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Original Article

Prevalence, Antibio-Resistance and Risk Factors for *Salmonella* in Broiler Turkey Farms in the Province of Khémisset (Morocco)

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ABSTRACT

The problem of contamination of poultry by *salmonella* in addition to the increase of antimicrobial resistance in *Salmonella* is of a great importance both in the field of public health as well as in the socio-economic sector of the country because of the damage it can cause, but no studies have been conducted so far in Morocco to determine the risk factors of *Salmonella* contamination in broiler turkey farms. In order to determine the statute of the broiler turkey farms (n= 20) with respect to the contamination by *Salmonella*, three visits were paid to each livestock buildings. A batch of 10 pools of 5 droppings per breeding by visit were collected (n= 600) and analyzed. All *Salmonella* (n= 62) isolates were serotyped, confirmed by the presence of virulence gene (invasion) and tested for the resistance to 15 antimicrobial agents by the agar diffusion method. In parallel, an analytical study was carried out to investigate risks factors of *Salmonella* contamination in these farms. The rate of insulation of *Salmonella* is important (35 %) and the isolated serotypes are worrying: 36 *S.* Kentucky, 15 *S.* Saintpaul, 8 *S.* Parkroyal and 3 *S.* Ruzizi River. They have relatively high rates of resistance to tetracycline (79 %) and streptomycin (72.5 %), followed by resistance to nalidixic acid (37.1 %), ciprofloxacin (33.9 %), ampicillin (33.8 %), spectinomycin (32.3 %), trimethoprim (30.6 %) to trimethoprim-sulfamethoxazole (24.2 %), gentamicin (21 %), kanamycin (17.7 %) and amoxicillin + clavulanic acid (16.1%). Three strains of *S.* Agona expanded spectrum betalactamase producing which have a high level of resistance to ceftriaxone with a minimum inhibitory concentration (CMI) of 16 µg/ml. The variables associated with this contamination are related to the duration of crawlspace (p = 0,037), treatment with antibiotics (p = 0.001) and the contamination of turkeys poults (p = 0.002) dice implementation, The storage of manure inside the livestock building (p = 0.003), The conservation of turkeys inside the rearing building (p = 0,009) in the breeding season (p = 0.001) and age of turkeys sample (p = 0.01). The high level of antibiotic resistance of *Salmonella* isolates in the present study, showed the possible Significance of turkey as a source of multiple antimicrobial-resistant *Salmonella* for human infections.

Keywords: *Salmonella*, Risk factors, Turkey, Droppings, Contamination, Khemisset, Antibiotic-resistance

INTRODUCTION

The problem of contamination of poultry farms has a considerable importance both for the public health sector and for the socio-economic sector of the country because of the damage it can cause (Bailey *et al.*, 2001). However, if the contamination of poultry is possible at all levels of the food chain, the breeding period represents a critical step of bacterial infection (Mead, 1993).

Despite the fact that the turkey is responsible two times for the cases of human salmonellosis that chicken produce (Barkok and Addioui, 2010), up to now, in Morocco, no study has been made on the prevalence of the contamination of the turkey by *Salmonella* spp in

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the farms or on the risk factors associated with it. In effect, in Morocco, the number of breeders of turkeys is increased from 6 in 2000 to more than 422 in 2010 with a capacity for breeding of 9.85 million of turkey per band (Schwarz and Chaslus-Dancla, 2001). This increasing in production is partially due to the use of the antibiotics as a preventative and curative, in metaphylaxie and prophylaxis (Insofan, 2008). However the international network of food safety authorities (Van den Bogaard et al., 2000) has reported that the misuse and the non-controlled administration of antibiotics, have led to the selection of resistant bacteria. The resistance to antibiotics of zoonotic enteropathogens, mainly the Campylobacter and *Salmonella*, is all the more dangerous, in terms of human health, that these bacteria can be transmitted to humans through the food chain (McEwen and Fedorka-Cray , 2002 ; Evans and Sayers , 2000).

Our attempt in present study this work of research consists of, on the one hand he estimation of prevalence of the infectious by *Salmonella spp* in the farms of the turkey flesh in Khemisset province (north west of Morocco), and to evaluation of the antimicrobial resistance of these bacteria isolated from manure. On the other hand, we preceded the identification of potential risk factors associated with this contamination. In the course of this investigation, we have tried to identify the critical points involved in the contraction of *salmonella* throughout the production chain of the turkey flesh.

MATERIALS AND METHODS

Choice of sites of farms

This choice is based on the fact that the Khemisset province is located in the central Moroccan plateau with temperate climate, semi continental agricultural vocation par excellence, which is in favor of development of the farms of the turkey flesh in this region. This area comprises 32 rural communes and possesses 35 farms of the turkey flesh which 20 of them were functional to the same period of this study.

