Contamination of broiler turkey farms by *Salmonella* spp. in Morocco: prevalence, antimicrobial resistance and associated risk factors

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A. El Allaoui [(1)*], F. Rhazi Filali [(1)], N. Ameur [(2)] & B. Bouchrif [(3)]

(1) Microbiology and health team, Department of Biology, Environmental chemistry-biology laboratory, Université Moulay Ismaïl, Faculty of science, B.P. 11201, Zitoune Meknès, Morocco

(2) Department of water and food microbiology and food hygiene, National Hygiene Institute, 27 avenue Ibn Batouta, B.P. 769, Agdal 11400, Rabat, Morocco

(3) Microbiology and food hygiene laboratory, National Hygiene Institute, 27 avenue Ibn Batouta, Rabat, Morocco

*Corresponding author: alaouixsaraa@hotmail.com

Summary

The authors present a study that estimates the prevalence, sensitivity to antibiotics and distribution of *Salmonella* spp. serotypes in 20 broiler turkey farm buildings in the north-west of Morocco. Each farm was inspected three times for this purpose; one batch of 10 pools of 5 droppings per farm was sampled on each visit ($n = 600$) for analysis. The high isolation rate observed for *Salmonella* spp. (35%) and the serotypes isolated were alarming. The authors found 62 *Salmonella*-positive isolates and identified nine serotypes: *S.* Kentucky (21 isolates, 33.8%), *S.* Parkroyal (10 isolates, 16.3%), *S.* Agona (7 isolates, 11.3%), *S.* Saintpaul (6 isolates, 9.6%), *S.* Typhimurium (5 isolates, 8%), *S.* Enteritidis and *S.* Heidelberg

(4 isolates each, 6.4%), *S*. Newport (3 isolates, 4.8%) and *S*. Ruzizi (2 isolates, 3.2%). The *Salmonella* spp. antimicrobial resistance results showed that 93.5% (58/62) of the strains were resistant to at least one antibiotic. Multi-resistant strains (resistant to three or more antibiotics) accounted for 80.64% of the strains isolated. The percentage with resistance to ceftazidime (third-generation cephalosporin), ceftriaxone and cefotaxime was lower at 4.8%. Three strains of *S.* Agona with extended-spectrum beta-lactamase were detected, with a high level of resistance to ceftriaxone and a minimum inhibitory concentration of 16 µg/ml. The variables associated with contamination are linked to: the cleanout period ($p = 0.037$); antibiotic treatment ($p = 0.001$); infection of turkey poults at placement ($p = 0.002$); manure storage ($p = 0.003$); keeping sick turkeys in the turkey house ($p = 0.009$); the season ($p = 0.001$); and the age of the turkeys at the time of sampling ($p = 0.01$).

Keywords

Antibiotic – Antimicrobial resistance – Broiler turkey – Contamination – Farming – Khemisset – Morocco – Risk factor – Salmonella.

Introduction

The problem of contamination of poultry farms by *Salmonella* spp. is of considerable importance for both public health and the socioeconomic fabric of the affected country because of the damage it can cause (1). Although poultry meat contamination is possible at any point in the food chain, the rearing period represents a critical stage for bacterial infection (2).

Despite the fact that turkey meat is responsible for twice as many cases of salmonellosis in humans as chicken products (3), until now no study has been conducted in Morocco on the prevalence of *Salmonella* spp. contamination on broiler turkey farms or the associated risk factors. The number of turkey farmers in Morocco rose from six in 2000 to more than 422 in 2010, with a batch production capacity of 9.85 million (4). This rise in output is partly due to the

preventive and curative use of antibiotics (metaphylactic and prophylactic) (5); the use of antibiotics has reduced turkey mortality, which has encouraged farmers to set up further rearing operations. However, the International Food Safety Authorities Network (6) reported the abusive use and uncontrolled administration of antibiotics, resulting in the selection of resistant bacteria. The antimicrobial resistance of zoonotic enteropathogens, principally *Campylobacter* spp. and *Salmonella* spp., is all the more dangerous for public health because these bacteria can be transmitted to humans via the food chain (7, 8).

