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Research Article

OCCURRENCE OF SALMONELLA SPP. SEROTYPES ISOLATED FROM THE POULTRY EXPLOITATIONS IN N'DJAMENA AND DOBA TOWNS-CHAD

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ABSTRACT

The aim of this study was to assess the prevalence of different serotypes of *Salmonella* spp. strains in poultry farming in N'Djamena and Doba towns. The strains were isolated on Hektoen agar medium, then identified through API 20E gallery, and serotyped by the direct agglutination technique on slide according to Kauffmann and White scheme. From a total of 1090 samples analyzed, 40 *Salmonella* strains corresponding to a prevalence of 3.67 % were identified, and regrouped into 11 serotypes: *Salmonella* Idikan (20%, n=8), *Salmonella* Anatum (15%, n=6), *Salmonella* Mbandaka (12.5%, n=5), *Salmonella* Limete, *Salmonella* Infantis, *Salmonella* Derby (10%, n=4), *Salmonella* Virchow (7.5%, n=3), *Salmonella* Gallinarum, *Salmonella* Choleraesuis (5%, n=2), *Salmonella* Paratyphi A, *Salmonella* Enteritidis (2.5%, n=1). Each sample taken apart contained at least one serovar, indicating that poultry farming in Chad is seriously affected by *Salmonella*. Moreover, *Salmonella* Paratyphi A and two strains of *Salmonella* Choleraesuis and peculiar respectively infecting man and pig were also isolated, probably due to fecal contamination of poultry food or water. These results reveal for the first time, a wide distribution of *Salmonella* spp. serovars in modern and traditional poultry farming sectors in N'Djamena and Doba in Chad.

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INTRODUCTION

Animal's reservoirs of *Salmonella* are numerous, of which livestock are found in industrialized countries (Rostagno *et al.*, 2006; Sánchez-Vargas *et al.*, 2011). Salmonellosis infections are among the most regular diseases of food reported in the world (WHO, 2000) and is mainly transmitted by ingestion of contaminated food or water. The causative agents are transmitted by several pathways, but chickens of commercial farms have been identified as one of the most important one (WHO, 2000). Considering this factor, FAO and WHO have made risks assessments related to the presence of *Salmonella* in broiler chickens (Salm-Surv, 2005). These assessments have enabled an overview of the available knowledge on *Salmonella*.

Although there is little specific data on the burden of food borne diseases associated with *Salmonella* in chicken, it is estimated that this load is important. The risk, however, varies according to control measures and methods implemented

throughout the chain, from primary production up to the final preparation for consumption (FAO/WHO, 2009).

Salmonella is one of the main microbiological contaminants responsible for food borne infections in Europe. More than 99 000 cases of human salmonellosis have been reported in Europe (EFSA, 2012). In France, the number of Collective Food Borne Toxi-Infections (CFBTI) attributed to *Salmonella* is in constant decline since 2002, and have remained stable between 2009 and 2010 (Weill and Hello, 2011). In 2010, this bacterium originate from 141 CFBTI homes, corresponding to 1357 cases of human infection of food origin (Lailler *et al.*, 2012). The majority of foods infected have been reported to be chicken and eggs (Parrys *et al.*, 2002; Flint *et al.*, 2005). The presence of these micro-organisms in the chicken also affects the trade, with deep economic impacts. The impact on human health and the associated costs, the disruption of trade and the cost of the implementation of effective measures to control have obliged the Commission of the Codex Alimentarius

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(2007) to prioritize the development of guidelines on the control of *Salmonella* in chickens.

The identification and characterization of *Salmonella* remain thus essential for the epidemiological survey of contamination along the influenza chain, and the control of this pathogen. Very little data are available at national level on the distribution of *Salmonella* serotypes in poultry. However, some disturbing information has risen up a tail of sailing on the epidemiology of salmonellosis in laying farms eggs chickens (Djim-Adjim et al., 2013).

Therefore, the objective of this study was to identify the different *Salmonella* spp. serotypes and investigate on their distribution in the poultry farms of N'Djamena and Doba towns.

We hypothesized that *Salmonella* spp. serotypes are widely distributed in the poultry farms of N'Djamena and Doba towns. Results on these investigations are discussed.

