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Phenotypic behavior of 35 *Salmonella enterica* serovars compared to epidemiological and genomic data

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Abstract

The behavior of 35 different *Salmonella enterica* serovars was investigated in an *in vitro* gastro-intestinal tract (GIT) system. Virulence was expressed as the probability of infection, P(inf), i.e. fraction of the ON-culture invading into Caco-2 cells after GIT passage. Results show that the (average) P(inf) of *Salmonella* serovars ranges from 1.7 10^{-8} (*S.*Kedougou) to 5.3 10^{-5} (*S.*Typhimurium). In general, the P(inf) corresponds to available epidemiological and virulotypic data from literature. Still, individual exceptions exist and it is hypothesized that the public health risk from *Salmonella* is associated with exposure (prevalence, dose and/or acquired immunity) rather than difference in virulence.

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Keywords: Salmonella; phenotype, genotype, food safety risk

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1. Introduction

Despite a declining trend, *Salmonella* is with 82,694 confirmed human cases in 2013 still the second most important zoonosis in the EU. Moreover, *Salmonella* caused the largest number, 1170 (which is 22.5%), of reported food-borne outbreaks. The top three most frequent serovars in 2013 were Enteritidis, Typhimurium and monophasic Typhimurium, EFSA [3]. The Netherlands reported 992 laboratory confirmed cases of salmonellosis corresponding to an estimated number of 28,000 cases in the general population of 16.8 million people, Zomer et al. [17].

The Dutch food and consumer product safety authority (NVWA) monitors the presence of *Salmonella* according to EU-regulation 2073, 2005: Absence of *S.* Enteritidis and *S.* Typhimurium in 25g fresh chicken meat and absence of *S.* spp. in meat preparations (25g, or 10g in some cases). The NVWA investigates food products in their monitoring program, which on several occasions reveal serovars that differ from those most frequently found in human reported cases. For example, *S.*Heidelberg in broilers and chicken products, *S.* Derby in pork, *S.* Mbandaka in tahin, Zomer et al. [17], and *S.* Montevideo in Oaktree lettuce, Wijnands et al. [16]. In addition, infrequently found serovars have recently occurred in outbreaks, e.g. *S.* Thompson in salmon, Friesema et al. [4], and *S.* Heidelberg in a spaghetti meal, Rijckevorsel et al. [13]. The occurrence of *Salmonella* serovars in food products and outbreaks that differ from those regularly found in laboratory confirmed human cases raises questions about the current *Salmonella* regulation in relation to the potential hazard of these infrequently found serovars.

In this study we investigate the phenotypic behavior of 35 different *Salmonella* serovars in an *in vitro* gastro-intestinal tract (GIT) system as proxy for virulence. The results give insight in the virulence of infrequently found serovars relative to the more frequently found laboratory confirmed serovars from human cases in The Netherlands. In addition, the phenotypic *in vitro* GIT results are put into perspective of molecular virulence properties of these serovars previously described in literature. Knowledge about the relative phenotypic virulence of different *Salmonella* serovars in relation to epidemiological and genomic data may provide guidance to policy makers for future regulation strategies.

2. Materials and methods

Table 1 shows the 35 different *Salmonella* serovars and their source selected for the *in vitro* gastrointestinal passage experiments. All strains were obtained from the National Institute for Public Health and the Environment (RIVM) in The Netherlands. The RIVM obtains these strains from different institutes, companies and laboratories within The Netherlands for (sero)typing.

2.1. Gastro-intestinal passage

The gastro-intestinal passage system was prepared following Oliveira et al. [10]. In short, 1 ml overnight (ON) culture of *Salmonella* was mixed with 9 ml simulated gastric fluid (SGF). Samples were incubated during 30 min at 37 °C, after which 1ml SGF-mixture was taken for microbiological analysis. From the remaining SGF-sample, 4 ml was mixed with 40 ml simulated intestinal fluid (SIF) and incubated during 2 hours at 37 °C under microaerophilic conditions and shaking at 100 rpm. After incubation, the samples were used for attachment and invasion assays with fully differentiated Caco-2 cells. These mimic the small intestinal epithelium, Pinto et al. [12]. The plates were inoculated with 40 µl SIF-mixture per well, and incubated at 37 °C in a humidified atmosphere of 96% air and 5% CO2 during 1 hour for the attachment assay. After aspiration of the medium, the cells were washed three times with sterile PBS and either lysed with 1% Triton in PBS, or treated with ECM supplemented with 0.3% gentamycin (50 mg/mL) for the invasion assay. The Triton-lysate was used for determining the number of attached *Salmonella*. For the invasion assay, the plates were incubated during 3 hours at 37 °C in the humidified atmosphere as mentioned before. After incubation, the cells were treated with PBS and 1% Triton in PBS as described before. The lysate was used for enumeration of the invaded *Salmonella*.

The point estimate for the fraction of invaded *Salmonella* was calculated by dividing the expected number of *Salmonella* (ml^{-1}) after the invasion assay by the expected number of cells in the ON-culture (ml^{-1}) following Pielaat et al. [11]. The resulting fraction was used as proxy for the virulence and expressed as the probability of infection,