The goal of this research was to estimate the prevalence of infection by *Salmonella* spp. on broiler turkey farms in the province of Khemisset (north-west Morocco) and to assess the antimicrobial resistance of the bacteria isolated in droppings. The authors also identified the potential risk factors for such contamination. The study highlighted the critical points associated with *Salmonella* contamination throughout the broiler turkey production chain.

Materials and methods

Choice of production sites

The province of Khemisset was selected because of its location on the Moroccan central plateau, characterised by the region's semi-continental, temperate climate, which is ideal for agriculture and highly suitable for the development of broiler turkey farms. This zone, made up of 32 rural communes, has 35 broiler turkey farms, 20 of which were operating at the time of the study.

Sampling

The study was conducted during the course of 2011 on 20 farms in the province of Khemisset, with a total production capacity of 439,000 broiler turkeys per batch. The 20 farms were chosen at random. They each comprised between two and nine buildings. One building was selected at random on each farm and the study was therefore conducted on 20 buildings (1 per farm). To determine the

status of each batch of poultry in terms of *Salmonella* spp. contamination, samples were taken ($n = 600$) during three visits to each farm (in the week prior to the removal of the previous batch, during the placement of turkey poults and before removal of the batch under study). In order to cover a large surface area in each building, 10 pools of five fresh droppings per farm per visit were sampled in sterile pots. This number of samples can be used to determine the status of an infected population with a risk of error of 5% (9). During the study, a questionnaire that had been validated by veterinarians with poultry farming experience was completed with the farmer in order to elucidate the potential risks associated with contamination. The questionnaire covered various issues, including location and environment, infrastructure, equipment, operations, hygiene, personnel, and feeding.

Detection of *Salmonella*

The bacteriological analysis was performed according to the AFNOR (French Standardisation Association) standard in force (NF U 47-100).

Colonies characteristic of *Salmonella* spp. were confirmed using biochemical criteria on the following mediums: Kligler iron agar (Biokar Diagnostic, France), Simmons citrate agar (Oxoid, United Kingdom), Mannitol mobility nitrate agar (Biokar Diagnostic, France), urea-indole medium (bioMérieux, France), lysine decarboxylase (Scharlab, Spain), orthonitrophenyl-β-galactoside (ONPG, Oxoid, United Kingdom) and oxydase (In-Vitro Diagnostics, United States of America). API 20E® gallery identification (bioMérieux, France) was conducted to corroborate the previous confirmation.

Serological and molecular identification of *Salmonella*

Serological confirmation was carried out using a slide agglutination test at the national hygiene institute of Morocco using polyvalent anti-O, anti-H and anti-Vi serums (Diagnostic Pasteur, Paris, France) and using the Kauffmann-White classification (10).

Molecular confirmation of the *Salmonella* spp. isolated was carried out using the nested polymerase chain reaction (PCR) technique in order to amplify a 1 kb fragment of DNA of the InvA gene of the chromosome (no. M90846.1 of *Salmonella* Typhimurium) specific to *Salmonella* spp. (11). The following protocol was applied: initial denaturing for one minute at 95°C followed by 30 cycles, denaturing for 45 seconds at 95°C, hybridisation for 30 seconds at 58°C, an extension for 45 seconds at 72°C and a final extension at 72°C for 10 minutes. The PCR product was analysed by electrophoresis on 1.5% agarose gel.

Antimicrobial resistance

The antibiogram was produced by the Mueller-Hinton agar method using antimicrobial susceptibility disks (Bio-Rad). The results were interpreted with reference to the rules and recommendations of the French microbiology society antibiogram committee. The following antibiotics were tested: ciprofloxacin (Cip; 5 µg), ceftazidime (Caz; 30 µg), amoxicillin clavulanate (Amc; 30 µg), ceftriaxone (Cro; 30 µg), spectinomycin (Spt; 100 µg), gentamicin (Cn; 30 µg), nalidixic acid (Na; 30 µg), chloramphenicol (C; 30 µg), tetracycline (Te; 30 µg), sulfamethoxazole-trimethoprim (Sxt; 25 µg), streptomycin (S; 10 µg), ampicillin (Amp; 10 µg), cefotaxime (Ctx; 30 µg), kanamycin (K; 10 µg) and trimethoprim (Tmp; 5 µg).