MATERIALS AND METHODS

Study Area

The study was conducted in two towns of Chad: N'Djamena, which is the political capital city in the centre, and Doba, the oil city in the south of the country. Twenty-six poultry farms production were selected: 10 flesh chickens farms in N'Djamena during the periods of intensive activity (November 2014 to January 2015), and 16 traditional chickens farms in Doba during the rainy (June to August 2014) and dry (March to May 2015) seasons. The bacteriological analyses were carried out in the general bacteriology Laboratory of the Institute for Research in Breeding for the Development (IREDD) of N'Djamena, Chad.

Bacteriological analyses

The isolation and confirmation of bacteria were carried out following the reference method NF/EN ISO 6579: 2002 (Anon, 2002), while the serotyping was performed using the agglutination technique on blade according to Kauffmann and white scheme (Kauffmann, 1966).

Isolation of *Salmonella* spp.

Each pre-enriched sample to 1/10 with water buffered peptone was homogenized on a vortex for 2 mn, then left for revivication at ambient temperature for 30 mn, before incubation at 37°C for between 18-20 h. The Rappaport Vassiliadis Soyabean (RVS) (Ref: 7730A) medium (10 ml) was inoculated with 0, 1 ml pre-fortified sample, while 1 ml was instead used to inoculate 10 ml of the Mueller-Kaufmann Tetrathionate (MKTTn) (Ref: 9221A) medium. Whereas the RVS medium was incubated at 42°C, the MKTTn medium was incubated at 37°C for 24 hours. The selective media Hektoën agar (Réf: 51050) and Xylose Lysine Desoxycholate (XLD) (Ref: 51049) stocked from the fortified products were incubated at 37°C for 24 h. Five characteristic colonies were selected and transferred onto Hektoen agar medium for the first purification, before the second 24 hours later (Anon, 2002).

Pre-identification of *Salmonella* spp.

Each pure culture was subjected to Gram stain and oxidase test (Korsak et al., 2004). Gram negative bacilli and oxidase

positive were recognized as non-pathogenic enterobacteria and discarded. On the reverse, Gram negative bacilli and oxidase negative, presumably enterobacteria were differentiated by sub-cultured on Kligler-Hajna agar (Ref: 51059) slope (CNR *Salmonella*, 2009). The typical *Salmonella* cultures corresponded to an alkaline slant (red), or an acidic pellet (yellow), with gas formation for approximately 90% of cases, and hydrogen sulphide production darkness agar.

Identification of *Salmonella* spp.

The biochemical identification of *Salmonella* was performed by culturing the inoculum on the API Gallery® 20E (Bio-Mérieux). The principle is based on the inoculation of microtubules with a suspension that rehydrates the environments. Incubation was conducted at 37°C in a bacteriological oven for 24 hours during which the biochemical reactions such as decarboxylation, fermentation and deamination are supposed to take place to release spontaneous colored products that are revealed by addition of reagents (Swanson and Collins, 1982). The identification of *Salmonella* was obtained using the API catalog® 20E, according to the French Society of Microbiology (FSM) instructions.

Serotyping of *Salmonella* spp

Salmonella strains were serotyped by direct agglutination technic on blade, based on the association of O-somatic and flagellar H antigens, according to Kauffmann and White diagram (Kauffmann, 1966; Institute of Food Technologists, 2006; Grimont et al., 2007).

The determination of serotypes is the combination of the antigenic formula corresponding to the "O" and "H" antigens expressed in various agglutination tests on blade of pure culture as obtained in 24 hours on ordinary agar medium. Prior to determination, *Salmonella* strains were checked out not to be in phase R (phase where self-agglutination occurs). For strains unable to undergo auto-agglutination, agglutination test was successively conducted with O-polyvalent (OMA, OMB, OMC) and O-monovalent anti sera, then H-anti (HMA, HMB, HMC and H1) sera, according to Kaufmann-white diagram (Kauffmann, 1966). *Salmonella* strain with antigen against the tested antiserum formed agglutinates visible to the naked eye.

In practice, the polyvalent OMA and OMB sera are supposed to enable the determination of 99% *Salmonella* strains. The use of O-monovalent anti-sera enable the specification of the group to which belongs the *Salmonella* strains. The Kauffman-White-minor table (Grimont and Weill, 2007; Guibourdenche et al., 2010) was used to determine the antigenic formula and reading of serotyping results.

Data analysis

The quantitative data were saved using the Excel software (Microsoft Office Corporation 2010) and analyzed with the Statistical Package for the Social Sciences, 17.0 version (SPSS). The Chi-square of person was used to evaluate and compare the frequences and distribution rates of different serotypes (Huneau et al., 2007).

