

## Characteristics of poultry farms and assessment of their level of contamination by *Salmonella* spp. and *Escherichia coli* in the towns of N'Djamena and Doba in Chad

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### Summary

The main purpose of this study is to evaluate the characteristics of poultry farms and the level of contamination of chickens farmed in the towns of N'Djamena and Doba by *Salmonella* spp. and *Escherichia coli*. Information, based on both formal and informal surveys, was collected from ten broiler chicken farms in N'Djamena and sixteen traditional farms in Doba, mainly small establishments with low levels of investment. A total of 1,090 samples were taken from these farms over three periods, each of three months, from June to August 2014 then from March to May 2015 for Doba, and from November 2014 to January 2015 for N'Djamena. The NF EN ISO 6579 method and the seeding of a specific eosin methylene blue (EMB) agar were used to

isolate 105 bacteria strains, including 65 (5.96%) strains of *E. coli* on five farms, giving a contamination level of 19.23%, and 40 (3.67%) strains of *Salmonella* on ten farms, with a contamination level of 38.46%. These high prevalences highlight, for the first time to the authors' knowledge, the contamination levels of the broiler chicken and traditional chicken value chains by *Salmonella* and *E. coli* in N'Djamena and Doba.

### **Keywords**

Chad – Poultry farm – Chicken – Contamination – *Escherichia coli* – Prevalence – *Salmonella* spp.

### **Introduction**

Foodborne diseases are one of the leading causes of morbidity and mortality worldwide. The World Health Organization (WHO) estimates that 2 million people die each year from diarrheal infections (1, 2). Among these, colibacillosis and salmonellosis are a real or potential problem in the majority of developing countries (3, 4, 5). They are a source of unease in many sectors and a continuing worry for the general public. They are mobilising consumption, production and marketing channels for products of animal origin and are a cause of concern for researchers responsible for public health (1).

Several strains of *E. coli* have been identified as the aetiological agent for infantile diarrhoea in humans, and for diarrhoea, gastroenteritis, urinary infections, meningitis and septicaemia in animals (2). In animals they are also responsible for mortality, poor performance and various reproductive disorders, and prevention costs are high. It is worth noting that, in the United Kingdom, the impact of avian colibacillosis results in an annual loss of six million euros (4). Salmonellosis is also of considerable importance in the veterinary and medical fields. It results in economic losses from declining production, seizures and the costs of prevention and control, as well as the high incidence of collective food poisoning, in a context where consumers now demand perfect food hygiene levels. Controlling contamination of

poultry meat by *Salmonella* has become vital for consumers and an economic argument for the industry (6).

Meanwhile, the emergence and continuing spread of antimicrobial resistance represents a public health problem. Resistance could compromise the effectiveness of treatments for human diseases, because it is now known that multi-resistant bacteria can be transferred directly from animals to humans and that the spread of resistant genes represents a real threat (7). Infection is also commonly associated with the consumption of meat and meat products, especially poultry-based products. Indeed, poultry plays a dominant role as a vector of transmission of human salmonellosis and colibacillosis (8).

In Chad, poultry numbers were officially estimated at 11 million in 1984, rising to 24 million in 1997 (9), representing annual growth of 10%. A recent review of the poultry sector in Chad, validated by the Food and Agriculture Organization of the United Nations in 2010, estimated poultry numbers at 47.8 million (10). These flocks are divided between semi-industrial farms and traditional family farms, and dominated by *Gallus gallus* domestic chickens (11).

Poor biosecurity practices on family farms and some inadequately maintained commercial units, coupled with limited access to veterinary services and medicinal products, are conducive to the persistence and spread of certain avian pathogens (12).

Very few reliable statistical data are available at national level on the prevalence of contamination by *Salmonella* and *E. coli*. Nevertheless, there are indications of a worrying spread of salmonellosis among laying hens on farms (13).

Therefore, this study sets out to determine the main characteristics of poultry farms in N'Djamena and Doba, as well as to evaluate the level of poultry contamination by *Salmonella* spp. and *E. coli* on these farms. The prevalence of infection and the distribution of strains in the different types of farms are also discussed.

## Materials and methods

### Study sites

The study was conducted in two towns in Chad: N'Djamena, the capital, and Doba, an oil town. The farms surveyed are located in urban and peri-urban areas of these two cities.