Escherichia coli (ATCC 25922) was used as a control strain. The multiple antibiotic resistance index was calculated using the following formula:

$$\text{Multiresistance index} = \frac{\text{number of antibiotics to which the organism is resistant}}{\text{total number of antibiotics tested}}$$

The amoxicillin clavulanate disk was positioned alongside the Ctx disk in order to observe the ‘champagne cork’ image indicating the production of extended-spectrum beta-lactamase (ESBL) (12). The minimum inhibitory concentrations (MIC) of the antibiotic used were measured using Etest strips according to the manufacturer's recommendations (13).

Statistical analysis

The tested batch was declared to be contaminated if at least one pool of droppings tested positive for *Salmonella* spp. The variable studied is therefore binary and indicates the presence or absence of *Salmonella* spp. A chi-square test with calculation of the odds ratio with a confidence interval of 95% was performed to test the link between this variable and each explanatory variable using the Statistica 6.0 software (Statsoft Ltd, Chicago, IL, USA). The questionnaire data were processed using the Sphinx Plus^2 software (V. 4.5.0.19).

Results

Prevalence of contamination

All the isolated strains that tested negative for lactose, urease, indole, ONPG and oxydase, and positive for glucose, lysine, citrate, mannitol, gas and hydrogen sulphide (H_2S), were tested using the API 20E method and subjected to PCR DNA amplification using the specific primers already described. The results show that all strains have a band common to the control strain (*S.* Typhimurium penta-resistant type ACTeStSul) with a size of around 1 kb (Fig. 1). *Salmonella* spp. bacteria were isolated in 35% of the 60 groups of turkeys analysed (21/60). In 25% of these (5/21) the bacterium was present in at least five of the 10 samples analysed. In the remaining 65% of groups (39/60) the bacterium was not detected (Table I).

Distribution of serotypes

Sixty-two serotypable *Salmonella* spp. isolates were obtained, within which nine different serotypes were identified. The most frequently isolated serotype was *S.* Kentucky (21 isolates, 33.8%) followed by *S.* Parkroyal (10 isolates, 16.3%), *S.* Agona (7 isolates, 11.3%), *S.* Saintpaul (6 isolates, 9.6%), *S.* Typhimurium (5 isolates, 8%), *S.* Enteritidis and *S.* Heidelberg (4 isolates each, 6.4%), *S.* Newport (3 isolates, 4.8%) and *S.* Ruzizi (2 isolates, 3.2%) (Tables II & III).

Antimicrobial resistance

The antimicrobial resistance results for the *Salmonella* spp. strains (Table II) showed that 93.5% (58/62) of the strains were resistant to at least one antibiotic, while the multi-resistant strains (resistant to three or more antibiotics) represented 80.64% of the total. There were rather high levels of resistance to Te (79%) and S (72.5%), followed by resistance to Na (37.1%), Cip (33.9%), Amp (33.8%), Spt (32.3%), Tmp (30.6%), Sxt (24.2%), Cn (21%), K (17.7%) and Amc (16.1%) (Table III). The authors did, however, note low resistance to Caz, Cro and Ctx (4.8% in each case).

One hundred per cent ($n = 21$) of *S.* Kentucky strains were resistant to Na, Cip, S and N; three strains of ESBL-producing *S.* Agona had a high level of resistance to Cro, with a MIC of 16 µg/ml. The highest level of multiple resistance to antibiotics was found in a strain of *S.* Kentucky and a strain of *S.* Agona (0.64) (Table II).