RESULTS

In general, all the bacterial colonies isolated (40) presented the biochemical profiles such as Glucose (+), Lactose (+), ONPG

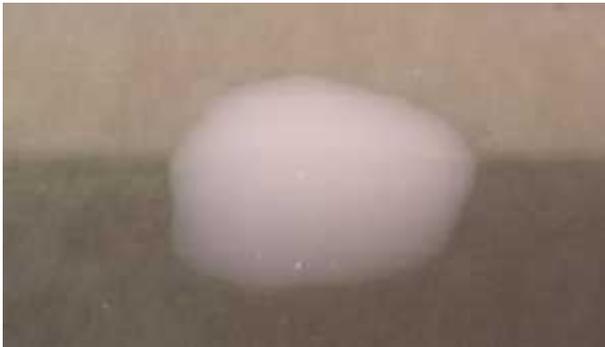
(-), urease (-), indole (-), with hydrogen sulphide (H₂S) production.

Antigenic characterization of Salmonella spp. Strains

Figure 1 illustrates aspects of *Salmonella* spp. strains in suspension within the antiserum test. The strains possessing antigens able to recognize antibodies present in the antiserum have formed aggregates (Fig 1A). In contrast, strains with antibodies unable to recognize these antigens took a milky appearance without agglutination (Fig 1B).



A



B

Figure 1 *Salmonella* spp. in the presence of antiserum: A) agglutination; B) no agglutination

Distribution of Salmonella spp. Serotypes

Serotyping of isolated *Salmonella* spp. strains enabled identification of eleven (11) different serotypes (Table 1), including: *S. Idikan*, the majority serotype (20%), *S. Anatum* (15%), *S. Mbandaka* (12.5%), *S. Limete*, *S. Infantis* and *S. Derby* (10%), *S. Virchow* (7.5%), *S. Gallinarum* and *S. choleraesuis* (5%), *S. Paratyphi A* and *S. Enteritidis* (2.5%).

Globally, the serotypes *Paratyphi A* and *Mbandaka* were not present in Doba, while in N'Djamena, serotypes *Enteritidis*, *Virchow*, *Gallinarum*, *Choleraesuis* and *Derby* were absent. The number of serotypes expressed in percentage was mostly higher in Doba than in N'Djamena. Significant differences (p<0.05) were observed respectively for the *Derby* serovars, *Mbandaka*, *Anatum* and *Idikan*, between Doba and N'Djamena samples. The proportions were respectively higher for *Mbandaka* (12.5%), *Anatum* (10%) and *Idikan* (17.5%) in N'Djamena, and for *Derby* (10%) in Doba.

A broad distribution was also noticed for serotypes *Idikan*, *Anatum* and *Mbandaka*, found in more than half of the infected farms and in equivalent proportions for almost all type samples from N'Djamena (Table 2). Moreover, the serotypes *Infantis*, *Limete* and *Paratyphi A* were found only in one farm for one

type of sample each. No serotype was found in the floor droppings.

Table 1 Distribution of serotypes per town

N°	Antigenic formula	Serotypes	Number of isolates (%)		P-value
			Doba	Ndjamaena	
1	1, 4, 12: f,g	<i>S. Derby</i>	4 (10)	0 (0)	0.045
2	6,7: r 1,5	<i>S. Infantis</i>	3 (7.5)	1 (2.5)	0.134
3	6,7: c 1,5	<i>S. Choleraesuis</i>	2 (5)	0 (0)	0.168
4	1,9,12	<i>S. Gallinarum</i>	2 (5)	0 (0)	0.168
5	1,2,12: a	<i>S. Paratyphi A</i>	0 (0)	1 (2.5)	0.287
6	6,7: r 1,2	<i>S. Virchow</i>	3 (7.5)	0 (0)	0.087
7	1,4,12: b 1,5	<i>S. Limete</i>	3 (7.5)	1 (2.5)	0.134
8	1,9,12: g,m	<i>S. Enteritidis</i>	1 (2.5)	0 (0)	0.335
9	6,7: z ₁₀ e,n,z ₁₅	<i>S. Mbandaka</i>	0 (0)	5 (12.5)	0.012
10	3,10: e,v 1,6	<i>S. Anatum</i>	2 (5)	4 (10)	0.041
11	1,13,23: i 1,5	<i>S. Idikan</i>	1 (2.5)	7 (17.5)	0.011