#### N'Djamena

N'Djamena is a century-old city, which borders Cameroon in the south-west of the country. It is located at the confluence of the country's two great rivers – the Logone and the Chari – and comprises ten districts (*arrondissements*). The most recent general census of the population and the environment recorded a population of 993,492 (14). According to the Ministry for Livestock directorate for studies and statistics, in N'Djamena there are 57 poultry farms, only 27 of which are in operation, with an estimated total annual throughput of around 30,000 birds.

#### Doba

Doba is the administrative capital of the Logone Oriental region, situated on the banks of the Pendé river, in the south-central part of the country at latitude North 8.660° and longitude East 16.850°. The town of Doba is divided into four *arrondissements* and has a population of 18,052 inhabitants (14). Poultry farming in the region is predominantly family-based, with a total of 3,911,139 birds and an estimated average of around 27 chickens per household (11).

The farms surveyed are located in the urban and peri-urban zones of N'Djamena and Doba.

### Sampling

In N'Djamena, the current study focused on ten of the 27 poultry farms, all of which were private. Farmers were approached indiscriminately to obtain agreement to study their farms, but many declined the offer, so the farms that were included in the study were those that had

volunteered. All the participating farms were concentrated in the first and ninth *arrondissements* and refusals related mainly to the fear of losing birds following the introduction of cloacal swabs.

In Doba, 16 family farms, including 10 in the town and 6 in Nangkesse (a village on the outskirts, around 3 km from the town centre), were surveyed. The *arrondissements* covered by the survey each had a minimum of one farm per district.

### **Formal and informal surveys**

The study used a participative approach in two stages:

- in the initial stage, an awareness-raising exercise was carried out to ensure acceptance by the farmers to be surveyed, followed by an inventory of farms
- a second visit then took place to take samples on the poultry farms.

The different points covered by the survey included: farm location and environment, infrastructure, farming equipment, hygiene measures (judged according to different criteria such as disinsectisation, deratting, equipment cleaning frequency, the observance of hygiene instructions by the personnel, washrooms, water, soap and special clothing), personnel, feeding, the use of veterinary medicinal products and the origin of feed. The survey form and the results are available from the corresponding author.

### **Samples**

All samples were taken by the same (previously trained) operators in three distinct campaigns, each lasting three months, with one in N'Djamena and two in Doba. Each sample was accompanied by a follow-up form mentioning the name of the operator, the date, type and time of the sample, the breed and age of the animals, their origin, the temperature on the day and any signs of disease.

## Samples in N'Djamena

In total, 545 samples were taken between November 2014 and January 2015 – a period of intense activity in the sector. On each farm, cloacal swabs were taken, together with samples of drinking water, feed and droppings on the bedding.

The dropping samples comprised three pools of two 5-gram samples of fresh droppings taken from one third of the total surface area of the building. Drinking water samples comprised three pools of two samples of 5 ml of water taken from three different watering troughs. Feed samples comprised three pools of two samples of 5 grams of feed taken from three different feeding troughs. All the pooled samples were placed in sterile stomacher bags. The cloacal samples consisted of 40 cloacal swabs (one sterile swab per chicken), which were then placed together in a sterile stomacher bag. The samples were placed in an insulated box with ice and icebags to maintain a temperature of 4°C, then delivered to the laboratory within a period of four hours, placed in the refrigerator and processed the following morning.

## Samples in Doba

The samples were taken during two separate campaigns. Two hundred and seventy-five samples were taken during the first campaign from June to August 2014, during the rainy season. A further 270 samples were taken during the second campaign from March to May 2015, during the dry season.

All the samples were taken from the cloaca. Twenty cloacal swabs (one sterile swab per chicken) were taken on each farm, then placed in sterile stomacher bags.

As in N'Djamena, the samples were placed in an insulated box with ice and icebags to maintain a temperature of 4°C, then delivered to the laboratory within a period of four hours, refrigerated and processed the following morning.

## Bacteriological analysis

Analyses were carried out at the general bacteriological laboratory of the Institute for Livestock Research for Development (IREL) in N'Djamena. The bacteria were isolated using the NF EN ISO 6579 method to detect *Salmonella*, and seeding of a specific eosin methylene blue (EMB) agar (Deben Diagnostics Ltd) was used to identify *E. coli*.

