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MINIREVIEW

Salmonella Serogroup C: Current Status of Vaccines and Why They Are Needed

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Nontyphoidal *Salmonella* (NTS; i.e., *Salmonella enterica* organisms that do not cause typhoid or paratyphoid) are responsible for 94 million infections and 155,000 deaths worldwide annually, 86% of which are estimated to be foodborne. Although more than 50 serogroups and 2,600 serovars have been described, not all *Salmonella* serovars cause disease in humans and animals. Efforts are being made to develop NTS vaccines, with most approaches eliciting protection against serovars Typhimurium and Enteritidis (serogroups B [O:4] and D [O:9], respectively), as they are widely considered the most prevalent. Here, we show that serogroup C (O:6,7, O:6,8, or O:8 epitopes) is the most common serogroup in the United States, and the prevalence of serovars from this serogroup has been increasing in Europe and the United States over the last decade. They are also the most commonly isolated serovars from healthy cattle and poultry, indicating the underlying importance of surveillance in animals. Four out of the 10 most lethal serovars in the United States are serogroup C, and reports from African countries suggest that strains within this serogroup are highly antibiotic resistant. Serogroup C consists of highly diverse organisms among which 37 serovars account for the majority of human cases, compared to 17 and 11 serovars for serogroups B and D, respectively. Despite these concerning data, no human vaccines targeting serogroup C NTS are available, and animal vaccines are in limited use. Here, we describe the underestimated burden represented by serogroup C NTS, as well as a discussion of vaccines that target these pathogens.

Salmonella enterica is a facultative intracellular pathogen responsible for a high burden of mortality and morbidity worldwide. The species *Salmonella enterica* contains six subspecies, with 99.5% of all isolated strains belonging to *S. enterica* subspecies *enterica* (also known as subspecies I). Further classification into serogroups relies on differences in the surface O antigens, of which individual serovars are distinguished by additional typing of the flagellar H antigen and biochemical tests (1). While almost 2,600 serovars and more than 50 serogroups have been described so far, only a few of these cause disease in humans and animals. Human host-restricted *S. enterica* serovars Typhi, Paratyphi A, and Paratyphi B cause typhoid and paratyphoid enteric fevers (2). These systemic diseases represent an annual estimated burden of 27 million cases and more than 200,000 deaths worldwide, with sub-Saharan Africa and Asia accounting for 46% and 32% of typhoid fever cases, respectively (3). Other *Salmonella* serovars have a broader host range and mainly cause gastroenteritis in animals and humans; they are referred to as nontyphoidal *Salmonella* (NTS). NTS infections cause an estimated 94 million cases and 155,000 deaths worldwide each year (2).

In 2013, diarrheal diseases were the second leading cause of loss of disability-adjusted life-years (DALYs) among communicable diseases (the leading cause was lower respiratory infections) (4). Approximately 80 million (86%) of human NTS infections worldwide are estimated to be foodborne (5). Moreover, multiple outbreaks related to contact with infected domestic or wild animals have been reported (6, 7). In 2010, the World Health Organization (WHO) estimated that nontyphoidal *Salmonella* was the leading cause of foodborne deaths worldwide (8). NTS thus represents a major public health concern, especially with the increasing number of antibiotic-resistant isolates being reported (9, 10). There are currently three vaccines licensed for use in humans, all targeting typhoidal *Salmonella*: the live attenuated oral vaccine *S.* Typhi Ty21a, Vi capsule polysaccharide vaccine, and Vi polysaccharide conjugated with tetanus toxoid (11–14). Despite extensive efforts, no human vaccine targeting NTS has yet been licensed. NTS vaccine developers are mainly targeting serovar Typhimurium (serogroup B, carrying the O:4 antigen) and/or serovar Enteritidis (serogroup D, carrying the O:9 antigen). Although these serovars are some of the most prevalent NTS, other serovars, particularly those belonging to serogroup C, represent underevaluated health and economic burdens for both humans and animals. Serogroup C *Salmonella* serovars are further subdivided into groups C1 (presence of O:6,7 epitopes, i.e., both O:6 and O:7 epitopes present) and C2 (presence of O:6,8 or O:8 epitopes). One serogroup C1 *Salmonella* serovar, Paratyphi C, can also express the Vi capsule. This review provides an overview of the burden and clinical syndromes produced by serogroup C NTS, and it describes the existing vaccine strategies against these serovars. In particular, we have analyzed raw serogroup/serovar distribution data published by U.S. and European public health laboratories, with a focus on *Salmonella* serogroup C infections.

