SUPPLEMENT ARTICLE

Incidence of Nontyphoidal *Salmonella* in Food-Producing Animals, Animal Feed, and the Associated Environment in South Africa, 2012–2014

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Background. Nontyphoidal salmonellosis continues to pose a global threat to human health, primarily by causing food-borne illnesses, and food-producing animals are the principal reservoirs of many pathogenic serovars. To identify key control points and generate information that may enable future estimation of the transmission routes between the environment, animals, and humans, we examined data on *Salmonella* isolates in South Africa.

Methods. Samples were obtained from livestock and poultry on farms, meat at abattoirs, raw materials at feed mills, animal feed, and environmental sources (eg, poultry houses, abattoirs, feed mills, water) from 2012 to 2014 in compliance with each establishment's protocols conforming to International Organization for Standardization (ISO) (ISO/TS 17728, ISO 18593:2004 and ISO 17604:2003) standards. Isolation and serotyping of *Salmonella* were performed according to the scope of accreditation of the respective laboratories conforming to ISO/IEC 17025:2005 standard techniques.

Results. *Salmonella* was isolated from 9031 of 180 298 (5.0%) samples, and these isolates were distributed among 188 different serovars. *Salmonella* Enteritidis was the most frequent isolate, with 1944 of 180 298 (21.5%) originating from poultry on farms, poultry meat, and poultry houses, followed by *Salmonella* Havana, with 677 of 180 298 (7.5%), mostly from environmental samples. Serovars that are uncommonly associated with human disease (*Salmonella* Idikan, *Salmonella* Salford, and *Salmonella* Brancaster) were isolated at higher frequencies than *Salmonella* Typhimurium, a common cause of human illness. Environmental samples accounted for 3869 of 9031 (42.8%) samples positive for *Salmonella*.

Conclusions. We describe the frequent isolation of *Salmonella* of a wide variety of serovars, from an array of animal feeds, food animals, and food animal environment. As prevention of human salmonellosis requires the effective control of *Salmonella* in food animals, these data can be used to facilitate *Salmonella* control in food animals and thereby prevent human infections.

Keywords. *Salmonella*; environment; feed; meat safety; livestock.

Nontyphoidal *Salmonella* (NTS) infections in humans are infections caused by *Salmonella enterica* of serotypes other than Typhi and Paratyphi A. Human infection with NTS remains a global public health concern despite advances in sanitary measures, water treatment, and food safety standards over the last decades [1].

The control of NTS infections requires the control of NTS in food animals, which in turn requires control of NTS in animal feed and the food animal environment. The majority of human NTS infections are foodborne, but each year infections are also acquired through direct or indirect contact with animals in homes, veterinary clinics, zoological gardens, farm environments, and public or private settings [2].

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Infections in humans generally manifest as a diarrheal illness. However, invasive NTS disease is common among infants and children, the elderly, and immunocompromised individuals and is especially common in Africa [3]. By contrast, salmonellosis in food-producing animals (livestock, poultry) and other animal hosts manifests as a self-limiting diarrheal illness resulting in long periods of latent carriage with occasional fecal shedding [4]. Fecal shedding by food-producing animals is the leading source of contamination of feed, water, and the environment, whereas intestinal carriage often leads to contamination of carcasses at slaughter. It is clear that salmonella contamination in livestock and poultry has a direct effect on the global marketing of the respective food-producing animals and animal-derived food products [5].

Salmonella bacteria can survive for several weeks in a dry environment or even for several months in water [6, 7], from where they can be easily be recovered using standard microbiological techniques. A robust and rigorous monitoring system combined with aggressive and thorough control actions (eg, mandatory heat treatment of feed, cleaning and disinfection of the environment, rodent control) when NTS are isolated is the key factor in a successful NTS monitoring program in food animals, with success measured by the successful control and elimination of NTS in animal feed, food animals, and the food animal environment. Consequently, NTS is a target of an integrated surveillance system of foodborne pathogens taking a "One Health" approach and implemented along the farm-to-fork continuum. Human susceptibility widely varies depending on diet and immunocompetence among other factors; consequently, all *Salmonella* serovars are pathogenic to humans as there is a range of infectious doses for the serovars.

