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Short communication

Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* serotypes isolated from laying hens and broiler chicken farms in N'Djamena, Chad

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ABSTRACT

This study aimed at updating knowledge on the prevalence and antimicrobial resistance characteristics of *Salmonella* isolated from poultry in the province of N'Djamena, Chad. The results collected during this study provide the first baseline data on the prevalence of contamination by *Salmonella* in laying hens and broiler chicken farms in N'Djamena. All samples were collected from sixteen poultry farms over two periods of six months each: from August 2010 to January 2011 and from September 2011 to February 2012. Diagnostic methods used during this study allowed to isolate eighty four *Salmonella* strains, belonging to twenty seven different serotypes. The most frequent serotypes were *Salmonella* Colindale (19%) followed by *S.* Minnesota (18%) *S.* Havana and *S.* Riggil (each 6%), *S.* Kottbus and *S.* Amager (4.7%), *S.* Idikan, Mississipi, and Muenchen (3.6%). Other serotypes were poorly represented. The majority of these serotypes were susceptible to all antibiotics tested (CLSI Standards), except some *S.* Colindale isolates that exhibited a decreased susceptibility to fluoroquinolones, *S.* Limete resistant to three antibiotics and *S.* Minnesota isolates resistant to five different antimicrobial classes. The different serotypes and antibiotic resistance profiles that were observed highlight the substantial diversity of *Salmonella* in Chad, the contribution of avian isolates to human salmonellosis and *Salmonella*'s capacity to colonize all types of environment worldwide.

1. Introduction

Salmonella infection is a major health concern and continues to have a serious economic impact worldwide (Humphrey, 2006; Dione et al., 2011). It is estimated that *Salmonella* serotypes cause 93.8 million human infections and 155,000 deaths annually through the world (Majowicz et al., 2010). Annually in the US, foodborne salmonellosis is responsible for over 600 deaths and 1.4 million illnesses. The costs for medical care and loss of productivity can range anywhere from $464 million to $2.3 billion (Galanis et al., 2006; Hendriksen et al., 2011). In Europe, the number of human cases was reported to be greater than 100,000 in 1997. From 2007 to 2009, the total of *Salmonella* outbreaks

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decreased by 23.6%, from 2,253 outbreaks in 2007 to 1722 outbreaks in 2009 (EFSA, 2011). In France, *Salmonella* serovars were isolated from 64% of foodborne outbreaks between 1996 and 2005 (O'Brien and De Valk, 2003).

Salmonella genus contains only three species, namely: *Salmonella enterica, Salmonella bongori* and *Salmonella subterranean*. *Salmonella enterica* can be subdivided into at least six subspecies and composed of more than 2600 serovars. *Salmonella bongori* has no subspecies (Millemann et al., 2010). Although several serovars belonging to the genus *Salmonella* have been identified, most animal and human salmonellosis are caused by only a few non-typhoidal serovars (O'Brien and de Valk, 2003). Some serotypes are associated with specific animal reservoirs, while others are known to infect a wide variety of animals including humans (Jones et al., 2008).

Most studies have indicated that food vectors identified as the main source of non-typhoidal salmonellosis are contaminated poultry meat (chicken, turkey, etc.) and raw or under-cooked eggs and derived products. World Health Organization (WHO), through the Salm-Surv data reported that from 2001 to 2005, the most commonly isolated *Salmonella* serotypes worldwide were Enteritidis (65% of isolates), Typhimurium (12%) and Newport (4%) (Hendriksen et al., 2011). According to Web-based surveillance and global *Salmonella* distribution, from 2000 to 2002, *S.* Enteritidis and *S.* Typhimurium represented respectively 26% and 25% of the isolates in Africa (Galanis et al., 2006).

More often on farms, poultry flocks can be infected by horizontal transmission through infected litter, faeces, feed, water, equipment, diseased chicks and rodents contaminated with *Salmonella*. They can also be infected by other animals, wild birds and personnel (Poppe, 2000).

In the two last decades, antimicrobial resistance in *Salmonella* has become a major problem in public health worldwide (Threlfall, 2002). It is known that in developing countries the rise of antimicrobial resistance is increasingly under surveillance, but in African countries, particularly in regions of Central Africa, these problems remain very complex. This is because in these countries there is little or no information available on bacterial resistance (Vlieghe et al., 2009).