Risk factors

The questionnaire data and the results of statistical analyses linked seven of the 26 factors tested with the presence of *Salmonella* spp. in buildings at the end of the rearing period ($p < 0.05$), namely the rearing season, the length of the cleanout period, treatment with antibiotics on placement, the age of the turkeys at the time of sampling, manure storage within or outside the farm, infection of turkey poults by *Salmonella* spp. at the time of placement and keeping infected turkeys in the building. These factors represent potential risks for the contamination of farms with *Salmonella* spp. (Table IV)

Discussion

Prevalence of *Salmonella*

The rate of contamination of farms by *Salmonella* spp. revealed by this study (35%) is higher than the prevalence observed in chicken droppings in previous studies (sampling of faeces on 25 farms) (14), and greater than the rates reported by Cardinale *et al.* (15) in Senegal (28.6%) (sampling of faeces on 70 farms). However, this prevalence

concords with that found several years ago in certain European countries. In Belgium, the prevalence was estimated at 36% (sampling of faeces on 122 farms) (16). In France, in 1986, the prevalence was found to be 53% (sampling of faeces on 180 farms), while in Turkey, in 2001, the prevalence was in the order of 43.3% (17). However, in more recent years, probably because of control programmes, contamination by *Salmonella* spp. seems to have declined in most European countries. On chicken farms, the recorded prevalence is between 1% and 13.6% (16). Research conducted in Nigeria based on samples of chicken droppings in poultry buildings showed isolated *S.* Paratyphi at levels of 12.5% and other serotypes of *Salmonella* spp., such as *S.* Enteritidis, *S.* Typhimurium and *S.* Gallinarum at lower levels (18). In India, cloacal swabs from chickens revealed *Salmonella* levels of 14.7%, represented by *S.* Enteritidis, *S.* Typhimurium, *S.* Gallinarum and *S.* Paratyphi B (19). In Morocco, *Salmonella* is one of the main causes of foodborne diseases, representing 42.8% of cases of bacterial origin, with *Staphylococcus aureus* responsible for 37%, *Clostridium botulinum* for 1.7% and *E. coli* for less than 1% (20). It is, however, impossible to establish a direct link between the contamination of farms by *Salmonella* spp. and foodborne diseases in Morocco, because the majority of the Moroccan population is unaware of the risks of foodborne diseases, which are only reported in severe cases.

Distribution of serotypes

The distribution of serotypes revealed a predominance of *S.* Kentucky ($n = 21$; 33.8%), followed by the serotypes *S.* Parkroyal ($n = 10$; 16.3%), *S.* Agona ($n = 7$; 11.3%), *S.* Saintpaul ($n = 6$; 9.6%), *S.* Typhimurium ($n = 5$; 8%), *S.* Enteritidis and *S.* Heidelberg ($n = 4$; 6.4%), *S.* Newport ($n = 3$; 4.8%) and *S.* Ruzizi ($n = 2$; 3.2%). This distribution is similar to that reported by Yan *et al.* (21) in the United States of America, which indicated that the serotypes most frequently found in chickens are *S.* Heidelberg, *S.* Kentucky, *S.* Hadar, *S.* Typhimurium and *S.* Thompson. In Europe in 2006, Chemaly *et al.* (22) reported that the serotypes most frequently found in chickens were *S.* Hadar, *S.* Infantis, *S.* Virchow, *S.* Typhimurium and

S. Enteritidis, which are found in foodstuffs and in humans in both developed and developing countries (23, 24, 25). In Europe, *S.* Enteritidis, *S.* Infantis, *S.* Newport and *S.* Typhimurium are the serotypes most frequently associated with human salmonellosis (26). In the United States of America, the Centers for Disease Control and Prevention (CDC) reported that *S.* Enteritidis, *S.* Typhimurium and *S.* Newport, in that order, are the serotypes most frequently reported by public health laboratories (27). In this research, the serotype that was the most widespread in the poultry farms under study was *S.* Kentucky, representing 33.8% of all the serotypes analysed. This is an alarming rate because, according to previous international studies conducted in France, Denmark, Nigeria, the United Kingdom and the United States of America, published in 2011, the sudden and worrying emergence of *S.* Kentucky is accompanied by multiple resistance to almost all families of antibiotics (28). In Senegal, the *S.* Kentucky bacterium was isolated in prepared food (29). In Malaysia this serotype was isolated in the carcasses of broiler chickens, as well as in samples taken in slaughterhouses and processing units, in addition to droppings and the bedding of breeding stock (30). All of these results are indicative of vertical contamination in the turkey meat production chain, from upstream to downstream. In the present study, *S.* Parkroyal was isolated on broiler turkey farms at a rate of 16.3%; in Poland, the Parkroyal strain was isolated in chicken carcasses at a rate of 0.18% (1/568) and in pigs at a rate of 3.03% (1/33) (31). In Bulgaria, the serotype was isolated in broiler chicken carcasses with a contamination rate of 26.29% (86/327) (32). *Salmonella* Agona, which was isolated in 11.3% of cases in the present study, was isolated for the first time in Ghana (33). The presence of this serotype was subsequently detected in many countries around the world, in samples taken from humans and animals (34).