Sampling

The study was conducted during 2011 on 20 farms representing 86 livestock buildings located in the Khemisset province and whose total production capacity is 439000 turkey flesh per band. The statistical unit corresponded to the lot by building visited by each farm. In order to determine the status of the batch of poultry screw-to-bolt of the contamination by *Salmonella*, samples have been carried out (n= 600) in three visits (during the week preceding the removal of the previous band, to the establishment of turkey poults and before the removal of the lot studied). In effect, to sweep a large area of the building, ten pools of 5 fresh droppings by livestock per visit were collected in sterile pots, this number of sample allows you to determine with 95% certainty, an infected population to 5% (Popoff, 2009). In the course of this study, a questionnaire previously validated by poultry veterinary related to different headings (location and environment, infrastructure, equipment, operation, hygiene, staff and power supply) is filled with the rancher aimed at determining the assumptions of risk associated with this contamination.

Search for *Salmonella*

The bacteriological analysis is carried out according to the French Association for Standardization (AFNOR) in force V08-052. The characteristic colonies of *Salmonella* have been confirmed from biochemical criteria on the following media: Hajna-Kligler (Biokar Diagnostics, France), Citrate of Simmons (Oxoid, England), Mannitol-mobility (Biokar Diagnostics, France), Uree-Indole (bio MerieuxR SA, France), Lysine decarboxylase (SCHARLAB, Barcelona), ONPG (Oxoid Limtedt, England) and oxidase (In vitro diagnostics, USA). An identification on a gallery API 20E (biomerieuxr SA, France) was carried out to ensure the previous confirmation.

Serological Identification and molecular *Salmonella*

The serological confirmation was made by the tests of agglutination on blade in the National Institute of Hygiene in Morocco by using sera versatile anti O, anti H and anti Vi (Diagnostic Pasteur, Paris, France) according to the diagram of Kaufmann-White scheme (Bouthors *et al.*, 1998).

The molecular confirmation of *Salmonella* isolated is carried out by the technique of gene Polymerase Chain Reaction (PCR). PCR amplification was performed using primer pairs (F-5'tatcgccacgttcgggcaa3' and R-5'tcgcaccgtcaaaggaacc3'). The amplicon sizes of invA, were 275 bp. Amplification was performed in a 25 µl final volume, with a reaction mixture containing 1 µl bacterial DNA; 5 µl green GO Taq buffer (5x); 100 µM each eoxynucleoside triphosphates (dNTPs), 0.125 µM each primers, and 0.5 U GO Taq DNA polymerase (Bio-Rad). Amplification was conducted in the thermocycler (Verity, Bio-Rad). The PCR cycling program of the virulence gene invA consisted of denaturation at 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 second, 52°C for 30 s, 72 °C for 45 s, and a final extension period at 72 °C for 7 min. PCR products (4 µl) were resolved by electrophoresis in 1.5–2% (w/v) agarose gels and visualized under ultraviolet transillumination after ethidium bromide staining. A wide-range molecular-weight DNA marker (100-bp DNA ladder, Promega) was used on each gel as a standard. *Salmonella* Typhimurium ATCC 14028 was used as control for all PCR detection (Figure 1).

Antibiotic Resistance

The antibiogram was done by the method of diffusion on Mueller-Hinton agar using disks of antibiotics (Bio-Rad). The interpretation of the results was made by referring to the rules and recommendations of the Committee of antibiogram of the French Society for Microbiology. The antibiotics tested are ciprofloxacin, Ceftazidime, amoxicillin + clavulanic acid, ceftriaxone, Spectinomycin, gentamycin, nalidixic acid, chloramphenicol, tetracycline, sulfamethoxazole-trimethoprim,

streptomycin, ampicillin, cefotaxime, kanamycin and trimethoprim. A screening test for the detection of expanded Spectrum Betalactamase producing (ESBL) was carried out by the double disc diffusion test (using cefotaxime, ceftazidime and amoxicillin/clavulinic acid discs) according to the CLSI criteria (CLSI, 2007). E. coli ATCC 25922 was used as a quality control strain. The Minimum Inhibitory Concentration (MIC) of ceftriaxone for strain Agona producing ESBL were also determined by Etest strips (AB Biodisk) (Pfaller et al., 1999). Multiple Atibiotic Resistances (MARs) index for each resistance pattern was calculated by using the formula given below: MAR index = Number of resistance antibiotics/ total number of antibiotics tested.