GROUP C NTS DISEASE BURDEN

The highest burden of NTS infections (both gastroenteritis and bloodstream infections) has been estimated by the WHO to occur in sub-Saharan Africa (193 to 338 DALYs per 100,000 population), while developed regions such as North America and Europe

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TABLE 1 Worldwide serogroup distribution of NTS isolated from humans

Region	Country	% of isolates belonging to serogroup (epitope[s]): B (O:4)	C (O:7, O:8)	D (O:9)	Other	Yr(s) covered	Source or reference
Africa		30.9	19.5	44.6	5	2014	WHO GFN
	Ethiopia	16.9	28.6	47.9[a]	3	1982–2012	19
	Kenya	57.5	9.3	33.2	0	2002–2004	20
	Tunisia	10.8	45.7	24.1	14.4	1994–2004	21
Asia		26.2	27.3	45.4	1.1	2009	WHO GFN
	Taiwan	39	23	29	9	2004–2012	22
	China	56.9	6.0	14.6	21.0	2007–2012	23
Middle East	Saudi Arabia	19.6	33.5	34.2	12.6	2008–2011	24
Europe		32.7	8.4	42	16.9	2012	26
North America	United States	20.5	25.7	16.5	17.8	2012	25

[a] Reported data include information for *S.* Typhi as well as NTS isolates.

have a lower prevalence (50 to 67 DALYs per 100,000 population) (8). Invasive NTS are a leading cause of morbidity and mortality in sub-Saharan Africa, with 388 cases per 100,000 children aged 3 to 5 years, 7,500 cases per 100,000 HIV-infected adults, and mortality rates between 10 and 30% (15, 16). Although currently not considered a major concern in Asia, recent reports suggest an increase in the number of NTS infections in that region. The decrease in the *S.* Typhi prevalence in Vietnam observed between 1994 and 2008 was concomitant with an increase in NTS infections (17). Up to 7% of diarrheal infections in Vietnamese children have been attributed to NTS (18). However, the serovar distribution worldwide varies, leading to a difference in the most common serogroups (Table 1). In Europe in 2012, 42% of cases were serogroup D (almost exclusively attributed to *S.* Enteritidis), followed by serogroups B (32.7%) and C (8.4%). In the United States in 2012, 25.7% of all reported cases of salmonellosis were caused by serogroup C isolates, followed by serogroup B (20.5% of all cases). Serogroup D accounted for only 16.5% of all reported cases. Global trends over time have also varied between the United States (Fig. 1A) and Europe (Fig. 1B). In Europe, the prevalence of serogroup D has been in decline, from 69.1% in 2005 to 40.6% in 2013, whereas it has remained relatively constant in the United States from 1995 to 2012 (31.5% in 1995 to 28.1% in 2012). In contrast, serogroup B declined in the United States (38.7 to 27.6%) while an increase was observed in Europe (14.4 to 32.7%). A slow but continuous increase in the prevalence of serogroup C has been observed in both regions (22.5 to 34.7% in the United States and 5 to 8.6% in Europe), suggesting that this serogroup may become more relevant in the future.

Access to detailed serogroup data is more complicated in countries or regions without an established surveillance network, such as in certain parts of Africa. The WHO, with the aid of its member institutions within the Global Foodborne Infections Network (GFN; http://www.who.int/gfn/en/), has established a surveillance system for tracking *Salmonella*. As of January 2016, only 85 countries worldwide were participants in this system. Although the NTS burden on the African continent is poorly characterized, the limited data suggest that serogroup C is the third leading serogroup (19.5% of all cases reported by African countries), after serogroup D (44.6%) and serogroup B (30.9%). Data from independent studies performed in several countries suggest a high variability in NTS serogroup distributions between countries (19–26) (Table 1). In Ethiopia, serogroup C represents 28.6% of all serotyped isolates, compared to only 9.3% in Kenya (20). The majority of studies with African isolates did not determine serovars other than *S.* Typhimurium and *S.* Enteritidis. For example, a study of isolates from The Gambia showed that although 40% of typed bacteria were *S.* Typhimurium and 10% were *S.* Enteritidis,