To alleviate foodborne salmonellosis, comprehensive preharvest pathogen reduction strategies are implemented in most countries including South Africa. One such strategy is outlined in the Guidelines for the Control of *Campylobacter* and *Salmonella* in Chicken Meat (CAC/GL 78-2011), which have been in existence since 2011 [8]. Further guidelines for the control of NTS in beef and pork were recently proposed at the 45th Session of the Codex Committee on Food Hygiene [9]. In addition, the Office International des Epizooties in 2014 recommended the prevention and control of *Salmonella* in commercial pigs from farm to slaughter (OIE ad hoc Group on *Salmonella* in pigs, August 2014, http://www.oie.int/). In South Africa, regulatory control measures of NTS in food-producing animals are targeted at *Salmonella enterica* subspecies *enterica* serotype Enteritidis in poultry under Section 31 of the Animal Diseases Act (Act 35 of 1984). These prescribed measures are supplemented by a movement control protocol that is triggered by an outbreak of *Salmonella* Enteritidis infection in poultry or other birds. The microbiological monitoring of food falls under statutory regulations issued in section 15(1) of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972). Furthermore, the Meat Safety Act (2000) requires the application of risk-based preventive strategies and subsequent implementation of hygiene management programs to reduce, eliminate, or prevent potential hazards such as *Salmonella*.

Because *Salmonella* can be isolated from food, environmental sources, animals, humans, and apparently healthy carriers, all isolates are regarded as potentially pathogenic for all species [10].

In fact, *Salmonella* is now defined in the standard operating procedure for the microbiological monitoring of imported meat as a biological agent associated with serious illness or death, particularly those strains resistant to 1 or more critically important antimicrobials used in human medicine. Serotyping is recommended on all *Salmonella* isolates to subtype *Salmonella* for epidemiological purposes so as to facilitate understanding on the source of *Salmonella* infections in humans, food animals, feed, and food animal environments. To identify key control points, we examine the frequency of isolation of NTS serovars from 2012 through 2014 in livestock, poultry, the environment, and other nonhuman sources in South Africa. Such data will facilitate the development of strategies to control salmonellosis in the food pathway, eventually leading to the lowering of the incidence in humans.

METHODS

Sample Collection

The sampling period was from January 2012 through December 2014. Sampling is undertaken in accordance with ISO-18593: 2004 protocol [11] and guidelines of the Codex Alimentarius. Sampling was done in accordance to each establishment's standard protocol based on guidelines conforming to ISO standards [12, 13] for the microbiological monitoring of (1) livestock and poultry; (2) meat and environment (process hygiene and cleaning); [14], (3) carcass microbiological sampling [15]; and (4) environmental sampling.

On-Farm Livestock and Poultry

Monitoring of *Salmonella* in livestock was mainly through laboratory-based passive surveillance by the National Notifiable Disease Surveillance System from January 2012 through December 2014. Rectal swabs or fresh faeces were collected from cattle, sheep, pigs, and poultry and transported on ice packs or cooler boxes to the accredited laboratory [16] for processing. The temperatures of samples were confirmed to be <7°C at submission. In transit, rectal swabs were preserved in Amies' transport medium whereas feces were in fecal cups without transport media. In the case of dead animals, birds, or dead-in-shell eggs on the farm, additional postmortem samples were collected as part of a wider disease investigation. In addition to prospective data, retrospective data of outbreaks of *Salmonella* Enteritidis in poultry from 2012 through 2014 were obtained from the Epidemiology Section of the Department of Agriculture, Forestry and Fisheries (DAFF), South Africa.