### Detection of *Salmonella* spp.

This was carried out in four phases, according to the NF EN ISO 6579 method:

i) a pre-enrichment phase on a non-selective medium, where samples taken from the refrigerator are pre-enriched at 1/10 with buffered peptone water, vortex-homogenised for two minutes, left to revive at room temperature for 30 minutes, then incubated at 37°C for 18 to 20 hours

ii) an enrichment phase in selective liquid media, where 0.1 ml of pre-enrichment media is used to inoculate 10 ml of Rappaport Vassiliadis Soya broth (RVS) and 1 ml is used to inoculate 10 ml of Mueller-Kauffmann Tetrathionate broth (MKTT<sub>n</sub>). The RVS broth was then incubated at 42°C and the MKTT<sub>n</sub> at 37°C for 18 to 24 hours

iii) an isolation phase: the selective growth mediums Xylose Lysine Deoxycholate (XLD) and Hektoen were seeded with the products of enrichment using the quadrants method and incubated at 37°C for 24 hours. Subsequently, five characteristic colonies were sampled and seeded onto Hektoen agar for initial purification and, 24 hours later, onto nutrient agar for a second purification

iv) a biochemical identification phase during which the following growth mediums were seeded with the pure colonies, typical of *Salmonella*:

– Kligler iron agar: the typical cultures of *Salmonella* produced an alkaline slant (red) and an acid butt (yellow), with the formation of gas (around 90% of cases) and hydrogen sulphide (blackening of the agar)

- urea-indole medium: in this medium the characteristics of *Salmonella* were urease (-) and indole (-)
- Galerie<sup>®</sup> API 20E<sup>®</sup> gram-negative bacilli test strips (Bio-Mérieux) after seeding and incubation according to the manufacturer's recommendations, the reactions resulted in spontaneous colour changes, revealed by the addition of reagents. The analytical catalogue was used to read the results.

For all these identification phases, incubation was at 37°C for 18 to 24 hours.

#### Detection of *Escherichia coli*

The samples taken from the refrigerator were pre-enriched at 1/10 with buffered peptone water, vortex-homogenised for two minutes, left to revive at room temperature for 30 minutes and incubated at 37°C for 18 to 24 hours. EMB agar was then seeded with the pre-enriched solution using the quadrants method and incubated at 37°C for 24 hours. Subsequently, five characteristic colonies (dark violet between 2 and 3 mm in diameter, with black centres and a greenish metallic sheen in reflected light) were sampled and seeded onto nutrient agar for purification. The colonies obtained were confirmed by biochemical tests, first using Kligler iron agar, followed by Galerie<sup>®</sup> API 20E<sup>®</sup> gram-negative bacilli test strips.

#### Statistical analyses

The number of samples was determined using the OPEN-EPI 3.01 software. The database was created and managed using Access (Microsoft Office 2010). The quantitative data were compiled using Microsoft Excel 2010 and analysed using the Statistical Package for the Social Sciences, version 17.0 (SPSS). A Pearson chi-square test was used to evaluate and compare the prevalence of infection on the different farms (15).

## Results

### Animal characteristics

On the basis of the information collected on broiler chicken farms in N'Djamena and family farms in Doba, the breeds were found to include Hybro broilers (60%), Vedette (20%) and Decko (20%), aged between 35 and 60 days, in N'Djamena. In Doba, the age of the local breeds ranged from five to eighteen months. At the time of sampling, none of the birds showed clinical signs of disease.

### Characteristics of the surveyed farms

#### Farms in N'Djamena

The broiler chicken farms surveyed were on a modest scale, with an average of 1,197 chickens per batch. Investment appeared to be relatively low and, in 60% of cases, it resulted in a single farm building being erected per farm, while in 40% of cases, it resulted in two farm buildings per farm being erected. The density varied from three to sixteen birds per square metre. The building walls were made of either mud (40%) or masonry (60%), with sheet metal roofs.

The farms were equipped with feeding troughs, water troughs and manual charcoal heating, but no ventilation systems. The variation in humidity was not controlled in the buildings, resulting in production stoppages during severe heatwaves. Lighting was mainly by hurricane lamps or battery lamps and was rarely powered by an electricity generator. The floors were concrete screed covered with either straw litter (80% of cases) or wood shavings (20% of cases). The majority of farms (80%) were unfenced, giving free access to farmyard animals (goats, sheep, ducks, dogs, cats). The drinking water sources were largely boreholes (80%), with open wells on only 20% of farms.