In Chad, Central Africa, *Salmonella* strains and other major zoonotic bacterial pathogens are not often isolated and identified, and the resistance of these pathogens including *Salmonella* to commonly used antimicrobial is rarely assessed or not at all looked for. In addition, the use of antibiotics in farms is not subject to any regulation. This study represents therefore, the first Chadian investigation to gather knowledge on *Salmonella* infections in laying hen and broiler flock farms in N'Djamena. The aims of this study were (1) to determine the prevalence of *Salmonella* in these farms and (2) to update knowledge on *Salmonella* serotypes diversity and their antimicrobial resistance profiles.

2. Materials and methods

2.1. Study and survey design

Our study was carried out during two seasons, from August 2010 to January 2011 and from September 2011 to February 2012, and involved sixteen out of the 30 functional laying hen and broiler chicken farms identified in N'Djamena. Farms were selected on the basis of the free manager's choice to cooperate or not during our study. One farm was visited each weak. Each farm was visited only once and the manager was interviewed with the questionnaire at right time of sampling. All birds in each sampled farms were healthy. At the farm, information about structures and building numbers, production capacity, race, age and origin of birds were collected. The Information regarding the number of animals in the flocks, control of rodents, reptiles, and other domestic animals, hygienic and bio-security practices and the number of workers were also recorded.

2.2. Sample collection

Samples were collected in poultry farms each weekends. Because of limited economic means, we pooled individual samples to minimize study costs. In each farm, only one poultry flock was chosen for sampling. Eight pools of samples per farm were collected as follows: 3 pools of fifteen fresh droppings excreted by birds on litter, 3 pools of two sterile cloths for microbiological control of litter surfaces (AES Chemunex, Combourg, France), 1 pool of 6×5 mL of water from watering places and 1 pool of 6×5 g of food from feeders. Each pool of samples was collected in sterile pouches (AES Chemunex, Combourg, France), placed in a cool box with ice packs and transported to the laboratory for analysis.

2.3. Salmonella isolation and identification

All samples (dropping, sterile Cloths, food and water) collected were analyzed according to French Norm for *Salmonella* spp. NF ISO 6579/2002.

From each sample, 25 g was homogenized in 225 mL of buffered peptone water (AES Chemunex, Combourg, France) and incubated at 37 °C for 18–20 h to allow bacterial revivification.

One millilitre and 0.1 mL of the pre-enrichment samples were respectively transferred into 9 mL of Muller-Kauffmann Tetrathionate-Novobiocine broth (AES Chemunex, Combourg, France), incubated at 37 °C for 18–24 h and into 10 mL of Rappaport Vassiliadis Soja broth (AES Chemunex, Combourg, France), incubated for 24 h at 42 °C. Afterwards, the enriched samples from each tube were sown onto Hektoen and XLD agar (AES Chemunex, Combourg, France) and incubated at 37 °C for 24 h.

Presumptive *Salmonella* colonies were picked and grown on nutrient agar (AES Chemunex, Combourg, France) for purification, and confirmed by biochemical tests using the Microgen ID-GNA gallery for enterobacteria (AES Chemunex, Combourg, France). Potential *Salmonella* spp. strains were serotyped by glass slide agglutination using *Salmonella* polyvalent O and H antisera (Bio-Rad, Marne la coquette, France), according to the White-Kauffmann-Le Minor Scheme (Guibourdenche et al., 2010).

To decrease in cost and time, some *Salmonella* strains isolated during our second campaign were serotyped according to the Premi® Test *Salmonella* (PTS) method

(Check-Points BV, Wageningen, The Netherlands). The PTS principle is called multiplex ligation detection reaction to generate a collection of circular DNA molecules that are subsequently PCR amplified by means of a single pair of amplimers. The PCR products are then sorted by hybridization to low density DNA microarray. Positive hybridization is detected using a biotin label incorporated into one of the PCR primers (Wattiau et al., 2008).

2.4. Antimicrobial susceptibility tests

Antimicrobial susceptibility was tested using the disc diffusion method on Mueller-Hinton agar (Bio-Rad), according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2009). The 16 antibiotics tested were ampicillin (10 μg), amoxicillin + clavulanic acid (20, 10 μg), cephalotin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), sulphonamides (300 μg), cotrimoxazole (1.25/23.75 μg), gentamicin (10 μg), streptomycin (10 μg), kanamycin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), colistin (50 μg), nalidixic acid (30 μg), ofloxacin (5 μg), and enrofloxacin (5 μg). Zone diameters were read by the automated Osiris scanner (Bio-Rad) and interpreted with CLSI quidelines (CLSI, 2011). *Escherichia coli* ATCC 25922 was used as quality control.