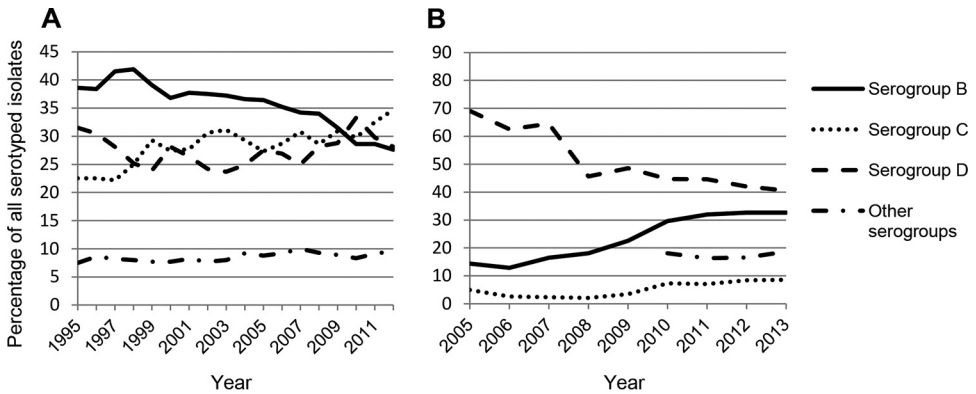

FIG 1 Frequency of nontyphoidal *Salmonella* serogroups associated with human infections, by year. (A) NTS serovars in the United States from 1995 to 2012. (B) NTS serovars in Europe from 2005 to 2013. Data were obtained from the U.S. Centers for Disease Control and Prevention and the European Surveillance System (TESSy).

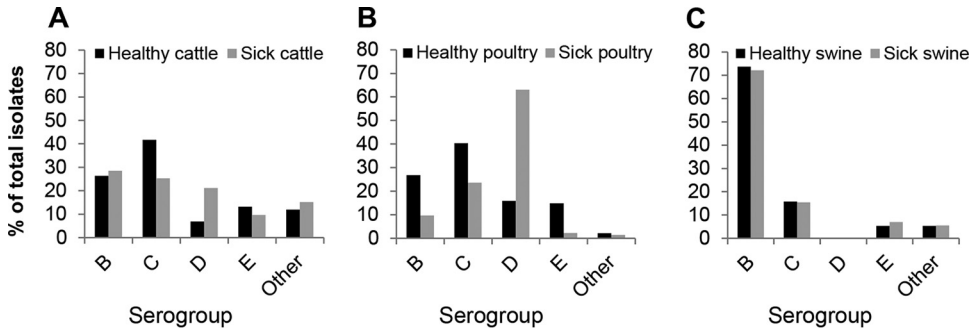

FIG 2 Serogroups of *Salmonella* isolates from animals in the United States in 2012. Data depict the 20 most common serovars for cattle (A), poultry (B), and swine (C), identified in healthy animals or sick animals and reported to the U.S. Centers for Disease Control and Prevention.

47% of all isolates belonged to other serovars that were not further serotyped (27). The presence of several country-specific isolates (such as *S.* Concord [serogroup C1] in Ethiopia, accounting for 34% of all identified strains) emphasizes the need for broader surveillance and more complete identification of *Salmonella* isolates in this region (19). In Asia, a similar lack of surveillance makes it difficult to gather information on serovar distributions. According to WHO GFN 2009 data for the entire Asian continent, serogroup C was the second leading cause of *Salmonella* infections, with 27.3% of reported cases, after serogroup D (45.4% of reported cases). In Taiwan, however, a very large study analyzed 18,280 human *Salmonella* isolates collected between 2004 and 2012 and found that 39% of isolates belonged to serogroup B, 29% to serogroup D, and 23% to serogroup C (Table 1) (22). The leading serovars in each serogroup were *S.* Typhimurium, *S.* Enteritidis, and *S.* Newport (serogroup C2), respectively. Two consecutive studies conducted on NTS isolated in Taiwan between 1993 and 2007 showed that while serogroup E was predominant in 1993 (32.4% of all cases), its incidence decreased to 17% in 2007 (28, 29). In the same period, the proportion of NTS infections caused by group C serovars increased from 15.4 to 26%. In Saudi Arabia, serogroups D (34.2% of reported cases), C (33.5%), and B (19.6%) accounted for the majority of *Salmonella* strains isolated from patients with gastroenteritis or diarrhea between 2008 and 2011 (24). Moreover, 37% of group C isolates were found to be resistant to at least one antibiotic, compared to only 12.6% of group D isolates.