The serotype *S.* Saintpaul, with isolation rates of 9.6% in the present study, caused an epidemic in 2008 that affected 43 American States. Out of a total of 1,442 reported cases of poisoning, at least 286 hospitalisations and two deaths due to this serotype were recorded (35). A study conducted in the European Union by Beutlich *et al.* (36) reported the serotype *S.* Saintpaul in turkey flocks (droppings) in

12 countries, reflecting the wide dispersion of this serotype. Several studies have highlighted the increasing multiple drug resistance of the *S*. Saintpaul serotype (e.g. 37).

The serotypes *S.* Ruzizi and *S.* Parkroyal, with low isolation rates, are not generally isolated in Morocco or more widely in North Africa. As far as the authors are aware, their isolation represents a first in this part of the world. They may have been imported in foodstuffs, turkey poults or eggs.

A comparison of rates of prevalence or the distribution of serotypes between various regions of the world should be undertaken with prudence, because the different methods of sampling and microbiological analysis used in each study must be taken into account. (In Morocco, prior to this research, no study had been conducted on the contamination of turkey farms by *Salmonella* spp.)

Antimicrobial resistance

As in the present study, Thong *et al.* (38) and White *et al*. (39), who studied *Salmonella* in meat, also reported high levels of resistance to tetracycline and streptomycin. Rates lower than those recorded in the present study were reported by Elgroud *et. al* (40) for *Salmonella* isolated in broiler chicken farms and slaughterhouses in Algeria. The high percentage of resistance to tetracycline and streptomycin in the present study reflects the frequency of their use, the period of utilisation and/or the improper use of these antibiotics (dosage, observance) by Moroccan farmers. As Te predominates in veterinary prescriptions, resistance to this molecule is quite widespread. This is generally due to a plasmid-mediated gene that can be acquired easily by bacteria (41). Strains resistant to C (12.9%) were also isolated during this study, although these antibiotics have been prohibited for several years in livestock production. This resistance could be maintained by association of the gene coding for this resistance with other resistance genes in plasmids or any other mobile genetic element, allowing it to be co-selected when the antibiotic is used (42). A high proportion of strains resistant to Na (37.1%) and to Cip (33.9%) was also observed in this study. The latter is the treatment of

choice for serious infections with *Salmonella* in adults (43). These rates are in line with other studies which highlighted increased resistance to Cip (11, 44, 45, 46) and to Na (47, 48). Resistance of these types (Cip^R and Na^R) may be due to certain fluoroquinolones in the feed and drinking water given to turkeys. In addition, Na^R could be due to chromosome mutations in the gene coding for DNA gyrase, resulting in efflux and/or a reduction in the membrane permeability (49) of bacteria. *Salmonella* resistant to Cip are generally resistant to other antibiotics (45) and are associated with high levels of morbidity and mortality. The *S.* Kentucky strain, which was 100% Cip^R in the present study, was isolated during the 2002–2006 period in French travellers returning from Egypt and from east and north-east Africa, with a MIC of >4 µg/ml (50). It was also isolated in Slovakia, where the original isolate which gave rise to the spread of the strain was also associated with travel to Egypt (51). In Morocco in 2006, the bacterium was isolated in a child hospitalised in a paediatric ward and displaying a high level of resistance to ciprofloxacin with a MIC of 4–16 µg/ml (52).