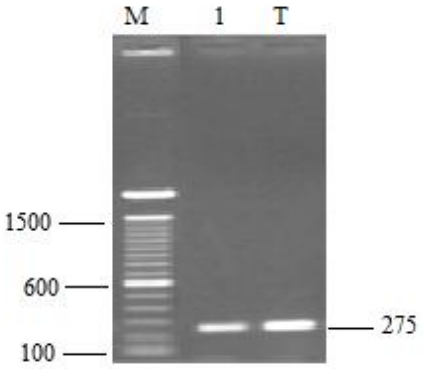

Figure 1: Agarose gel electrophoresis of amplicons generated by simple PCR using primers specific for *Salmonella v*irulence genes. Line 1: 275-bp invA amplicon; T: Positive control (S. Typhimurium penta-resistant ACTeStSul kind); M: 100-bp DNA ladder

Statistical Analysis

The batch (Composed of ten pool droppings) is declared contaminated if at least one pool of droppings is declared positive in *Salmonella*. To Test the connection of this variable with each explanatory variable, a test Chi^2 and the calculation of odds ratios with a 95% confidence interval were carried out using the software Statistica 6.0 (Statsoft Ltd. Chicago, III). The processing of data in questionnaire has been done thanks to the Sphinx $Plus^2$ (V. 4.5.0.19).

RESULTS

Prevalence of contamination

All strains isolated which were negative for the characters (Lactose, Urease, indole, ONPG and oxidase) and positive for the characters (Glucose, Lysine, Citrate, Mannitol-Mobilite , Gas and H2S) were tested by the system $API20^E$ and submitted to a DNA amplification by using primers specific already described. The results showed that all of these strains have a common band with the strain of control tested *Salmonella* Typhimurium pentaresistante type (ACTeStSul) to a size of approximately 275 pb (Fig1).

The bacterium *Salmonella* is isolated in 35% of the 60 batch analyzes (Table 1). For 25% of the batches of *Salmonella* positive, the bacterium is present in month 5 on 10 samples analyzed (Table1).

Distribution of serotypes

Sixty two isolates of *Salmonella* serotypables were obtained although 9 serotypes have been identified. The serotype Kentucky was the most frequently isolated (21 isolates, 33.8 %) monitoring of serotypes Parkroyal (10 isolates, 16.3%), Agona (7 isolates, 11.3%), Saintpaul (6 isolates, 9.6%), Typhimurium (5 isolates, 8%), *salmonella* Enteritidis and Heidelberg (4 isolates each, 6.4%), Newport (3 isolates, 4.8 %) and the serotype Ruzizi (2 isolates, 3.2 %) (Table 2).

Antimicrobial Resistance

The results of antimicrobial resistance of strains of *Salmonella s*pp (Table 2) showed that 93.5% (58 / 62) of the strains are resistant to at least one antibiotic whereas the multidrug-resistant strains (resistant to ≥ 3) constitute 80.64 per cent, showing levels of resistance high enough to tetracycline (79%) and streptomycin (72.5%) followed by the resistance to nalidixic acid (37.1%), ciprofloxacin (33.9%), ampicillin (33.8%), spectinomycin (32.3%), to the trimethoprim (30.6%), to the sulfamethoxazole-trimethoprim (24.%), to the gentamycin (21%), to the kanamycin (17.7%) and to the amoxicillin + clavulanic acid (16.1%) (Table2). However, we noted a rate of low resistance to ceftazidime, ceftriaxone and cefotaxime or 4.8 percent for each of the latter cases.

100 % (n= 21) of the strains of *S*. Kentucky are resistant to nalidixic acid, ciprofloxacin and to streptomycin (Table 2) and 42.8 percent of the serovars Agona are resistant to the Ceftazidime, ceftriaxone and cefotaxime with three strains of *S.* Agona producing an also ESBL which have a high level of resistance to ceftriaxone with a CMI of 16 µg/ml. The index of increasingly insensitive to antibiotics has indicated that a *S*. Kentucky and a *S*. Agona had the highest index (Mar = 0.64) (Table 1).

Risk Factors

From the data of the questionnaire and the results of statistical analyzes, 7 of the 26 factors tested are associated with the presence of *Salmonella* in the buildings at the end of the period of rearing ($P<0.05$). These factors may constitute potential risks of contamination of farms by *Salmonella spp.* (Table 3).

Table 1. Distribution (%) of batches studied depending on the number of fecal samples contaminated with *Salmonella* in Morocco

Item	Batch of *Salmonella*+										Batch of *Salmonella* –
	21/60 (35%)										39/60 (65%)
Number of pools *Salmonella*+	1	2	3	4	5	6	7	8	9	10	0
Number of batch	7	4	3	2	2	1	2	0	0	0	39

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Table 2. Antibiotypes profiles strains and multiple antibiotic resistance index of *Salmonella* (n=62) isolated from turkey droppings in Morocco.