In the United States, 74% of *Salmonella* outbreaks in 2012 were foodborne (25). Many of these were linked to contaminated animal products (30–32). Comparison of *Salmonella* isolates obtained from both healthy and sick farm animals indicates that isolates found associated with livestock are not necessarily the same as those that cause disease (Fig. 2). In cattle and poultry, serogroup C is the serogroup most commonly associated with healthy animals but only the second most common serogroup in diseased animals. It is worth noting that the serovar most commonly isolated from broiler meat is *S.* Kentucky (O:8,20), accounting for 49% of typed isolates between 1998 and 2010 (33). These findings suggest that serovars carried by farm animals, which can potentially cause outbreaks in humans via the food chain, are different from the serovars that cause disease in those animals. These silent infections in commercial livestock present difficulties in surveillance and disruption of transmission. These findings bear relevance to continued efforts in preventing transmission from animals to humans.

In Europe, serovars isolated from broiler-associated human salmonellosis cases were shown to belong to serogroups C (42.6%), D (42.2%), and B (14.45%) (12). In 2006, member states of the European Union (EU) implemented breeding flock control programs aiming for 1% or fewer positive poultry flocks for five target serovars: *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow, and *S.* Hadar. The latter three of these five target serovars belong to serogroup C, thus emphasizing the importance of this serogroup in animals destined for human consumption.

INVASIVENESS AND LETHALITY OF *SALMONELLA* SEROGROUP C

Although they generally produce gastroenteritis, salmonellae can become invasive and cause septicemia, as well as focal infections such as meningitis, endocarditis, or osteomyelitis (34, 35). A recent meta-analysis found that 18 to 21% of bloodstream infections in infants and adults in Africa were due to *Salmonella enterica* serovars, for which 87 to 97% were due to nontyphoidal *Salmonella* organisms (16). NTS is known to become invasive in about 5% of cases worldwide (this proportion increases to 12% in people 65 years or older), and the case-fatality rate can reach up to 47% in some regions (34, 36, 37). Certain NTS serovars have been associated with a higher mortality rate than others (Table 2). When examining case-fatality rates, 4 out of the 10 most lethal serovars belong to serogroup C and 2 to serogroup E (*S.* Muenster and *S.* Anatum), whereas there is only one serogroup D serovar (*S.* Dublin).

Overall, there is no significant difference in invasiveness at the serogroup level between serogroups B, C, and D or other groups

TABLE 2 Serogroup and associated mortality rates of the 10 deadliest serovars isolated in the United States between 1996 and 2006[a]

Rank	Serovar	Serogroup	Mortality rate (%)
1	Dublin	D	3
2	Muenster	E	2
3	Choleraesuis	C	1.8
	Cerro	K	1.8
5	Johannesburg	R	1.5
6	Tennessee	C	1.3
7	Manhattan	C	1
	Anatum	E	1
9	Bovismorbificans	C	0.9
	Adelaide	O	0.9

[a] Data obtained from reference 37.

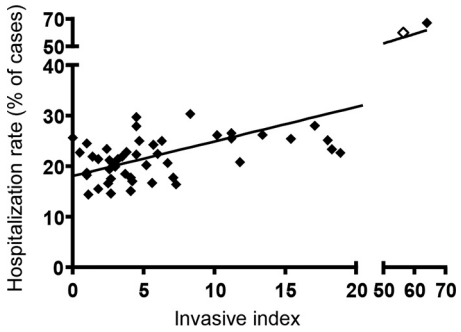

FIG 3 Correlation between invasiveness of a serovar and hospitalization rate. Hospitalization rates were defined as the percentages of salmonellosis patients who were hospitalized within 7 days following specimen collection. The invasive index was defined as the ratio of the number of specimens isolated from normally sterile sites and the total number of isolates of a serovar. Data for *S.* Choleraesuis (empty symbol) and *S.* Dublin (filled symbol) are shown on a separate scale, as they are much more invasive than other NTS. Data obtained from reference 37.

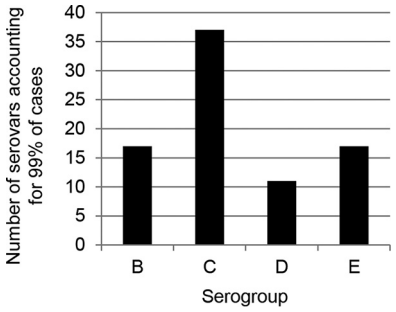

FIG 4 Number of serovars within each serogroup accounting for 99% of NTS cases in 2012. Data are from the U.S. Centers for Disease Control and Prevention.

