotypes, further complicating the burden of *Salmonella*-associated bacteremia, and illness [15]. Since the pathogenesis of *Salmonella*-induced sepsis cannot be studied in humans due to ethical reasons, development of an animal model, which mimics such disease, is critical. An animal model could provide important insights into how NTS infections disseminate and spread in mammalian hosts.

A central goal of all sepsis models is to reproduce clinically relevant pathogenesis that is similar to disease seen in humans. We focused on the development of an animal model of *Salmonella* induced bacteremia. A murine enteric fever model has previously been developed to evaluate *Salmonella* pathogenicity. Collins and Carter [16] have shown that irradiated germ-free mice developed diarrhea when they were orally challenged with non-typhoidal *S. enterica* and died within 5–8 days of challenge. However, these animals may not be representative of conventional animals as these mice were germ free and were irradiated during the course of the study. Furthermore, previous *Salmonellae* infection studies in mice have reported varying results regarding spread of the organisms, as well as of induction of bactermia in infected animals [17–22]. These studies postulated different mechanisms related to the absorption and spread of bacteria in infected mice (elaborated in Section 4). The mouse model has other limitations; including an insufficient volume of blood available to detect bacteria, important for studies of pathogenesis as well as diagnostic assay development. Since detection of low-level bacteremia in humans requires large blood volume samples for evaluation, we decided to explore a rabbit model to study *Salmonella* associated bacteremia. The rabbit animal model enabled evaluation of a larger volume of blood, which is required to translate information gained from animal studies to human studies.

Previous studies have shown that rabbits developed illness comparable to human salmonellosis [23]. We performed initial pilot studies in 2 New Zealand White (NZW) rabbits in which animals were inoculated with human clinical isolates of 10^{11} colony forming units per milliliter (CFU/ml) of non-typhoidal *S. enterica* bacteria via the orogastric route following previously reported oral inoculation techniques [23]. Although rabbits in this initial pilot study shed *Salmonella* in their feces, we could not detect bacteria by blood culture (data not shown). Additionally, none of the animals showed any clinical signs of disease (fever, diarrhea, dysentery, dehydration, lethargy and weight loss). Similar studies by others produced very low frequency [23] or no bacteremia [21,22] during the first few days of infection with oral inoculations.

We conducted another pilot study in 2 NZW rabbits where animals were inoculated with non-typhoidal *S. enterica* bacteria (10^{11} CFU/ml) via a different route (intraperitoneal – i.p.). Although rabbits in this study developed fever and weight loss, they did not display bacteremia (data not shown). We accordingly conducted another i.p. infection study with a higher dose of bacteria (10^{13} CFU/ml) in 2 NZW rabbits. Both rabbits developed fever, weight loss, diarrhea as well as bactermia (data not shown).

Herein we report the successful development of the NZW rabbit as an animal model of *S. enterica*, in which bacteremia was induced, producing pathology. This model adds to the current body of knowledge regarding NTS animal models, and could prove to be a useful tool for studying how NTS infections disseminate and spread in humans. The rabbit model may further help in developing improved and novel rapid diagnostic assays used for detecting low levels of bacteria in the blood of infected individuals.

2. Materials and methods

2.1. Animals

Specific pathogen free NZW rabbits (*Oryctolagus cuniculus*) were purchased from an approved vendor (Covance Research Products, Denver, PA). Rabbits used in this study were free of bacteria including pathogenic *Escherichia coli*, *Clostridium difficile*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Bordetella bronchoseptica*, *Treponema cuniculi*, *Clostridium piliforme*, cilia-associated respiratory bacillus, *Salmonella* spp., ectoparasites, and fungi. The study was conducted at the AAALAC-accredited animal facility overseen by the Program of Comparative Medicine at the University of Maryland School of Medicine. Animals underwent a 48-h acclimatization period at the animal facility before being included in this study. Ten female adult NZW rabbits (3.5–5 kg) were utilized in this study. Fecal samples from all rabbits were pre-screened and tested negative for presence of *Salmonella* spp. prior to study initiation. Additionally, these rabbits were observed for signs of diarrheal illness (diarrhea/dysentery) and were found to be free of such clinical signs. Animals were single-housed in cages maintained in an animal biosafety level 2 containment facility (ABSL-2), and appropriate measures were taken to ensure safe handling practices while working with the animals and bacteria. All animal procedures were carried out under ABSL-2 practices. All procedures were reviewed and approved by the Institutional Care and Use Committee and the Institutional Biosafety Committee of the University of Maryland School of Medicine. These protocols followed strict guidelines of the *Guide for the Care and Use of Laboratory Animals* [24] and the *Biosafety in Microbiological and Biomedical Laboratories* [25].