3. Results

3.1. Prevalence of Salmonella infection in laying hens and broiler chickens

The 16 farms studied corresponded to a very low investment and were modest (average capacity: 1000 subjects per band), generally with one or two buildings (13/16 farms). The density of birds ranged between 6 and 13 subjects/m^2 (10/14 farms) and the floor was covered by a litter of hacked straws of doubtful quality. Drinking water sources were from open wells or boring (12/16 farms). Some farms studied (10/16 farms) had a fence in grilling, but in poor condition with free access to stray animals (dogs, cats, ducks, rodents, reptiles..). A total of 128 pooled samples collected from 14 laying hen farms and 2 broiler chicken farms in N'Djamena, were analyzed for the presence of *Salmonella* and of these, 56 (43.75%) pooled samples were positive for *Salmonella*. The prevalence rate of *Salmonella* isolates from droppings, sterile Cloths, feeds and water are shown in Table 1.

3.2. Distribution and antimicrobial susceptibility of Salmonella serotypes

Out of a total of 84 *Salmonella* isolates, 27 different serotypes were identified. The most frequent serotypes were *Salmonella* Colindale (19%) and *Salmonella* Minnnesota (18%), followed by *S.* Havana and *S.* Riggil (each 6%), *S.* Kottbus and *S.* Amager (4,7%), *S.* Idikan, Mississipi, and Muenchen (3.6%). Other serotypes were poorly represented. The overall of 16 poultry farms tested were positive for *Salmonella* and the farms 3, 9, and 16 were the most contaminated (Table 2).

Among the 84 *Salmonella* isolates tested for resistance to 16 different antimicrobials, 28 (33.3%) isolates belonging to three different serotypes were resistant. This resistance concerned 12 isolates of *S.* Colindale that exhibited decreased susceptibility to fluoroquinolones, 1 *S.* Limete resistant to three antimicrobials and 15 *S.* Minnesota isolates resistant to five different antimicrobial classes (Table 2).

4. Discussion

4.1. Prevalence of poultry farm contamination

Before our study, there was no recent information available on *Salmonella* prevalence on Chadian poultry farms. Indeed, to discuss *Salmonella* prevalence in the N'Djamena area, it is necessary to look back to the 1960s and '70s to gather information from several studies by Vigier and et Chamoiseau (1967); Le Minor et al. (1969). These studies have widely shown the diversity and the dissemination of *Salmonella* strains in animal species during this time.

In the present study, 10.7% of *Salmonella* isolates were from samples collected from the two broiler chicken farms with 50 day-old flocks. This result is not representative of the N'Djamena broiler chicken population because of the small number of samples from broiler farms and should not be interpreted as the effective prevalence rate of *Salmonella* infections in these farms. However, it allows to underline the question of sanitary control and the high contamination of broiler chicken farms.

The prevalence rate of *Salmonella* isolates was also important in laying hen flocks, aged from 2 to 23 months according to farms (Table 2). This study showed that *Salmonella* excretion by birds occurs at all ages of rearing

Table 1
Prevalence of *Salmonella* per sample and poultry flock types.

Sample types	Number of pooled samples					
	Laying hen flocks ($n = 112$)		Broiler chicken flocks ($n = 16$)		Total ($n = 128$)	
	Examined	Positive (%)	Examined	Positive (%)	Examined	Positive (%)
Droppings	42	13 (11.60)	6	3 (18.75)	48	16 (12.50)
Litter samples	42	16 (14.29)	6	4 (25.00)	48	20 (15.63)
Feed	14	10 (08.93)	2	2 (12.50)	16	12 (09.37)
Water	14	8 (07.14)	2	0	16	08 (06.25)
Total	112	47 (41.96)	16	9 (56.25)	128	56 (43.75)

Table 2
Characterization of poultry and *Salmonella* strains isolated from16 farms around N'Djamena.