isolated in the United States between 1996 and 2006 (37). However, some serovars are more likely to become invasive than others, and several of these belong to serogroup C (2). In Ethiopia, 30% of isolates of the group C1 serovar *S.* Concord (responsible for 34% of all NTS infections in that country) have been isolated from blood, compared to 14% of *S.* Typhimurium isolates (38). One of the leading serovars in Asia, *S.* Choleraesuis (group C1) has been found to be invasive in up to 56% of cases (37, 39). In Taiwan, *S.* Choleraesuis has a much higher odds ratio (44:1) of being recovered from blood rather than feces, compared to other NTS serovars (40). *S.* Dublin (serogroup D) is also one of the most invasive NTS serovars, with 64% of strains isolated from sterile sites (37). There is also a positive correlation (Spearman coefficient of 0.42; $P < 0.002$) between the invasiveness of a serovar and the hospitalization rate due to infection by the serovar (Fig. 3). Targeting these invasive serovars would therefore be important to reduce health care costs as well as indirect economic burdens (e.g., due to lost work days). In the United States alone, the annual costs of all *Salmonella* infections have recently been estimated to be $3.3 billion (41). The economic burden of *Salmonella* infection in humans has been estimated by the European Food Safety Authority to be 3 billion euros in the EU (42).

MULTIDRUG RESISTANCE OF *SALMONELLA* SEROGROUP C

In addition to invasiveness, emerging antibiotic resistance is a major concern for the control of nontyphoidal *Salmonella*. Many studies have reported an increase in multidrug-resistant *Salmonella* isolates (20, 22, 24, 43–45).

Analysis of antibiotic resistance patterns of the four most frequently isolated serovars in Greece between 2011 and 2012 (*S.* Enteritidis, *S.* Typhimurium, *S.* Newport, and *S.* Hadar) showed that 17% of serogroup C isolates were resistant to more than four antibiotics, compared to only 9% of the serogroup B isolates and none of the serogroup D strains (46). In Ethiopia, 81% of isolates of the most common serovar, *S.* Concord (serogroup C1), were found to be multidrug resistant (19).

The intensive use of antibiotics on animal farms has been linked to the appearance and spread of antibiotic resistance genes among several bacterial genera and species, including *Salmonella* (47). Even in countries that had reduced their use of antibiotics in animals destined for the food industry, persistence of previously acquired antimicrobial resistance was observed, suggesting that consequences of antimicrobial misuse may not be easily reversed (47). A study of NTS isolates from animals in Senegal and The Gambia showed that 83% of group C2 isolates were resistant to more than four antibiotics, compared to 50% of group B isolates (27). Only one group D isolate was tested, and it was found to be sensitive to all antibiotics used in this study. Strikingly, isolates of *S.* Hadar (serogroup C2) were found to be resistant to up to nine different antibiotics. A recent report of two *S.* Newport isolates obtained from the stools of pilgrims attending Hajj in Saudi Arabia being resistant to the "last-resort" antibiotic colistin only reinforces the need for another strategy to control *Salmonella* infections and prevent transmission to humans (48).

DIVERSITY OF SEROGROUP C SEROVARS CAUSING HUMAN DISEASE

The overall number of serovars reported to have caused disease is highest for serogroup C; 228 different serogroup C serovars were isolated from human patients in the United States in 2012, compared to 106, 93, and 105 serovars of serogroups B, D, and E, respectively. When excluding serovars that cause less than 10 cases per year (accounting altogether for less than 1% of all cases), the remaining *Salmonella* infections due to serogroups B, D, and E were attributed to 17, 11, and 17 serovars, respectively (Fig. 4). However, infections due to serogroup C were caused by 37 serovars. The contributions of individual serovars within each serogroup is also striking (Fig. 5). While two (*S.* Enteritidis and *S.* Javiana) and three (*S.* Typhimurium, *S.* Heidelberg, and *S.* Saintpaul) serovars account for the majority of cases (80% or more) within serogroups D and B, respectively, seven serovars (*S.* Newport, *S.* Montevideo, *S.* Infantis, *S.* Muenchen, *S.* Bareilly, *S.* Braenderup, and *S.* Thompson) account for the same proportion within serogroup C cases. Serogroup E exhibits an intermediate distribution, with five serovars accounting for 80% or more of cases. These data suggest that successful vaccines against *Salmonella* serogroup C would need to elicit cross-protection against the entire serogroup rather than protection against a few individual serovars.