Serotype	Resistance profile	MAR index	Serotype	Resistance profile	MAR index
Kentucky	Cip, Spt, Na, Te, S, Amp, Cn	0.50	Saintpaul	Te, Sxt, S, Tmp	0.28
Kentucky	Cip, Spt, Na, Te, S, Amp, Amc, Sxt, Tmp	0.64	Saintpaul	Te, Sxt, S, Tmp	0.28
Kentucky	Cip, Spt, Te, S, Sxt, Tmp, K, Na	0.57	Agona	Caz, Amc, Cro, Sxt, S, Amp, Ctx, Tmp	0.57
Kentucky	Cip, Spt, Na , Te, S, Amp, K	0.5	Saintpaul	Te, Sxt, S	0.21
Kentucky	Cip, Spt, Na , Te, S, Amp, K	0.5	Saintpaul	Te, Sxt, S	0.21
Kentucky	Cip, Spt, Na , Te, S	0.35	Saintpaul	Amc, Sxt, S, Amp, Tmp	0.35
Kentucky	Cip, Spt, Na , Te, S	0.35	Saintpaul	te	0.07
Kentucky	Amc, Spt, Na , Te, S, Amp, Cn	0.5	Agona	Caz, Amc, Cro, Sxt, S, Amp, Ctx, Tmp, Cn	0.64
Kentucky	Cip, Spt, Na , Te, S, Cn	0.42	Typhimurium	Amp, C, Amc, Tmp	0.28
Kentucky	Cip, Spt, Na, Te, S, Cn	0.42	Typhimurium	Amc, S, Na	0.21
Kentucky	Cip, Spt, Na, Te, S, Cn	0.42	Typhimurium	Tmp	0.07
Kentucky	Cip, Spt, Na , C, Sxt, Stamp, Cn	0.57	Typhimurium	C, Amp	0.14
Kentucky	Cip, Amc, Spt, Na, Te, S, Amp, Cn	0.57	Typhimurium	sensible	0
Kentucky	Cip, Spt, Na ,Te, S, Tmp, Cn	0.50	Newport	S, Te, Tmp	0.21
Kentucky	Cip, Na, Te, S	0.28	Newport	Amp, c, k, te, Tmp	0.35
Kentucky	Cip, Amc, Spt, Na, Te, S, Amp, Cn	0.35	Newport	S, Te, Tmp	0.21
Kentucky	Cip, Spt, Na, Te, S, Cn	0.42	Entéritidis	Na, Te, K, Amp	0.26
Kentucky	Cip, Amc, Spt, Na, Te, S, Amp, Cn	0.57	Entéritidis	sensible	0
Kentucky	Na , Te, S, Sxt, Cip	0.35	Entéritidis	Te, K, Amp	0.21
Kentucky	Cip, Spt, Na, Te, S, Cn	0,42	Entéritidis	sensible	0
Kentucky	Cip, Na, Te, S	0.28	Agona	Amp, Cro, Cxt, S, Caz, Te	0.42
Parkroyal	Amc, S, C, Te, S, amp	0.42	Agona	Amp, Te	0.14
Parkroyal	Te, S,K	0.21	Agona	Amp, Te	0.14
Parkroyal	Te, Sxt, Tmp, S	0.28	Agona	Tmp, S, Sxt	0.21
Parkroyal	Te, Sxt, Tmp	0.21	Agona	Te	0.07
Parkroyal	Te, S,K	0.21	Heidelberg	sensible	0
Parkroyal	Te, Sxt, Tmp, s	0.28	Heidelberg	S, Te, C	0.21
Parkroyal	Te, S, K	0.21	Heidelberg	Te	0.07
Parkroyal	Amc, Spt, C, Te, S,Amp	0.42	Heidelberg	S, Te, C	0.21
Parkroyal	Te, S, K	0.21	Ruzizi	Te	0.07
Parkroyal	Te, S, K	0.21	Ruzizi	S, Te, Tmp	0.21

Cip (Ciprofloxacin; 5 µg), Caz (Ceftazidime; 30µg), Amc (amoxicillin + acid. Clavulanic ; 30 µg), Cro (Ceftriaxone; 30ug), Spt (Spectinomycin; 100 mcg), Cn (gentamycin; 30 µg), Na (nalidixic acid; 30 µg), C (Chloramphenicol ; 30 µg), Te (Tetracycline; 30 µg), Sxt (Sulfamethoxazole-Trimethoprime ; 25 µg), S (Streptomycin ;10 µg), Amp (Ampicillin ; 10 µg), Ctx (Cefotaxime ; 30µg), K (Kanamycin ; 10 µg), Tmp (trimethoprim ; 5 µg).