Salmonella Agona, which has been isolated from both humans and food, is resistant to a broad range of antibiotics (53, 54). This finding is reflected in the present study by a multiple resistance index for this serotype of 0.64, as well as by three ESBL strains with a high level of resistance to Cro with, for the first time in Morocco, a MIC of 16 µg/ml. This result is both worrying and alarming as Cro is widely indicated in the prophylactic treatment of human salmonellosis.

Resistance to Cn (21%) and to Spt (32.3%) is significant but remains less than that reported by Poppe *et al.* (55) in strains isolated on turkey farms in Canada. These levels of resistance could be due to the injection of day-old turkey poults with Cn, Spt and norfloxacin to prevent *E. coli* infection (55).

The high levels of multiple resistance to antibiotics may be due to the fact that all the strains involved came from environments (farms) where antibiotics are used on a regular basis. Novick *et al.* (56) and Nowroozi *et al.* (57) have demonstrated that the uncontrolled use of

antibiotics on poultry farms promotes the emergence of multi-resistant bacteria. In order to limit the selection and proliferation of multi-resistant strains, European Union regulation (EC) 1831/2003 prohibits the use of antibiotics as growth stimulators in animal feed (58).

Risk factors

A significant correlation was found between rearing during the hot season and the contamination of flocks by *Salmonella* spp. (OR = 6.31; CI of 95% between 2.01 and 19.79) (Table IV). It transpires that only one farm of the eight visited during the cold season was positive, while eight out of 12 farms visited during the hot season were contaminated. As traditionally reported in the literature (59), the hot season provides conditions more conducive to the proliferation of bacteria; in addition, excessive wetting of the bedding favours the development of microorganisms and insects, which could explain the role played by bedding in the transmission of *Salmonella* spp. (46).

A short cleanout period (Table IV) seems to contribute significantly to an increased risk of contamination (OR = 3.5; CI of 95% between 1.2 and 10.2). In fact, seven of the 10 farms with a cleanout period of less than 15 days were contaminated. Clearly, a long cleanout period allows the disinfectant more time to take effect (3), as turkeys were subject to more contamination after 40 days (OR = 5.25). This result is reinforced by several theories which suggest that treatment with antibiotics at the time of placement reduces contamination (age <40 days), while a long batch period results in more passages through the building and is consequently conducive to the presence of *Salmonella* spp.

The use of antibiotics from day one (OR = 6.82; $p = 0.001$) (Table IV) is a proven prophylactic method for controlling infection by salmonella in farmed birds (60). However, antibiotic treatment from the outset can slow the maturity of digestive flora in chickens (60).

A significant link was identified between the storage of manure inside the farm building and contamination by *Salmonella* spp. (OR = 8; CI

of 95% between 2.18 and 29.31; $p = 0.003$) (Table IV): farms where manure was stored outside had a lower level of contamination than those where manure was stored inside. This finding agrees with the results of Villate (61), who observed that spreading contaminated manure on pasture represented a double risk: contamination of water courses and direct contamination of the animals placed on these pastures. Manure must therefore be stored as far as possible from farm buildings and covered rapidly (3).

The contamination of turkey poults by *Salmonella* spp. at the time of placement, i.e. already infected upon arrival at the turkey farm, is a major risk factor (OR = 4.60; CI of 95% between 2.13 and 35.25; $p = 0.002$) (Table IV). Infected turkey poults increase the level of contamination of farm buildings through their droppings (3).

Keeping contaminated turkeys in a corner of the building (OR = 7.89), isolated from the rest of the flock only by a permeable partition, is conducive to contamination; the percentage of contamination is halved when sick birds are placed outside the building. Salmonella contamination of the air by sick animals (62) can spread throughout the flock. The bacteria can also be transmitted by animals (rodents, insects, birds, reptiles, flies, mites, etc.), workers, equipment and feed (cross-contamination).