VACCINES AGAINST SEROGROUP C NTS

Although there has been very little work on development of vaccines that can protect humans against *Salmonella* serogroup C

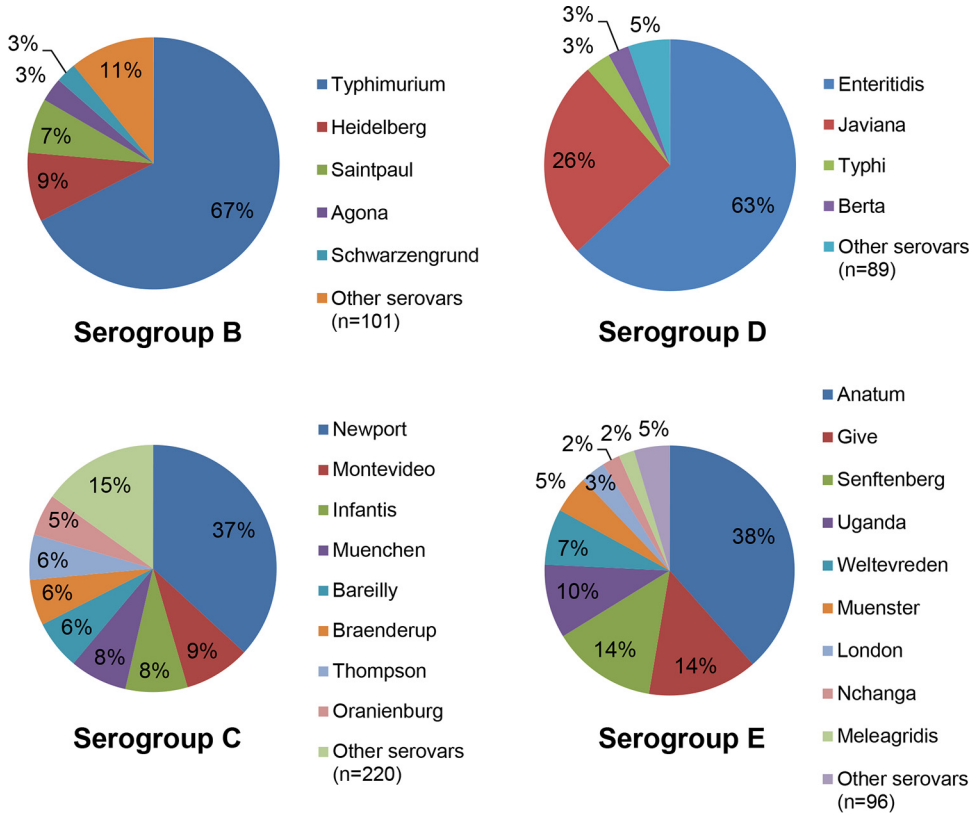

FIG 5 Contribution of individual serovars within serogroups isolated in the United States in 2012. Only serovars responsible for 2% or more of cases within each serogroup are represented individually. In 2012 in the United States, serovar I 4,[5],12:i:− accounted for 15.8% of all cases associated with serogroup B NTS. These data are included within the *S.* Typhimurium slice of the pie chart. Data are from the U.S. Centers for Disease Control and Prevention.

infections, there are a variety of vaccines licensed for use in animals. Lessons learned from these vaccines can help to guide development of human vaccines.

Animal vaccines. Several vaccines have been developed to protect swine against *S.* Choleraesuis (serogroup C1) (49–53). They all rely on a single approach, wherein live attenuated strains of *S.* Choleraesuis have been obtained by chemical mutagenesis, targeted gene deletions (such as Δ*cya* and Δ*crp*), or removal of the virulence plasmid. Several of these *S.* Choleraesuis vaccines have been licensed worldwide for use in animals (Table 3). The reason for targeting *S.* Choleraesuis in swine is due to its high carriage rate; it constituted 57.3% of isolates from swine in the United States in 2003 (54, 55). Moreover, this serovar is documented as among the most invasive *Salmonella* serovars. Protecting swine, along with poultry and other animals of agronomical importance, is an imperative under the recently introduced One Health Initiative (an initiative aimed at collaboration between physicians, veterinarians, and other scientific health and environmental professionals), another measure to reduce human infectious disease.

Only one vaccine targets serogroup C2: the SRP vaccine (siderophore receptor and porin), developed by Zoetis Inc. This is a subunit component vaccine under conditional license, and it contains purified outer membrane proteins from *S.* Newport. This vaccine formulation has been shown to induce antibody production in cattle; however, no significant difference in fecal shedding of *Salmonella* or symptoms of disease were observed between vaccinated and unvaccinated groups (56, 57). Surprisingly, it was observed that vaccinated cattle produced more milk than unvaccinated cattle, although the mechanism for this remains unclear (56).