Table 3. Factors associated with the contamination of farms turkey by *Salmonella spp.* (Test khi^2 à 5%).

Variables	Modality	% of contaminated batches	Pα	χ2	OR	95% CI (OR)	RR
Season	Cold	29.1	0.001	10.79	6.31	2.01-19.79	2.84
	Hot	72.2					
Duration of crawls pace	>15	33.3	0.037	5.45	3.5	1.2-10.2	1.91
	≤15	63.3					
Use of antibiotics on the first day	Yes	30.5	0.001	11.39	6.82	2.13-35.23	3.56
	No	75					
Age of turkeys at levy	>40	77.7	0.01	7.42	5.25	1.51-18,31	1.94
	≤40	33.3					
Storage of manure	Inside of the farm	80	0.003	11.32	8	2.18-29.31	2.4
	Outside of the farm	33.3					
Salmonella contamination of turkey poults diced the establishment	Yes	66.6	0.002	10,85	4.60	2.13-35.25	3.56
	No	18.7					
Conservation sick turkeys in the building	Yes	62.2	0.009	7,89	7.89	1.54-13.7	2.29
	No	28.1					

OR: Odds Ratio (ratio of the sides). 95% CI (OR): Confidence interval for Odds Ratio to 95% depending on the method of Woolf (method of logit). RR: Relative Risk. P<0.05: Variable significantly associated with infection by *salmonella.*

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DISCUSSION

Prevalence of *Salmonella*

The rate of contamination of turkey flocks by *Salmonella* found in this study (35%) was higher compared to the prevalences found in chickens (24 %) in Meknes Morocco by Chaiba and collaborators (2011) in the manure from chicken (25 farms) and by Cardinale and collaborators (2004) in Senegal (28.6 %) in the manure (70 farms). However, this prevalence is in agreement with those found there a few years ago in some countries of Europe. Thus, in Belgium, the prevalence was estimated at 36% (Levy of faeces in 122 farms) (Carli et al., 2001). In France, in 1986, the prevalence has been estimated at 53% (180 farms, on samples of faeces) whereas in Turkey, in 2001, the prevalence was of the order of 43.3 percent (Carli et al., 2001). Nevertheless, these last few years, and probably due to the performance of control programs, the contamination by *Salmonella* seem to decline in most of the European countries. Thus in poultry flocks, the prevalence recorded is between 1 and 13.6 percent (van Immerseel et al., 2005). A research conducted in Nigeria has shown that samples from poultry manure to the interior of buildings poultry have helped to isolate *S.* Paratyphi A to reason of 12.5 percent and other *salmonella* serotypes *S.* Enteritidis, *S.*Typhymurium and *S.* Gallinar um (Orji *et al.,* 2005). In India of cloacal swabs, made on chickens, have revealed a rate of *salmonella* from 14.7 percent represented by *S.* Enteritidis, *S.* Typhymurium, *S.* Gallinarum and *S.* Paratyphi B (Murugkar et al ., 2005). In Pakistan samples from eggs, meat, poultry manure, have helped identify 155 *S.* Enteritidis among the 206 *salmonella* isolated on 615 samples, either in 75% of cases infected (Akhtar *et al*., 2010).

Distribution of serotypes

The distribution of serotypes showed a predominance *S*. Kentucky (n=21, 33.8 %) followed by the serotypes Parkroyal (n=10, 16.3 %), Agona (n=7, 11.3 %), Saintpaul infection (n=6, 9.6 %), Typhimurium (n=5, 8 %), Enteritidis and Heidelberg (n=4, 6.4 %), Newport (n=3, 4.8 %) and the serotype Ruzizi (n=2, 3.2 %). This distribution is in accordance with the results of Yan and collaborators (2003) who reported the most frequent serotypes among the chicken in the United States are *S*. Heidelberg, *S*. Kentucky, *S*. Hadar, *S*. Typhimurium and *S*. Thompson. In 2006 , Chemaly in Europe, has also been found that The most common serotypes among the chicken of flesh are *S*. Hadar, *S*. Infantis, *S*. Virchow, *S*. Typhimurium and *S*. Enteritidis, which are found in foods and humans in developed and developing countries (Cetinkaya et al ., 2008; Fearnley *et al.*, 2011). Indeed, in Europe *S*.Enteritidis, *S*. Infantis, *S*. Newport and *S*. Typhimurium are the most frequently associated with human salmonellosis (EFSA, 2011). In the U.S, The Centers for Disease Control and Prevention (CDC) have reported that *S*. Enteritidis, *S*. Typhimurium and *S* .Newport, in that order, are the serotypes the most reported (*S*. Enteritidis, *S*. Typhimurium and *S*. Newport) by the public health laboratories (CDC, 2011). In this research, the serotype Kentucky is the most responded in the poultry farms studied, it represents 33.8 percent of the totality of serotypes analyzed. This rate is alarming, because, according to the international studies pre-established in France, Denmark, United Kingdom, United States, Morocco and Nigeria, published in 2011, the sudden emergence and worrying of *S.* Kentucky has shown an increasingly insensitive to almost all families of antibiotics (Cloeckaert and Bousquetmelou, 2011). In Morocco, between 2005-2008, the presence of *S*. Kentucky in the minced meat flood in the turkey, represented 20.5 percent (Karraouan *et* al., 2010), in Senegal the bacterium has been isolated from the cooked dishes (Bada-Alambedji et al., 2006). In Malaysia at the level of chicken carcases of flesh, as well as at the level of slaughterhouses and processing units in addition to the manure and litter of purebred breeding (Rusul *et* al., 1998).