Farms	Characteristics of poultry and *Salmonella* isolates				
	Age of birds and reared flock types	Number of isolates by sources (*n*)	Serotypes	Prevalence rate/farms	Antimicrobial resistance phenotypes
1	23 months/laying hens	Droppings (2), food (1) Litter (1) Litter (2)	*S.* Havana *S.* Kalina *S.* Idikan	7.14%	Pan-susceptible
2	4 months/laying hens	Litter (2) Litter (2) Litter (2), droppings (2) Food (1)	*S.* Muenchen *S.* Gamaba *S.* Amager *S.* Eppendorf	4.76% 11.9%	
3	7 months/laying hens	Droppings (2) Droppings (1) Litter (1) Litter (1)	*S.* Oakland *S.* Muenchen *S.* Gloucester *S.* Typhimurium		
4	6 months/laying hens	Water (1) Food (2)	*S.* Kottbus *S.* Colindale	3.57%	
5	8 months/laying hens	Food (3)	*S.* Mississipi	3.57%	
6	48 days/broiler	Droppings (3), litter (3), food (1)	*S.* Colindale	8.33%	NA[a], OFX[a], ENR[a]
7	50 days/broiler	Litter (1) Food (1)	*S.* Limete *S.* Colindale	2.38%	SXT, SSS, TET Pan-susceptible
8	12 months/laying hens	Water (2) Food (1)	*S.* Bokanjac *S.* Havana	3.57%	
9	7 months/laying hens	Droppings (4), water (1) litter (1) Litter (2) Food (2)	*S.* Colindale *S.* Colindale *S.* Wilhelmsburg *S.* Ried	11.9%	NA[a], OFX[a], ENR[a] Pan-susceptible
10	13 months/laying hens	Food (1), water (1) Food (1), litter (1)	*S.* Telelkebir *S.* Kinondoni	4.76%	
11	14 months/laying hens	Water (1) Droppings (1)	*S.* Winterthur *S.* Virchow	2.38%	
12	17 months/laying hens	Litter (1) Litter (1), water (2) Litter (1) Food (1) Litter (1)	*S.* Stanley *S.* Kottbus *S.* Winterthur *S.* Tennessee *S.* Enteritidis	8.33%	
13	4months/laying hens	Droppings (1)	*S.* Minnesota	1.19%	AMP, CHL, SSS, SXT, STR, TET
14	5 months/laying hens	Litter (2), water (1)	*S.* Minnesota	3.57%	
15	3 months/laying hens	Food (2), water (3) Water (1) Food (1)	*S.* Riggill *S.* Hull *S.* Havana	8.33%	Pan-susceptible
16	2 months/laying hens	Droppings (5), litter (6) Food (1)	*S.* Minnesota *S.* Idikan	14.28%	AMP, CHL, SSS, SXT, STR, TET Pan-susceptible
Total of *Salmonella* isolates		84		99.96%	

AMP: ampicillin; CHL: chloramphenicol; ENR: enrofloxacin; NA: nalidixic acid; OFX: ofloxacin; SSS: sulphonamides; STR: streptomycin; SXT: trimethoprim-sulfamethoxazole (cotrimoxazole); TET: tetracycline.
[a] Decreased susceptibility, pan-susceptible means susceptible to all antibiotics (16) tested.

period. This is certainly because of stress factors such as the onset of laying, high temperatures and the end of the production period (Humphrey, 2006). We found that the prevalence of *Salmonella* isolates was significantly higher in litter samples (35.7%) than in other pooled samples (faeces, feed and water). The relative resistance of *Salmonella* to desiccation (Davies and Wray, 1996) might justify the higher probability of isolating *Salmonella* from litter samples than from droppings, food and water samples, where the competitive flora is likely to be important. This finding may be explained by the choice of the bacteriological and sampling methods used in our study, which were very sensitive in flocks during our investigations for isolating *Salmonella* serotypes or by the combination of bad cleaning and disinfection of both equipment and surfaces such as floor, walls or use of untrained personnel.

According to Davies and Breslin (2003), the high contamination encountered in caged flocks was not due to a higher susceptibility of the sampling method but may rather be linked to a failure to properly clean and disinfect the poultry house or to an insufficient sanitary control. The majority of the infections in laying hens seem to be attributed to the persistent contamination of the farm. Several studies suggested that measures to limit vertical and horizontal transmissions include ensuring *Salmonella*-free feed and water, effective cleaning and disinfection of the farm, applying appropriate measures against animated and unanimated vectors (Humphrey, 2006; Wales et al., 2007). These findings seem to corroborate with the high

prevalence rate showed by our study and are in agreement with the general infrastructure of the poultry farms involved in our study which are not standardized and with their very low bio-security measures as the control against stray animals, rodents, reptiles and amphibians which were not well done. This suggests in term that sanitary measures at the flock level contribute to a significant reduction of the *Salmonella* contamination in poultry farms of N'Djamena.