Vaccines currently in development include two trivalent whole-cell killed vaccines. The first vaccine targets serogroups B, C1, and E (serovars Typhimurium, Mbandaka, and Orion, respectively), and the second vaccine targets serogroups B, C1, and D (serovars Typhimurium, Infantis, and Enteritidis, respectively). Both were shown to confer protection against colonization and shedding of *Salmonella* in animals. Colonization after vaccination with the Typhimurium-Mbandaka-Orion-based vaccine was reduced from 50% of nonvaccinated animals to 9% of vaccinated hens challenged with *S.* Typhimurium, from 58% to 8% when challenged with *S.* Mbandaka (group C1), and from 17% to no detectable colonization when challenged with *S.* Orion (group E) (58, 59). Cross-protection was found to be complete against other serogroup C (*S.* Infantis) and E (*S.* Zanzibar) serovars but was only partial against the serogroup B serovar Agona. One study found incomplete protection against rechallenge in chickens that had previously been infected with wild-type serogroup C1 serovars (60). Here, despite eliciting both humoral (specific IgA, IgM, and IgG) and cellular immune responses (especially CD4- and CD8-positive T cells), infection with *S.* Virchow failed to protect against rechallenge with the same strain.

Human vaccines. Most NTS vaccine efforts have been directed

TABLE 3 Vaccines against group C *Salmonella* currently licensed or in development

Vaccine name	Type	Principle	Serovar(s) (serogroup) included	Usage	Company and/or reference
Licensed vaccines[a]					
Nitro-Sal	Live attenuated	Chemical mutagenesis	Choleraesuis (C1)	Swine	Arko Laboratories (Jewel, IA, USA)
Nobl	Live attenuated	Loss of virulence plasmid	Choleraesuis (C1)	Swine	51
Argus-SC	Live attenuated	Gene deletions (Δ*cya* and Δ*crp*)	Choleraesuis (C1)	Swine	50
Enterisol *Salmonella* T/C	Live attenuated	Public information not available	Choleraesuis (C1), Typhimurium (B)	Swine	Boehringer Ingelheim GmbH (Ingelheim, Germany)
Enterisol SC-54	Live attenuated	Loss of virulence plasmid	Choleraesuis (C1)	Swine	Boehringer Ingelheim GmbH
Suisaloral	Live attenuated	Attenuating gene mutation	Choleraesuis (C1)	Swine	IDT Biologika GmbH (Desau-Roslau, Germany); 53
Newport SRP	Component vaccine	Purified siderophore receptor and porin	Newport (C2)	Cattle	Zoetis Inc. (Florham Park, NJ, USA)
Vaccines in development[b]					
Salenvac	Killed vaccine	Inactivated whole-cell vaccine	Typhimurium (B), Mbandaka (C1), Orion (E)	Poultry	Intervet Schering-Plough Australia; 59
Unknown	Killed vaccine (with adjuvant)	Inactivated whole-cell vaccine	Typhimurium (B), Infantis (C1), Enteritidis (D)	Poultry	58
TBD	Live attenuated	Attenuating gene deletions	Paratyphi C (C1), Newport (C2)	Human	F. J. Fuche and S. M. Tennant, Center for Vaccine Development (unpublished data)
TBD	Conjugate vaccine	Core O polysaccharide conjugated to flagellin	Paratyphi C (C1), Newport (C2)	Human	G. Ramachandran and R. Simon, Center for Vaccine Development (unpublished data)

[a] Data include vaccines with conditional licenses.
[b] TBD, vaccine name to be determined.