All these results assume a vertical contamination in the production chain of the meat of the turkey, ranging from upstream to downstream. In our study *S***.** Parkroyal has been isolated at the level of the farms of the turkey flesh, with a proportion of 16.3 %, in Poland the strain was isolated from carcasses of chickens and pigs with respectively 0,001 % (n= 568) and 0.03 % (n= 33) (Andrzej and Wasyl, 2002). In Bulgaria its isolation has been made from the carcasses of chicken flesh with a rate 0.018 per cent (n= 53) (Valcheva *et* al., 2011).

S. Agona whose isolation rate is 11.3 per cent (Table 4) was isolated for the first time in Ghana (Guinea *et* al., 2011). Then this serotype has been screened in many countries around the world either in the levies of human origin is of animal origin (Clark et al., 1973).

The serotype Saintpaul whose isolation rate is 9.7 percent has caused in 2008 an epidemic which has been triggered in 43 American States. In effect, on a total of 1442 cases of intoxication declared, at least 286 hospitalizations and two deaths have been reported (Castro-del Campo et al., 2012). According to a study carried out by the European Union by Janine and collaborators (2010) *S.* Saintpaul infection has been detected in the flocks of turkeys (droppings) in 12 countries, reflecting the wide dissemination of this serotype with an increase in multidrug highlighted studied (Molla et al., 2007).

S. Ruzizi and *S.* Parkroyal, whose isolation rate is low, are not at all common among poultry, they probably originated from the environment and particularly of other animal species which have a free access to cattle farms, sheep, pigeons, reptiles, insects, rodents and frogs. These serotypes are not usually isolated in Morocco or even in North Africa. To our knowledge, their isolation is a first in this part of the world; we suggest that they may have been imported with the food, the turkey poults or in the eggs.

Generally, the comparison of the prevalence or distribution of serotypes among the various regions of the globe should be taken with caution, because it

takes into account the methods of sampling and microbiological analysis carried out for each case study.

Table 4. Resistance (%) to antibiotics of *Salmonella* strains isolated from turkey droppings in Morocco.

Serotypes	K	S	T	N	E	A	H	R	P	Total
Number	**21**	**6**	**5**	**3**	**4**	**7**	**4**	**2**	**10**	**62**
Cip	100	0	0	0	0	0	0	0	0	33.9
Caz	0	0	0	0	0	42.8	0	0	0	4.8
Amc	23.8	16.7	40	0	0	28.5	0	0	20	16.1
Cro	0	0	0	0	0	42.8	0	0	0	4.8
Spt	85.7	0	0	0	0	0	0	0	20	32.3
Cn	57.1	16.7	0	0	0	0	0	0	0	21
Na	100	0	20	0	25	0	0	0	0	37.1
C	4.8	0	40	33.3	0	0	50	0	20	12.9
Te	95.2	83.3	0	100	50	57.1	75	100	100	79
Sxt	19	83.3	0	0	0	42.8	0	0	30	24.2
S	100	66.7	20	66.7	0	57.1	50	50	90	72.5
Amp	38.1	16.7	40	33.3	50	71.4	0	0	20	33.8
K	14.3	0	0	33.3	50	0	0	0	50	17.7
Ctx	0	0	0	0	0	42.8	0	0	0	4.8
Tmp	19	50	40	100	3	14.3	0	50	30	30.6