Half of the farms (5/10) had more than one employee who had followed a course on biosecurity. The employees were well equipped on 6/10 of farms. On 2/10 farms, employees called on a veterinarian during their activities.

## Farms in Doba

Sixteen poultry farms were surveyed, all of them traditional with very low investment. Bird numbers varied between 8 and 140. In 25% of cases, the birds were free to range with no care, no feed and no proper shelter. In 37.5% of cases, the poultry were housed in makeshift shelters made of large pots containing soil, straw, or stacked mud bricks, or else in a corner of the kitchen during the night, then set free in the morning with a few handfuls of cereal or legume seeds distributed in the morning and/or evening. The remaining 37.5% benefited from a wire netting enclosure and had access to a chicken house made of durable materials, as well as care and feed.

### **Prevalence of farm contamination by *Escherichia coli* and *Salmonella* spp.**

In both towns, a total of 1,090 samples, collected from 26 broiler poultry farms and traditional farms, were analysed. In total, 40 samples (3.67%) from 10 farms (38.46%) proved positive for the presence of *Salmonella* spp., and 65 samples (5.96%) from five farms (19.23%) were found to be positive for *E. coli* (Tables I and II).

Only four out of the ten farms studied in N'Djamena proved positive for *Salmonella* spp., representing a contamination level of 40% (Table I) and three were positive for *E. coli*, giving a contamination level of 30% (Table II). However, in Doba, in the first of the two campaigns, 6 of the 16 farms were found to be positive for *Salmonella* spp., representing a contamination level of 37.5%, and one for *E. coli*, giving a contamination level of 6.25%. In the second campaign, no contamination of farms by *Salmonella* spp. was observed. However, two samples were positive for *E. coli* (0.74%) on one farm (6.25%). Overall, for the two campaigns, 6 of the 16 farms studied proved positive for *Salmonella* spp., equivalent to 37.5%, with 21 positive samples among the 545 analysed, giving an isolation rate of 3.85% (Table I). Two of the farms (12.5%) were positive for *E. coli*, with a total of three positive samples, giving an isolation rate of 0.55% (Table II).

The results for the prevalence of contamination by type of farm are presented in Table III. This table shows that the difference in prevalence observed between farm types is highly significant, both for *E. coli* ( $p < 0.001$ ) and for *Salmonella* spp. ( $p < 0.001$ ).

All sample types were positive for at least one bacterium (Table IV), with the exception of fresh droppings, which proved to be negative for *Salmonella*. Most sample types had a low isolation rate, with the majority of isolates coming from the cloacal swabs, which had a high isolation rate of 71.42%.

It should be noted that, for the cloacal swabs, a highly significant difference between the two towns was observed ( $p < 0.001$ ) both for *E. coli* and for *Salmonella* spp., with higher isolation rates for *E. coli* (69.2%) in N'Djamena and for *Salmonella* spp. (52.5%) in Doba.

For feed and drinking water, significant differences ( $p = 0.002$  and  $p = 0.005$ ) were observed only for *Salmonella* spp. The isolation rates were higher in N'Djamena for both feed (17.5%) and water (15%). However, no significant difference was observed between the two towns for fresh droppings ( $p = 0.423$ ).

## Discussion

### Characteristics of the farms

The characteristics of the farms in N'Djamena are very similar to those observed in a study conducted on 30 farms in the *wilaya* (district) of Constantine in Algeria (5) and in a relatively recent study carried out on 16 farms in N'Djamena (13). As for the farms in Doba, they match the description of traditional farming systems in Chad, as described in a government report on the country's poultry sector (11).

### Prevalence of infection

#### Prevalence at the farm level

On the farms included in the study, 19.23% were contaminated with *E. coli* and 38.46% were contaminated with *Salmonella* spp., giving an

overall prevalence of contamination of 57.69%. This rather high prevalence points to severe contamination of broiler chicken farms in N'Djamena and traditional farms in Doba. More than half the farms were positive for the presence of bacteria, doubtless because of poor hygiene and a lack of proper infection-prevention measures for farm buildings and equipment (16, 17, 18). Several studies carried out in industrialised and developing countries (19, 20, 21) revealed quite high prevalences of *Salmonella* in broiler chicken farms, corroborating this study.