against *S.* Typhimurium and *S.* Enteritidis, as they are recognized as the main causes of gastroenteritis in developed countries such as the United States and of invasive disease in sub-Saharan Africa (2, 16). However, according to our reanalysis of existing data, in the United States serogroup C is one of the most prevalent serogroups and was the most common serogroup in 2012 (Fig. 1). Therefore, in an effort to prevent *Salmonella*-induced gastroenteritis, as well as invasive *Salmonella* infections, a vaccine that targets serogroup C *Salmonella* along with *S.* Typhimurium and *S.* Enteritidis is desirable. However, due to the diversity within serogroup C, cross-protection against seven serovars from that serogroup would be required to prevent >80% of *Salmonella* serogroup C infections. There are several vaccines in development against invasive NTS, such as *S.* Typhimurium and *S.* Enteritidis. A discussion of the *S.* Typhimurium and *S.* Enteritidis candidate vaccines is beyond the scope of this review and has been discussed elsewhere (14, 35). No licensed human vaccine against serogroup C NTS currently exists. Here at the Center for Vaccine Development of the University of Maryland School of Medicine, efforts to develop a live attenuated vaccine are under way, based on well-characterized attenuating gene deletions (14). Another strategy that has proven successful in generating functional immunity and protection against *S.* Typhimurium and *S.* Enteritidis relies on conjugate vaccines in which flagellin serves as a carrier for core O polysaccharide (61). We expect that, based on differences in their O polysaccharides, we would need vaccines against both serogroups C1 and C2 to elicit broad protection against all serogroup C serovars. Ultimately, one could combine these live attenuated or conjugate vaccines to protect against serogroups B, D, C1, and C2, therefore preventing the majority of salmonellosis cases (up to 88% of cases in the United States).

Vaccines in development should ideally generate both antibody- and cell-mediated immunity, as T cells are required for ultimate resolution of *Salmonella* infections (62–64). It was recently reported that cell-mediated immunity might be crucial for protection against NTS in individuals coinfected with malaria, a common comorbidity in parts of Africa and Asia where these pathogens are endemic (65). In that study, the protection elicited by an *S.* Typhimurium live attenuated vaccine was transiently lost as CD4 and CD8 T cells levels decreased during acute malarial episodes, despite elevated antibody titers. This loss of protection was shown to be reversible upon resolution of malaria, as CD4 and CD8 T cell levels recovered to premalaria levels, suggesting that antibodies alone were not sufficient to protect against *S.* Typhimurium. It was also shown in mice that despite generating high antibody titers, intraperitoneal immunization with three doses of formalin-killed *S.* Bovismorbificans (serogroup C2) did not protect against homologous challenge (66). Protection against challenge with a 100× 50% lethal dose (LD_{50}) of virulent *S.* Bovismorbificans was, however, achieved by immunizing the mice twice with a live attenuated derivative strain (carrying an *aroA* deletion) via either the intraperitoneal route or the oral route: 75% of mice survived in either case. Attempts to generate a vaccine against *S.* Choleraesuis in the late 1980s demonstrated that an *S.* Typhimurium-based vaccine carrying the attenuating mutation Δ*galE* conferred protection to BALB/c mice, whereas the corresponding *S.* Choleraesuis vaccine did not (67). The lack of protection was later suggested to be due to the inability of the vaccine strain to colonize the livers and spleens of vaccinated mice (68). The type of immunity generated is therefore an important factor for consideration when developing vaccines against serogroup C *Salmonella*.

CONCLUSIONS

NTS infections are the leading cause of DALYs in the United States among major foodborne pathogens (*Campylobacter* spp., *Escherichia coli* O157, *Listeria monocytogenes*, *Clostridium perfringens*, *Toxoplasma gondii*, and norovirus) (69). Among pathogens that cause gastroenteritis in developed countries, NTS is also the leading cause of hospitalization (0.6% to 3.9% of all NTS cases) and death (37 per 100,000 cases). Moreover, NTS infections are responsible for an underestimated burden of sequelae: 1 to 2.8% of cases can lead to reactive arthritis, and 5.7 to 16.6% of infections are thought to induce postinfectious irritable bowel syndrome (69, 70). A multivalent NTS vaccine would not only protect against acute gastroenteritis but also protect against these secondary diseases, therefore reducing the costs associated with long-term health care.

Vaccination of animals is an important way to protect both humans and animals, since most human disease sources are linked to animal-related food products. However, vaccinating only farm animals might not be sufficient to prevent disease in humans. A major outbreak of *S.* Thompson in the Netherlands in 2012 was attributed to smoked salmon (71). Multiple outbreaks have also been linked to fruits and vegetables. Cases of contamination after handling pet food have also been reported (6). Moreover, following introduction of vaccination and improved sanitation in swine in Taiwan, a decrease in human cases due to *S.* Choleraesuis was observed between 2004 (4.3% of all serogroup C infections) and 2007 (0.84%) (72). However, that decrease was associated with an increase in infections due to other serogroup C serovars (from 16.5% of all serogroup C cases in 2004 to 33.7% in 2007). This suggests that targeting only one serovar may lead to emergence of other serovars, indicating that a multivalent vaccine that can protect against all serogroup C *Salmonella* (and possibly all NTS) is needed for complete protection.

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