Conclusion

The results of this study show a high level of salmonella contamination in broiler turkey farms in the region of Khemisset (35%). The study identified the most frequently isolated serotypes, namely *S.* Kentucky, *S.* Parkroyal, *S.* Saintpaul, *S.* Typhimurium, *S.* Agona, *S.* Enteritidis and *S.* Heidelberg, as well as their percentage of all isolates. Data concerning the antibiotics used on broiler turkey farms are very sparse in Morocco. Nevertheless, epidemiological studies show the emergence of salmonella that are resistant to quinolones in Morocco. It is therefore time to give greater emphasis to food safety and to rationalise the veterinary and medical use of fluoroquinolones in order to limit the emergence of mutants with multiple resistance to quinolones. If the potential risk factors

associated with the contamination of turkey farms by *Salmonella* are taken into account, control strategies could be formulated based on the following: maintaining a long cleanout period, storing manure outside the farm, ensuring that turkey poults are not already infected with salmonella at the time of placement, keeping sick turkeys in the turkey house, rearing during the favourable season, and administering antibiotic treatment on arrival.

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Table I

The distribution of contaminated faecal samples taken from 20 broiler turkey farms in north-west Morocco

Three sets of samples were taken from each of the 20 farms (two from the batches under study and one from the previous batch). A set of samples was considered to be contaminated if at least one pool of droppings tested positive

	Sample sets positive for *Salmonella* 21/60 (35%)									
Number of positive pools* (per sample set)	1	2	3	4	5	6	7	8	9	10
Number of sample sets	7	4	3	2	2	1	2	0	0	0

*Each set of samples contained 10 pools. Each pool contained five droppings

Table II
Antibiotic sensitivity profiles of 62 strains of *Salmonella* isolated during a study covering 20 broiler turkey farms in north-west Morocco

Serotype	Resistance profile	Serotype	Resistance profile
Kentucky	Cip, Spt, Na, Te, S, Amp, Cn	Agona	Te
Kentucky	Cip, Spt, Na, Te, S, Amp, Amc, Sxt, Tmp	Agona	Caz, Amc, Cro, Sxt, S, Amp, Ctx, Tmp
Kentucky	Cip, Spt, Te, S, Sxt, Tmp, K, Na	Agona	Caz, Amc, Cro, Sxt, S, Amp, Ctx, Tmp, Cn
Kentucky	Cip, Spt, Na, Te, S, Amp, K (2)	Saintpaul	Te, Sxt, S, Tmp (2)
Kentucky	Cip, Spt, Na, Te, S (2)	Saintpaul	Te, Sxt, S (2)
Kentucky	Amc, Spt, Na, Te, S, Amp, Cn	Saintpaul	Te
Kentucky	Cip, Spt, Na, Te, S, Cn (5)	Saintpaul	Amc, Sxt, S, Amp, Tmp
Kentucky	Cip, Spt, Na, C, Sxt, S, Amp, Cn	Typhimurium	Amp, C, Amc, Tmp
Kentucky	Cip, Amc, Spt, Na, Te, S, Amp, Cn	Typhimurium	Amc, S, Na
Kentucky	Cip, Spt, Na, Te, S, Tmp, Cn	Typhimurium	Tmp
Kentucky	Cip, Na, Te, S	Typhimurium	C, Amp
Kentucky	Cip, Amc, Spt, Na, Te, S, Amp, Cn	Typhimurium	Sensitive
Kentucky	Cip, Amc, Spt, Na, Te, S,	Newport	S, Te, Tmp (2)

	Amp, Cn		
Kentucky	Na, Te, S, Sxt, Cip	Newport	Amp, C, K, Te, Tmp
Kentucky	Cip, Na, Te, S	Enteritidis	Na, Te, K, Amp
Parkroyal	Amc, S, C, Te, S, Amp	Enteritidis	Sensitive
Parkroyal	Te, S, K (5)	Enteritidis	Te, K, Amp
Parkroyal	Te, Sxt, Tmp, S (2)	Enteritidis	Sensitive
Parkroyal	Te, Sxt, Tmp	Heidelberg	Sensitive
Parkroyal	Amc, Spt, C, Te, S, Amp	Heidelberg	S, Te, C (2)
Agona	Amp, Cro, Ctx, S, Caz, Te	Heidelberg	Te
Agona	Amp, Te (2)	Ruzizi	Te
Agona	Tmp, S, Sxt	Ruzizi	S, Te, Tmp

Antibiotics:

Amc: amoxicillin clavulanate
Amp: ampicillin
C: chloramphenicol
Caz: ceftazidime
Cip: ciprofloxacin
Cn: gentamicin
Cro: ceftriaxone
Ctx: cefotaxime
K: kanamycin
Na: nalidixic acid
S: streptomycin
Spt: spectinomycin
Sxt: sulfamethoxazole-trimethoprim
Te: tetracycline
Tmp: trimethoprim

Table III
Percentage of *Salmonella* spp. strains resistant to various antibiotics during a study covering 20 broiler turkey farms in north-west Morocco

Serotypes	K	S	T	N	E	A	H	R	P	Total
No. of strains	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

Salmonella **strains**: K (*S.* Kentucky), S (*S.* Saintpaul), T (*S.* Typhimurium), N (*S.* Newport), E (*S.* Enteritidis), A (*S.* Agona), H (*S.* Heidelberg), R (*S.* Ruzizi), P (*S.* Parkroyal)

Antibiotics:
Amc: amoxicillin clavulanate
Amp: ampicillin
C: chloramphenicol
Caz: ceftazidime
Cip: ciprofloxacin
Cn: gentamicin

Cro: ceftriaxone
Ctx: cefotaxime
K: kanamycin
Na: nalidixic acid
S: streptomycin
Spt: spectinomycin
Sxt: sulfamethoxazole-trimethoprim
Te: tetracycline
Tmp: trimethoprim

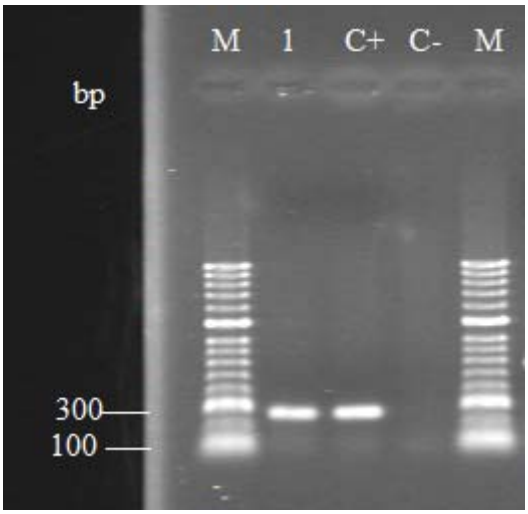
Table IV
Factors associated with the contamination of broiler turkey farms by *Salmonella* spp. (χ^2 test at 5%) during a study covering 20 broiler turkey farms in north-west Morocco

Variables	Modality	Percentage of batches contaminated (*n* = 60)	Pα	χ^2	OR	CI of 95% (OR)	RR
Rearing season	Cold	29.1	<0.001	10.79	6.31	2.01–19.79	2.84
	Hot	72.2					
Cleanout period (days)	>15	33.3	<0.037	5.45	3.5	1.2–10.2	1.91
	≤15	63.3					
Treatment with antibiotics on arrival	Yes	30.5	<0.001	11.39	6.82	2.13–35.23	3.56
	No	75					
Age of turkeys on sampling	>40 days	77.7	<0.01	7.42	5.25	1.51–18.31	1.94
	≤40 days	33.3					
Manure storage	On the farm	80	<0.003	11.32	8	2.18–29.31	2.4
	Outside the farm	33.3					
Contamination of turkey poults by *Salmonella* at the time of placement	Yes	66.6	<0.002	10.85	4.60	2.13–35.25	3.56
	No	18.7					
Keeping 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, 95%
CI (OR): confidence interval for the odds ratio of 95% using the Woolf methods (logits)
RR: relative risk
$p < 0.05$: variable significantly associated with infection by *Salmonella*

Fig. 1

Agarose gel electrophoresis of amplicons generated by single gene amplification (PCR) of *Salmonella* isolated during a study covering 20 broiler turkey farms in north-west Morocco, using virulence gene specific primers



Lane 1: amplicon of the gene invA (275 bp)
Lane C+: penta-resistant *Salmonella* Typhimurium, type ACTeStSul
Lane C–: negative control
Lane M: DNA ladder of 100 bp