In Africa, surveys in Senegal found a prevalence of *Salmonella* and *E. coli* of 84.4% (22). In Algeria, a prevalence of 36.66% was reported for *Salmonella* on 30 broiler chicken farms (5). Another study conducted between 2000 and 2001 on droppings in 70 poultry farms in Senegal found a prevalence of 28.6% for contamination by *Salmonella* (23).

In this study, taking into account each type of farming system, the highest prevalence (77.15%) was found on semi-industrial farms. Studies carried out in Senegal on frozen chicken legs imported from Europe (22) showed that 2.1% of 95 boxes of chicken legs were contaminated by *Salmonella*, and 45.26% by *E. coli*. This study also showed that local chickens contained a high level of pathogenic bacteria such as *Salmonella* and *E. coli*. These results, just like those recently referred to, could be explained by defective hygiene practices on poultry farms (24, 25).

#### Prevalence at the animal level

The prevalence of contamination of chickens in the present study is 3.67% for *Salmonella* spp. and 5.96% for *E. coli*. Non-typhoid *Salmonella* (NTS) is widespread, colonising a broad range of animals, including mammals, amphibians, reptiles, birds and insects (26). There is, however, very little information concerning environmental reservoirs of NTS and modes of contamination, especially in Africa (27). *E. coli*, on the other hand, forms part of the commensal flora of the digestive tract of poultry. It is therefore spread by bird faeces, and poultry can be contaminated by various sources (birds, rodents, insects,

wildlife, water, dust, environment) (28). *E. coli* is generally considered to be an agent of secondary infection (29). It is most probably for these reasons that other studies have reported higher percentages of *E. coli* contamination (68.33%) than those found in the present study (22). In addition, the presence of *E. coli* is used as an indicator of poor hygiene conditions (22). The high prevalence indicates that there is insufficient compliance with strict hygiene rules on the surveyed farms. It transpires that on some farms, the buildings are unfenced, meaning that poultry come into contact with farmyard animals, and the buildings are surrounded by filth. On other farms, poultry are allowed to wander freely with no care or shelter. The presence of *E. coli* is also the best indicator of faecal contamination when searching for pathogenic enterobacteriaceae such as *Salmonella* (22). The latter were found in 3.67% of samples. These levels are lower than those reported by Djim-Adjim in Chad (43.75%) (13) and those obtained by Fofana in Senegal (62.56%) (22), but still higher than those reported by Elgroud in the wilaya of Constantine in Algeria (1.66%) (5). This level of contamination is in line with the prevalence observed in the European Union for broiler chickens (between 0% and 18%) (1). These figures were reported by various studies and surveillance programmes and it is quite possible that the variations reflect differences between sampling schemes and between the samples used from one country to another (1).

Moreover, the prevalence of contamination of chickens by *Salmonella* was estimated at 69.8% in France (30), 41.3% in Turkey (31) and 28.6% (70 farms studied using faecal samples) in Senegal in 2000–2001 (23). In recent years, probably thanks to control programmes in the European Union, *Salmonella* contamination seems to have declined in most European countries. There appears to be a significant correlation between farm size and contamination of flocks by *Salmonella* spp. Studies in the Netherlands (32) and Belgium (33) also revealed that the risk of farm contamination by *Salmonella* spp. increases in line with the size of the farm and the sampling period.

It should be noted that, during the current study, contamination levels were higher around the cloaca (71.42%) than in droppings and water (10.48%) or feed (7.62%). These findings could be linked to the

abundant excretion of bacteria caused by stress factors such as high temperatures and water vapour deficits that are prevalent in the farms in the study (34, 35, 36).

The high level of contamination detected within caged flocks of birds was not necessarily linked to the sensitivity of the sampling and analysis methods, but might rather be associated with inadequate cleaning and disinfection of the buildings used to house chickens or a lack of hygiene controls (37, 38). It is currently accepted that most cases of contamination of chickens appear to result from persistent contamination on the farm. *Salmonella* contamination of the previous batch of birds has been shown to be a potential source of infection for the following batch within the same poultry farm (39). It has also been reported that vertical and horizontal transmission play an important role in *Salmonella* contamination of flocks on poultry farms (33).