S: *S.* Saintpaul, K: *S.* Kentucky, A: *S.* Agona, T: *S.* Typhimurium, I: *S.* Infantis, E: *S.* Enteridis, H: *S.* Heidelberg, R: *S.* Ruzizi, P: *S.* Parkroyal. Cip (Ciprofloxacin; 5 µg), Caz (Ceftazidime; 30µg), Amc (amoxicillin + acid. Clavulanic; 30 µg), Cro (Ceftriaxone; 30ug), Spt (Spectinomycin; 100 µg), Cn (gentamycin; 30 µg), Na (nalidixic acid; 30 µg), C (Chloramphenicol; 30 µg), Te (Tetracycline ; 30 µg), Sxt (Sulfamethoxazole-Trimethoprime ; 25 µg), S (Streptomycin; 10 µg), Amp (Ampicillin; 10 µg), Ctx (Cefotaxime; 30 µg), K (Kanamycin; 10 µg), Tmp (trimethoprim; 5 µg).

Antimicrobial Resistance

In comparison with our results (Table 4), Chen and collaborators (2004), Thong and collaborators (2002) and White and collaborators (2001) have also reported rates of high resistance to the three antibiotics tetracycline (73.8%), streptomycin (57.9 %) and sulphonamides (63.3 %) among *Salmonella* isolated from the meat. However, lower rates than those recorded in our study have been indicated by Elgroud and collaborators (2008) in Algeria among *Salmonella* isolated from farms and slaughterhouses of broiler chickens. This high percentage reflects the frequency and length of the use of these antibiotics. As well, the tetracycline has a predominant place in the veterinary requirements; the resistance to this antibiotic is fairly answered. It is generally due to a gene plasmid which can be acquired easily by bacteria (Aarestrup et al., 2007). Strains resistant to chloramphenicol were also isolated during this study, although these antibiotics are banned for a few years in animal production. The maintenance of this resistance could be explained by the association of the gene responsible for this resistance with other resistance genes in plasmids or any other mobile genetic element, which enables it to be co-selected while in use of antibiotic (McMurry et al., 1994). We note also a high proportion of strains resistant to nalidixic acid and ciprofloxacin. This antibiotic represents the choice treatement for serious infections with *salmonella* in adults (Bouchrif et al., 2008). These rates of present study are in accordance with other studies which have emphasized the rise of resistance to ciprofloxacin (Cailhol et al., 2006 ; Cui et al ., 2008; Lecoanet, 1992; Yang et al., 2010) and to nalidixic acid (Van and al ., 2007; Kwai and Shabnam,2011). These resistances (Cip^R and Na^R) may be due to the use of some fluoroquinolones in food and water used in watering of the turkeys. In addition the resistance (Na^R) can be explained by the chromosome mutations in the gene coding for the DNA gyrase, a phenomenon of efflux pump and /or a decrease of the membrane permeability (Cloeckaert and Schwarz, 2001). The *Salmonella* resistant to ciprofloxacin are generally resistant to several antibiotics (Cui et al., 2008), they are associated with morbidity and mortality. Consequently, *S.* Kentucky which is 100 percent Cip^R in our study has been isolated, during 2002-2006, at home of French travellers returning from the east and northeast Africa with a CMI > 4µg/ml (Oliveira et al., 2002), among travellers returning from Egypt (Weill *et al*., 2006) and the Emirates in a patient who is being cured of cancer (Michael et al., 2005). In Morocco, in 2006, the bacterium was isolated from a hospitalized child to the pediatric service with a high resistance to ciprofloxacin presenting a CMI of 4-16 µg/l (Bouchrif, 2009).

S. Agona is also among the human isolates or food which represent a broad resistance to antibiotics (Majtan et al., 2006; Michael et al., 2005). The findings of our study is translated on the one hand, by hand with three ESBL strains which have a high level of resistance to ceftriaxone with a MIC, for the first time in Morocco from 16 µg/ml . These results are worrying and alarming: the indication of

ceftriaxone in the treatment of prophylaxique of human infections *salmonella* is very common.

The resistors the gentamycin (21%) and spectinomycin (32.3%) are important but are still lower than those reported by Poppe and collaborators (1995) among strains isolated from turkey farms in Canada. These resistors can be explained by the injection of gentamicin, spectinomycin, and norfloxacin to turkey poults for preventing infection of *E. coli* (Poppe et al., 1995).

The interpretation or explanation of the high rates of the multiresistance to antibiotics can be attributed to the fact that all strains come from offending environments (farms) or the antibiotics are in everyday use. In fact, Novick (1985) and Nowroozi et al (2004) have shown that the uncontrolled use of antibiotics in poultry production has increased the emergence of multidrug-resistant bacteria. In order to limit the selection and multiplication of multidrug-resistant strains, the European commission (EC) "regulation No 1831/2003" in European Union has banned the use of antibiotics as growth stimulus in animal husbandry.