Table 2 Distribution of serotypes within N'Djamena farms

Semi-industrial farms	Origin sample	Serotypes	Number of isolates
1	Cloac	<i>S. Infantis</i>	1
		<i>S. Mbandaka</i>	1
	Food	<i>S. Anatum</i>	1
2	Food	<i>S. Mbandaka</i>	2
		<i>S. Paratyphi A</i>	1
	Cloac	<i>S. Idikan</i>	2
		<i>S. Limete</i>	1
3	Water	<i>S. Idikan</i>	3
		<i>S. Anatum</i>	1
	Water	<i>S. Anatum</i>	1
Water		<i>S. Idikan</i>	2
	Water	<i>S. Mbandaka</i>	2
Water		<i>S. Anatum</i>	1
	Total		19

In Doba only one type of sample was encountered (Table 3). The serotypes *Derby* and *Virchow* were the most widely distributed, followed by *Infantis*, *Limete*, *Anatum* and *Gallinarum* found each in two of the six contaminated farms. The weakly distributed were *Choleraesuis*, *Enteritidis* and *Idikan*.

Table 3 Distribution of serotypes within Doba farms

Traditional farms	Origin sample	Serotypes	Number of isolates
1	Cloac	<i>S. Derby</i>	1
		<i>S. Infantis</i>	1
2	Cloac	<i>S. Virchow</i>	1
		<i>S. Limete</i>	1
		<i>S. Infantis</i>	2
3	Cloac	<i>S. Choleraesuis</i>	2
		<i>S. Derby</i>	1
		<i>S. Virchow</i>	1
		<i>S. Anatum</i>	1
4	Cloac	<i>S. Enteritidis</i>	1
		<i>S. Limete</i>	2
		<i>S. Anatum</i>	1
5	Cloac	<i>S. Virchow</i>	1
		<i>S. Gallinarum</i>	1
		<i>S. Gallinarum</i>	1
6	Cloac	<i>S. Derby</i>	1
		<i>S. Derby</i>	1
Total		<i>S. Idikan</i>	1
			21

DISCUSSION

Results obtained from this study have revealed 40 *Salmonella* spp. strains identified in N'Djamena and Doba farms, representing an overall prevalence of 3.67%, which falls within the 0%-18% range prevalences observed within the European

Community (Anonymous, 2006). However, this finding was lower than 43.75% and 62.5% prevalences reported respectively in N'Djamena-Chad (Djim-Adjim et al., 2013), and in Dakar-Senegal (Fofana, 2004), but remained higher than 1.66% obtained in Constantine-Algeria (Elgroud et al., 2009). From the serotyping experiments *S. Idikan* (20%, n=8), followed by *S. Anatum* (15%, n=6), *S. Mbandaka* (12.5%, n=5), *S. Limete*, *S. Infantis* and *S. Derby* (10%, n=4), *S. Virchow* (7.5%, n=3), *S. Gallinarum* and *S. Choleraesuis* (5%, n=2), *S. Paratyphi A* and *S. Enteritidis* (2.5%) were identified with an isolate each.

The number of serovars identified was important (11 serovars for 40 strains). They were potentially pathogens and different from those isolated by Djim-Adjim et al. (2013), and Elgroud et al. (2009). This can be explained by the fact that some serotypes may appear or suddenly disappear from one year to another (Ghafir, 2006). On the other hands, this distribution was very close to the results of a community study conducted in Europe, which reported *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Mbandaka* and *S. Hadar* as the most frequent serovars in chicken of flesh (Devos, 2007), although *S. Typhimurium* and *S. Hadar* were not revealed in our samples. *S. Idikan* and *S. Anatum* were isolated from more than half of the farms and in equivalent proportions for almost all samples type. This may suggest that the strains of these serotypes would be indigenous to these farms and their environment. This situation could be linked to the bad hygienic practices, and lack of prophylactic measures applied to rearing buildings and equipments (Garber et al., 2003; Wales et al., 2007; Murase et al., 2001).

It has been reported that when livestock is infected by *Salmonella*, bacteria may persist if the cleaning and disinfection measures are not adequate (Lahellec et al., 1986; Baggessen et al., 2000). Studies in N'Djamena indicated the presence of *S. Idikan* on samples eggs laid without shells and contaminated by chicken manures (The Minor et al., 1969). These observations were also found in 2 other farms of laying eggs chicken in N'Djamena (Djim-Adjim et al., 2013). However, there is still little information about the environmental reservoirs of *Salmonella*, particularly in the African context (Morpeth et al., 2009).