Other studies have shown that the introduction of *Salmonella*-free chicks, following vaccination of parental flocks against *Salmonella*, is an effective way of controlling vertical transmission in a flock, but it is not enough to prevent environmental contamination of poultry if no hygiene measures are implemented in parallel (40). Several studies have also suggested that measures to limit horizontal contamination on farms should include cleaning and effective disinfection of buildings and appropriate control measures against mobile and immobile vectors of bacteria (16, 17, 18, 40, 41).

Such contamination is probably linked to the very rudimentary overall condition of infrastructure and equipment on the farms covered by the study, which were not up to the required hygiene and biosecurity standards, highlighting the need to set up epidemiological surveillance and appropriate control controls for poultry farms in the surveyed areas.

## Conclusion

This study characterises semi-industrial farms in N'Djamena and traditional farms in Doba. It provides an indication of the patterns of contamination by *Salmonella* and *E. coli* in the broiler chicken sector in N'Djamena and on traditional farms in Doba (Chad). It is clear that

the size of the sample, in terms of the number of broiler farms and traditional farms in N'Djamena and Doba, is a major limiting factor, preventing the authors from ensuring the representativeness of the study results. Nevertheless, these data reveal a pattern of prevalence and persistence of contamination, highlighting the need for a range of precautions in the management of poultry farms. It would be useful to repeat this study on a larger scale with a more representative sample of the poultry population in Chad to confirm or refute this pattern.

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**Table I**  
**Prevalence of *Salmonella* spp. infection**

Sites	Farms			Samples		
	No. of farms	Positive	%	Analysed	Positive	%
<b>N'Djamena (broilers)</b>						
Single campaign	10	4	40	545	19	3.49
<b>Sub-total</b>	<b>10</b>	<b>4</b>	<b>40</b>	<b>545</b>	<b>19</b>	<b>3.49</b>
<b>Doba (traditional)</b>						
First campaign (rainy season)	16	6	37.5	275	21	7.64
Second campaign (dry season)	16	0	0	270	0	0
<b>Sub-total</b>	<b>16</b>	<b>6</b>	<b>37.5</b>	<b>545</b>	<b>21</b>	<b>3.85</b>
<b>Total</b>	<b>26</b>	<b>10</b>	<b>38.46</b>	<b>1,090</b>	<b>40</b>	<b>3.67</b>

**Table II**  
**Prevalence of *Escherichia coli* infection**

Sites	Farms			Samples		
	No. of farms	Positive	%	Analysed	Positive	%
<b>N'Djamena (broilers)</b>						
Single campaign	10	3	30	545	62	11.38
<b>Sub-total</b>	<b>10</b>	<b>3</b>	<b>30</b>	<b>545</b>	<b>62</b>	<b>11.38</b>
<b>Doba (traditional)</b>						
First campaign (rainy season)	16	1	6.25	275	1	0.36
Second campaign (dry season)	16	1	6.25	270	2	0.74
<b>Sub-total</b>	<b>16</b>	<b>2</b>	<b>12.5</b>	<b>545</b>	<b>3</b>	<b>0.55</b>
<b>Total</b>	<b>26</b>	<b>5</b>	<b>19.23</b>	<b>1,090</b>	<b>65</b>	<b>5.96</b>

**Table III**  
**Prevalence of contamination on traditional and semi-industrial farms**

Bacterial species	No. of strains by farm (%)		P-value
	Traditional	Semi-industrial	
<i>Escherichia coli</i>	3 (4.6)	62 (95.4)	< 0.001
<i>Salmonella</i> spp.	21 (52.5)	19 (47.5)	< 0.001

**Table IV**  
**Distribution of strains by sample type**

Sample type	No. of strains by pool					
	<i>Escherichia coli</i>			<i>Salmonella</i> spp.		
	Doba	N'Djamena	P-value	Doba	N'Djamena	P-value
Cloacal swabs	3	45	< 0.001	21	6	< 0.001
Feed	0	1	0.825	0	7	0.002
Drinking water	0	5	0.609	0	6	0.005
Fresh droppings	0	11	0.423	0	0	-