4.2. Distribution of Salmonella serotypes

Salmonella isolated from 56 positive samples were of 27 different serotypes. Among the 84 *Salmonella* isolated during our study, the distribution of serotypes is very heterogeneous and unique because of their large diversity. During our study, the most frequent serotypes worldwide, *Salmonella* Enteritidis and Typhimurium (EFSA, 2011), were poorly isolated (Table 2).

To our knowledge, *S.* Colindale, the most prevalent serotype in our study, was first described in 1955 from a woman suffering from acute diarrhoea in London (Audrey and Holt, 1955). Vigier and et Chamoiseau (1967), described also two isolates of *S.* Colindale from human faeces and water samples in N'Djamena. In a recent report from Gambia, *S.* Colindale is described as the most common (21.42%) serotype of NTS in cases of enteric infection under the age of 5 years (Dione et al., 2011). We believe that in animal infections, particularly in poultry, this is the first description of this serotype. In poultry farms sampled, birds were asymptomatic carriers. Indeed *Salmonella* can persist in the chicken caecum or ovaries without triggering clinical signs in the host. The asymptomatic *Salmonella* carrier state in poultry has serious consequences for food safety and public health due to the risk of food poisoning following consumption of contaminated products.

Salmonella Minnesota was the second most frequent serotype (18%) in our study and isolated from 3 laying hen farms. *S.* Idikan was described in the past by Vigier and et Chamoiseau (1967), from eggs of hen laid without shell and soiled by the droppings. Five isolates of *S.* Riggil and 4 isolates of *S.* Amager were isolated from 2 different farms. They were isolated in the past by Le Minor et al. (1969) from reptiles, known as the important reservoirs of *Salmonella* species. Our study revealed 3 isolates of *S.* Muenchen from droppings and swabs samples in 2 different farms. This old serotype has been implicated in outbreaks associated with consumption of raw alfalfa sprouts in Madison, USA (Proctor et al., 2001). The other serotypes were variously represented in the different poultry farms.

4.3. Antimicrobial resistance of the Salmonella isolates

Our study revealed that the majority of *Salmonella* serotypes were susceptible to all antibiotics tested, except some *S.* Colindale isolates that exhibited decreased susceptibility to fluoroquinolones, *S.* Minnesota isolates resistant to five different antimicrobial classes and *S.* Limete isolate resistant to three antibiotics as listed in Table 2. *Salmonella* Colindale isolates resistance concerns its decreased sensitivity to Nalidixic acid, Ofloxacin and Enrofloxacin. In the past, *Salmonella* Colindale strains did not seem to harbour any specific resistance pattern (Dione et al., 2011). Nevertheless, one isolate was described as carrying the *qnrB1* gene encoding for resistance to fluoroquinolones, associated with a resistance to ampicillin and cefotaxime (Murray et al., 2008). *S.* Limete resistant to Sulphonamides, Trimethoprim and Tetracycline. As well, *Salmonella* Minnesota isolates that were Multi-drug resistant to Ampicillin, Chloramphenicol, Trimethoprim, Sulphonamides, Streptomycin and Tetracycline. To our knowledge no previous study has reported a MDR of *S.* Minnesota. This MDR phenotype could be attributed to MDR genes located in the genetic structure named SGI1 (*Salmonella* Genomic Island 1) associated with antimicrobial resistance in some S. Typhimurium phage types and gram negative organisms (Mulvey et al., 2006).

It has to be noticed that, even if in most cases, salmonellosis is a self-limited disease, in some complicated cases, antibiotic treatment is required for patient recovery. The usual treatment is then either a beta-lactam (i.e. ampicillin) or a fluoroquinolone (i.e. ciprofloxacin) or sulphonamides + trimethoprim (cotrimoxazole). Even if in this study, we detected very few resistances, they are of public health concern as they affected the first line empiric treatment of invasive salmonellosis.

5. Conclusion

The results of our study provide representative information concerning prevalence rate (43.75%) and resistance on *Salmonella* contamination in broiler and laying hen farms of N'Djamena. In this study, we revealed that the sixteen poultry farms tested for *Salmonella* were all positive. We have also shown the great diversity among the *Salmonella* serotypes isolated and a small proportion of antimicrobial resistant isolates during our present study. These results highlight the necessity to create more environmental and personnel hygiene awareness among Chadian farmers, because many managers do not understand the good practice of sanitary measures. We point out that the comparison with human *Salmonella* isolated at the same time, based on some molecular methods to assess the possible contribution of avian *Salmonella* to human salmonellosis is under way.

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