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Abattoir Surveillance

Monitoring for *Salmonella* at abattoirs is undertaken under the auspices of the Meat Safety Act (2000), which stipulates that all meat must be free of hazardous substances and therefore declared safe and fit for human consumption. Approximately 50 g of meat from randomly selected sites on the carcass of livestock, poultry, or food-producing wildlife (ostrich or crocodile) were aseptically removed for microbiological processing. Additionally, randomly selected carcasses were also swabbed for *Salmonella* isolates using sponges as per standard practice described [11].

Animal Feed and Raw Materials

At least 25 g of animal feed (ie, bone meal, blood meal, meat meal, carcass meal, feather meal, fish meal, and cat and dog food) or raw materials (eg, flour, lucerne, maize, soya, sunflower, tallow, water, and yeast) were collected and subjected to selective and enrichment techniques for *Salmonella*.

Environmental Samples

Litter and environmental swabs were collected using moistened cotton boot covers worn during routine poultry house activities such as egg collection and changing feed. Swabbed matter from boot covers was pooled into sterile bags and transported for processing. Egg shells and droppings from hatcheries were collected from multiple sites and pooled. Dust and wood shavings from at least 6 different locations were collected by using a feather picked up in the house to sweep enough dust into the sampling bottle. Samples from feed mills, abattoirs, and associated production equipment were obtained by swabbing multiple sites with moistened cotton swabs. When available, litter beetles, insects, and frogs were collected as part of a wider environmental monitoring. Environmental water from farms or poultry houses was sampled [17].

Culture and Identification of *Salmonella* Isolates

Isolation and serotyping of *Salmonella* were performed according to the scope of accreditation of the respective laboratories conforming to ISO/IEC 17025:2005 [16].

Samples were tested according to ISO 6579:2002 [18, 19]. All confirmed *Salmonella* isolates were serotyped according to the White–Kauffmann–Le Minor scheme [20] using polyvalent O and H antisera (BD Diagnostics, Gauteng, South Africa).

Descriptive statistics (Microsoft Excel) were used to calculate the frequency (percentage) of isolation of each serovar per source.

RESULTS

Frequencies of *Salmonella* Serovars

A total of 180 298 samples were distributed as follows: 42 331 (2012), 64 190 (2013), and 73 777 (2014) from animals, animal feed, environmental sources, carcasses, and nonanimal sources were received at the specialized *Salmonella* diagnostic unit for processing between January 2012 and December 2014.

The prevalence of *Salmonella* isolation by year was 1954 of 42 331 (4.6%) in 2012; 3631 of 64 190 (5.7%) in 2013, and 3446 of 73 777 (4.7%) in 2014. Overall, *Salmonella* was isolated from 9031 of 180 298 (5.0%) samples, encompassing 188 *Salmonella* serovars. *Salmonella* Enteritidis was isolated from 1944 of 9031 (21.5%) samples; *Salmonella* Havana from 677 of 9031 (7.5%); followed by *Salmonella* Idikan, *Salmonella* Salford, and *Salmonella* Brancaster in decreasing order of prevalence. *Salmonella* Typhimurium was isolated from 361 of 9031 (4.0%) samples (Table 1). *Salmonella* Seftenberg, *Salmonella* Montevideo, *Salmonella* Ohio, *Salmonella* Muechen, *Salmonella* Schwarzengrund, *Salmonella* Anatum, *Salmonella* Mbandaka, *Salmonella* Hadar, *Salmonella* Infanits, and *Salmonella* Orion completed the list of the 16 most frequently encountered serovars in the period under study. Of 9031 samples positive for *Salmonella*, 2094 belong to 162 (23.2%) uncommon serovars.