S. Mbandaka was present in drinking water and poultry food. The food provided to animals may be contaminated directly either by raw material or indirectly during the manufacturing process, the storage, transport or distribution. In 2002, the most frequently reported serotypes in the European Community (EC) in chicken exploitations were *Enteritidis* (42%), *Mbandaka* (8.8%), *Livingstone* (6.4%), *Typhimurium* (4.5%) and *Senftenberg* (3%) (European Commission for Health, 2005).

S. Infatis, *Limete*, *Derby*, and *Virchow* were isolated from the animal cloacs. In the infested farms, some animals showing no visible sign of disease are known as carriers. These asymptomatic animals are one of the main sources of microbial contamination of food. Their intestine is the privileged place of colonization and persistence by *Salmonella* (Virlogeux-Payant et al., 2012).

In an European Food Safety Authority (EFSA) report, it has been stated that *S. Infantis* and *S. Virchow* are the most frequent serovars in chicken supply chain, after *S. Hadar*

(Anonymous, 2007). The emergence of *S. Infantis* in Israel in the case of human infections since 2007 was correlated with a higher isolation frequency of this serovar in poultry (Gal-Mor et al., 2010).

Contrary to several studies conducted throughout the world, of which the majority isolated serotypes were *S. Enteritidis* and *S. Typhimurium* (Aboun et al., 2003; European Food Safety Authority Journal, 2011; Ottomo et al., 2007), the present study has revealed only one *S. Enteritidis* strain isolated from chicken cloac. This low level of contamination has been reported by the CNR the strains of human origin isolated between 2002 and 2010 (Jourdan-Da Silva and Hello, 2012). This decrease is likely due to the impact of the control measures and management applied over the past few years in avian sector.

It is important to note that during the course of our study, it was isolated from the cloac of traditional chickens a *S. Gallinarum* strain that causes typhoid (Carli et al., 2001; Shivaprasad and Barrow, 2008). This could be due to a lack of a health monitoring survey program for operation system. A survey conducted in 5 laying hens farms in Batna-Algeria has highlighted 4 contaminated farms with 3 different serotypes, namely *Typhimurium*, *Gallinarum-Pullorum*, and *Wien* (Ayachi et al., 2010). A related work on the breeding of local poultry in sub-Saharan Africa has pointed out a sero-prevalence to *S. Gallinarum* with 5.8% to 22.3% (Boko et al., 2012). One strain of *S. Paratyphi A* and two strains of *S. choleraesuis* were isolated, specific to man and pig intestines, their presence being related to fecal contamination (Bornet, 2000).

Some serovars affect only some hosts, although most of the serovars can cause infections in a wide variety of animals. Pigs are the sensible hosts to numerous serotypes of *Salmonella* and constitute the main reservoir of *S. Choleraesuis* (Kauffmann, 1966; Proux et al., 2001). Although strongly linked to pigs, the serotype *Choleraesuis* is transmissible to poultry and the contamination is through the digestive tract (Gransart, 1998). The emergence of a salmonellosis depends on the virulence of the strain (Van Asten and Van Dijk, 2005) and the host physiology.

All strains of *Salmonella* are potentially pathogenic to man, but some are particularly pathogens, such as *S. Typhi*, *S. Paratyphi A*, *S. paratyphi C* and *S. Sendai* causing typhoid (ICMSF, 1996 ; D'Aoust, 2001 ; NRCSS, 2004).

The study has demonstrated that all inputs are potential sources of salmonellosis. In poultry farming chickens infected by oral route, the intestine is the privileged milieu of colonization and persistence of *salmonella*.

The whole of these epidemiological data highlights the real impact of *Salmonella* infections that is not typhoid invasive in Africa and the associated risk factors. This implies the need of improving our current knowledge on environmental risk factors that would underline the propagation and the contamination processes. The results of this study come to elucidate the dark epidemiological table of *Salmonella* infections in the African context, and particular in Chad where no system of alert or effective health monitoring exist.

CONCLUSION

The body of data generated in this study is to our knowledge, the first epidemiological information related to the contamination of streams broilers and traditional chickens in Chad. The most frequently isolated serotypes have been revealed as *S. Idikan*, *S. Anatum*, *S. Mbandaka*, *S. Limete*, *S. Infantis*, *S. Derby*, *S. Virchow* which constitute worrying indicators of the threat due to multi-serotypical *Salmonella* infections within the Influenza sector in Chad. This situation is probably due to the lack of health monitoring programs, but also to lack of required hygienic and biosecurity measures. This work thus evidences the need of establishing an epidemiological survey as well as appropriate sanitary measures to be applied to control *Salmonella* infections in Chad.

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