Risk Factors

It has been proved that the season is associated with the contamination of flocks by *Salmonella spp* (OR= 6.31; I.C to 95 %] 2.01 -19.79 [) (Table 3). In fact, only 1 out of 8 farms visits during the cold season was positive, while 8 out of the 12 farms visited during the hot season, were contaminated. Relevant to what has been conventionally described in the literature (Annan-Prah et al., 1998) , it is obvious that the hot season offers, on the one hand, conditions of high temperature promoting the multiplication of the bacteria, on the other hand, the excessive humidity of the litter makes it favorable to the development of micro-organisms and insects, it is as well as the litter may intervene in the transmission of *Salmonella* spp. (Lecoanet, 1992).

A short duration of crawlspace (OR=3.5, I.C to 95% %] 1.2 -10.2 [) (Table 3) seems to contribute significantly to increase the risk of contamination. Actually, 7 out of 10 farms which have undergone a sanitary vacuum less than 15 days, are contaminated. It is obvious that a long duration of crawlspace allows you to prolong the action of disinfectant (Barkok and Addioui, 2010). As well, the turkeys are subject to contamination after 40 days (OR= 5.25). This result is supported by several assumptions according to which the treatment by the antibiotics in the establishment reduces contamination (age < 40 days), the length of the duration of the band generates more passages in the building, and therefore constitutes a potential vector of *Salmonella* (Cardinale et al., 2004).

The use of antibiotics on the first day (OR = 6.82; P=0.001) (Table 3) has proved to be a medium prophylaxique of fight against infection with *Salmonella* of birds in farms (Bousser, 1985). But the establishment of an antibiotic treatment at startup can slow down the maturity of the digestive flora of turkey poults (Kimura et al., 2004).

The storage of manure to the inside of the farm (OR= 8, I.C to 95% %] 2.18-29.31 [, P= 0.003) (Table 3) showed a strong significant association to the contaminations by S*almonella:* the farming or the storage of manure that are done outside the farm has shown a rate of contamination lower than farming or storage that is done inside. This observation is in agreement with the results of Villate (2001) who has found that the flow of manure contaminated on the pastures, presents a dual risk: the contamination of water and that of direct contamination of the animals placed on this parcel. Thus, the manure must be stored as far as possible from livestock buildings and buried quickly (Barkok and Addioui, 2010).

The Contamination by *Salmonella* poults takes place as soon as the sick turkeys are put inside the farm, that is to say, farm: the turkeys that have been already contaminated will be a significant risk factor (OR= 4.60, I .C to 95% %] 2.13 -35.2 [, P= 0.002) (Table 3), In other words, the turkeys contribute to the increase in the level of contamination of livestock buildings through their droppings (Barkok and Addioui, 2010).

The conservation of sick turkeys in a corner of the rearing building (OR=7.89), isolated only from the rest of the group by a partition not hermetic, promotes the contamination, to the extent that the percentage of contamination is reduced twice when the sick birds are placed outside of buildings. That is to say, the contamination of the air by the sick animal will spread among the turkeys (Chemaly, 2006). In addition to the contamination transmitted either by the animals (rodents, insects, birds, reptiles, flies, mites…) or by the employees because of cross-contamination.

CONCLUSION

The results found in the course of this study have shown a high rate of contamination by *Salmonella* in poultry farms in turkey flesh in the region of Khemisset (35 %). It has informed us, on the one hand, on the serotypes most frequently isolated, namely *S*. Kentucky, *S*. Parkroyal, *S*. Saintpaul, *S*. Typhimurium, *S*. Agona, *S* .Enteritidis and *S*. Heidelberg, as well as their percentage in relation to the totality of the isolated serotypes, This allows us to make comparisons at the national and international scale with other studies . On the other hand, it has shown us the increasing frequency of strains with multiple resistance to antibiotics especially those used in the therapy of human *salmonella* infections, which allows you to foresee the types of contamination transfer and resistance factor between the bacteria. The data concerning the antibiotics used in farms of the turkey are very limited in Morocco, epidemiological studies showed an emergence of resistance to quinolones of *Salmonella* in Morocco. It is therefore time to give more importance to food security and to rationalize the use of fluoroquinolones in veterinary and medical practice, to limit the emergence of mutants resistant to quinolones. The potential risk factors to this contamination will eventually develop strategies for targeted control over these factors: this is the duration of crawlspace, the storage of manure, the contamination

by *Salmonella* as soon as the turkeys are put inside the farm, the conservation of sick turkeys in the livestock building, the season for breeding and the antibiotic treatment.

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