Distribution of *Salmonella* Serovars by Source

The *Salmonella* Enteritidis–positive samples originated mainly from poultry, poultry meat, and the poultry-rearing environment. Of 832 *Salmonella* Enteritidis isolates from meat products, all were from poultry (Table 1). Of 615 *Salmonella* Enteritidis isolates from the farm, all were from broilers, layers, eggs and dead-in-shell embryos (Table 2). Of the 461 environment samples positive for *Salmonella* Enteritidis, these were mainly from egg shells, poultry house dust, droppings, and shavings sampled in poultry houses. Of the 677 *Salmonella* Havana isolates, 551 (81.3%) were isolated from the environment, with 677 (7.5%) from poultry houses, abattoirs, and feed mills, collectively. Of 483 *Salmonella* Salford isolates, 458 (94.8%) were from animal feed and specifically the raw materials (Table 1). Of 9031 *Salmonella* isolates, 1096 (12.1%) were from animal feed, 673 (7.5%) were from feed materials, and 38 (0.4%) were from dog or cat food.

A greater proportion of *Salmonella* Typhimurium isolates, 194 of 361 (53.7%), originated from the environment, followed by 108 (29.9%) from farm animals, 26 (7.2%) from animal feed, and 25 (6.9%) from meat. Of 9031 *Salmonella* isolates, 3869 (42.8%) were from the environment. Of 3869 environmental *Salmonella* isolates, 2642 (69.3%) were from poultry house samples that included dust, shavings, droppings, and egg shells; 622 (23.5%) were from abattoirs and 584 (22.1%) were from feed mills. A total of 492 (5.4%) of the samples were from undefined sources obtained as part of a wider environmental investigation.

Retrospective Trends of *Salmonella* Enteritidis Outbreaks

Data from the Epidemiology Section, DAFF, South Africa, showed that there were 14 outbreaks in poultry of *Salmonella* Enteritidis in 2012, 23 in 2013, and 23 in 2014 (Figure 1).

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Table 1. Frequencies of Major *Salmonella* Serovars Isolated From Food-Producing Animals, Meat, Animal Feed, the Environment, and Other Nonhuman Sources in South Africa, 2012–2014

Salmonella Serovar	Farm Animals	Meat	Animal Feed	Environment	Other Sources	Total
Enteritidis	615	832	3	461	33	1944
Havana	10	51	55	551	10	677
Idikan	9	112	79	295	12	507
Salford	3	0	458	22	0	483
Brancaster	7	47	93	297	1	445
Typhimurium	108	25	26	194	8	361
Senftenberg	78	15	33	208	17	351
Montevideo	6	93	37	131	41	308
Muenchen	24	106	11	126	13	280
Ohio	2	63	19	145	53	282
Anatum	10	91	27	113	25	266
Schwarzengrund	13	13	220	26	3	275
Mbandaka	6	26	73	101	6	212
Hadar	13	30	10	145	5	203
Infantis	6	22	71	28	56	183
Orion	4	3	78	65	10	160
Other (162 serovars)	166	251	514	961	202	2094
Total	1080	1780	1807	3869	495	9031

DISCUSSION

Relatively few African countries report their surveillance data and as such, very limited information on NTS is available for the continent [21]. Nevertheless, previous data on isolates from South Africa veterinary diagnostic laboratory revealed the most common *Salmonella* serovars, in descending order from 1996 to 2006, to be the following; *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Isangi, *Salmonella* Infantis, *Salmonella* Dublin, *Salmonella* Heidelberg, *Salmonella* Virchow, *Salmonella* Newport, *Salmonella* Muenchen, *Salmonella* Hadar, *Salmonella* Anatum, *Salmonella* Arizonae, and *Salmonella* Schwarzengrund [21–23].

Salmonella Typhimurium was isolated from a variety of sources including livestock and poultry on farm, but no further characterization studies were undertaken to determine the presence of invasive ST313 strains believed to be restricted to Africa [21]. Potential environmental or zoonotic sources (domestic or wild animal) of invasive *Salmonella* Typhimurium sequence type 313 have been partially investigated with limited application of epidemiological investigation tools; hence, speculation has focused more on direct or indirect human-to-human transmission routes where asymptomatic carriage potentially plays a role [24]. Although it has previously been shown that the invasive *Salmonella* Typhimurium ST313 is adapted to a niche of immunosuppressed human immunodeficiency virus– or malaria-infected patients or is restricted to human infection in Africa [24], a recent study has shown a similar invasive phenotype in chickens [25], indicating a possible reservoir of these strains.

In the current study, *Salmonella* serovars were isolated from a wide variety of sources including imported meat, animals, feed, meat, and environmental sources, indicating diverse potential sources for human infection. The isolates were distributed among 188 *Salmonella* serovars, with many serovars represented rarely or only once. Despite uncertainty about the true burden of human illness posed by uncommon *Salmonella* serovars, the emergency of new virulent pathogenic strains and antimicrobial resistant strains of *Salmonella* continually evolve [26]. Therefore, all *Salmonella* are considered as potential pathogens that warrant institution of control measures to safeguard human health. Animals are known principal reservoirs of NTS, and fecal shedding is the principal source of environmental contamination. Intestinal carriage often leads to carcass contamination at slaughter with, or possibly a subsequent, contamination of the abattoir environment [5]. The use of animal waste as fertilizer for crops or raw materials destined for producing animal feed is common practice by some farmers. It is plausible that such raw materials may get contaminated through this practice, leading to subsequent contamination of the feed mill environment. Further, epidemiological tracking of strains at the molecular level along the process chain would support this hypothesis.

Following *Salmonella* challenge, *Salmonella* fecal shedding and immune response are time sensitive and dose and serotype dependent. The long periods of latent intestinal carriage of NTS serovars (which may be accompanied by intermittent or transient fecal shedding) and strong immunological responses induced during intestinal persistence make it difficult to diagnose some NTS serovars in livestock [2, 4]. As such, the

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Table 2. Distribution of Sources of *Salmonella*-Positive Samples Processed at the Diagnostic Laboratories in South Africa, 2012–2014

Category	Actual Sources	No. of Isolates	Total
Farm animals	Poultry (broilers, layers, breeding stock, dead-in-shell, etc)	1073	
	Cattle	7	1080
Meat	Poultry meat (carcasses, cuts, byproducts)	1728	
	Beef	31	
	Pork	4	
	Crocodile	9	
	Ostrich	8	1780
Animal feed	Feed (bone meal, fish meal, blood meal, etc)	1096	
	Raw materials (soya, yeast, sunflower, maize, etc)	673	
	Dog/cat food	38	1807
Environment	Poultry houses (droppings, dust, shavings, egg-shells, fluff, etc)	2642	
	Abattoirs	622	
	Feed mills	584	
	Water	21	3869
Other sources	Undefined	492	
	Litter beetles	2	
	Frog	1	495
Total			9031

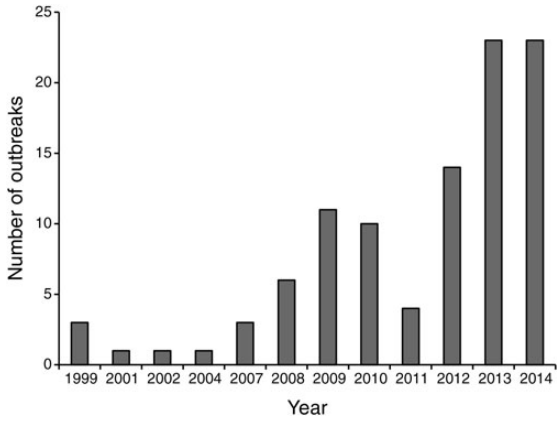

Figure 1. Number of outbreaks of *Salmonella enterica* subspecies *enterica* serovar Enteritidis in poultry reported in South Africa for the period 1999–2014. Source: Epidemiology Section, Department of Agriculture, Forestry and Fisheries, Directorate of Animal Health, South Africa.

monitoring of NTS in livestock is undertaken through routine random sampling outbreak investigation and/or routine animal disease diagnosis. Because NTS in animals usually manifests as a subclinical disease, successful preharvest *Salmonella* controls with rigorous sampling and consequential actions resulting from sample results are needed. Research should target gaining a deeper understanding of *Salmonella* carriage and transmission dynamics in animal species, including early detection methods of new serovars or strains. This will aid in the development of strategies to reduce preharvest fecal shedding and subsequent carcass contamination at slaughter. A gap in the standardization of methodologies and evaluation criteria for surveillance methods across sectors and departments within South Africa [27] should also be addressed. However, surveillance data for NTS in livestock and poultry in South Africa were reported for the period of 1999–2006, where *Salmonella* Typhimurium was the most frequently isolated in poultry and *Salmonella* Dublin was second in poultry, but highest in cattle [22]. We therefore focused on the period from 2012 through 2014 in the current study, due to lack of data for livestock in subsequent years after 2006; however, it will be interesting to carry out a traceback on the available data of *Salmonella* isolates so as to identify trends and risk factors for the emergence of certain *Salmonella* serotypes.

Interestingly, more recent data show that the epidemiology of salmonellosis in poultry might have changed over the years, with new *Salmonella* serovars now topping the list [22]. This was not surprising as there was a sharp increase in reported *Salmonella* Enteritidis outbreaks between 2012 and 2014 (Figure 1), perhaps owing to improvements in the compulsory monitoring of *Salmonella* in poultry [28]. The opening of doors for the import of multispecies fresh meat over the years from various developed and developing countries could have altered the trends in *Salmonella* serotypes in South Africa. Active laboratory-based surveillance for *Salmonella* was introduced nationally through the Meat Safety Act (2000) with a view of protecting the consumer. This promulgates routine sampling throughout the year on consecutive days to assess the effectiveness of protocols to address issues pertaining to *Salmonella* contamination of carcasses at abattoirs and processing plants. It includes a requirement for abattoirs to have a functioning microbiologic sampling program as part of their hygiene management plan. Active surveillance is also undertaken in poultry breeding stocks, layers, hatchlings, and broilers as part of statutory requirements for the control of *Salmonella* [28]. Furthermore, this compulsory testing has now been extended to poultry houses, abattoirs, feed and raw materials, feed mills, or even imported poultry meat consignments. Monitoring the environment for *Salmonella* has not been reported before in South Africa. The data presented here implicate the environment as an important source of *Salmonella*, which therefore is one of the key control points requiring intervention. Poultry houses, feed mills, and abattoir therefore require regular decontamination after use to

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curtail the transmission of *Salmonella* to new flocks or carcass contamination at slaughter.

The frequency of the serovars in these parts of South Africa could have been biased due to nonavailability of data from state laboratories. Despite this, an average of 5.0% of the samples submitted to the laboratories tested positive for *Salmonella* over the 3-year study period. The implications of the relatively high prevalence of isolates from diverse sources on human health were not readily discernible. However, data from annual reports of the Group of Enteric, Respiratory and Meningococcal Disease Surveillance of South Africa [23] and the World Health Organization Global Foodborne Infections Network database [21] showed a progressive doubling in human cases due to NTS and, in particular, *Salmonella* Enteritidis between 2003 and 2013. However, the linkage between animal, environmental, and human strains can strictly be established by using molecular techniques such as pulsed-field gel electrophoresis as recently reported between strains from human and captive wild animals [29]. Widespread application of this technique will enable generation of a regional database for pulsotypes (PulseNet) that will facilitate rapid epidemiological investigations.

In conclusion, our data provide insights into potential sources of NTS in the farm-to-fork food pathway in South Africa. These can form a basis for instituting intervention strategies for the control of NTS. To achieve the optimal human and animal health outcome, collaboration and communication within and between veterinary and human microbiology entities involved in *Salmonella* prevention, surveillance, outbreak response, and research should be promoted. Moreover, a legally mandated public health information system sharing agreement between animal and human health disciplines within the "One Health" framework should be viewed as the best approach for effective control of salmonellosis.

Notes

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