Brief Original Article

Survey of *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria

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Abstract

Background: Avian salmonellosis affects the poultry industry in underdeveloped and in developed countries. The aim of this study was to identify the most common *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria according to the ISO 6579 method.

Methodology: A total of 294 samples were obtained from two flocks of 10,000 broilers and laying breeding reproducers. Samples included livers and spleens, drag swabs of bottom boxes of young chickens, cloacal swabs, and faecal samples of chickens. Additional samples were also taken from water, feed and dusty surfaces.

Results and conclusions: Only the cloacal swabs, poultry faeces and samples from dusty surfaces were positive for S*almonella* Typhimurium and *Salmonella* Livingstone with a detection rate of 12% and 1.6% respectively. The results showed evidence of legislative failure regarding biosafety within the poultry industry in the area of Batna, Eastern Algeria.

Key words: *Salmonella*, broilers, laying reproducers, ISO 6579 method

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Introduction

Salmonellosis is the main collective food-borne disease of human beings, caused by many serotypes of *Salmonella enterica*. Poultry products and derivatives have been implicated in *Salmonella* zoonoses in both developed and underdeveloped countries. Pullorum disease with *Salmonella* Pullorum represented a serious poultry health problem until huge resources were used to limit its expansion, but these efforts allowed the emergence of other *Salmonella* [1]**.** The development of food-borne disease in humans caused by *S.* Typhimurium, and *S.* Enteritidis following the consumption of contaminated eggs and egg products is mainly due to lapses in hygiene practices and the propagation of these bacteria in the poultry chain, especially in layers. This work was undertaken to investigate *Salmonella* serovars in the parental laying poults and broiler chicken poultry industry, to identify the prevalent *Salmonella* species, and to determine the bacteria's sensitivity to antimicrobial agents.

Material and Methods

Materials

Two poultry houses each containing 10,000 parental broiler chickens and laying poults (Hubbard F15 and Highline breeders) were studied. They received locally manufactured feed, and the feed and drinking water were automatically distributed. The animals were apparently healthy and no symptoms were observed.

Methods

Sampling was conducted according to the SNA Hatcheries guide for detection of *Salmonella* in breeding reproducers [2]. Sixteen livers and spleens were aseptically removed from day-old chickens and 120 cloacal swabs and 120 faecal samples were collected from the laying poults and 18 week-old broilers. Also collected were 4 x 200 ml of water aseptically from the watering system [3], 4 x 500 g of food obtained randomly from a bag on a truck during unloading [4], 24 swipes of building surfaces obtained using sterile tissue "chiffonnettes" at the level of rearing surfaces [5] and 4 bottom boxes

cleaned with sterile tissue pre-moistened [6]. All samples were immediately inoculated in 225 Buffered Peptone Water (BPW) bottles. Care was taken to prevent contamination by using aseptical methods. The samples were labelled and transported to the Laboratory of Microbiology where the analysis was performed during the four hours post sampling.

Microbiological analysis

Solid samples were transferred aseptically to sterile Stomacher bags and homogenized in 100 ml of (BPW) using a Seward 400 circulator Stomacher unit Brinkmann USA prior to static incubation at 37°C for 18-24 hours. The samples were next inoculated in Rappaport-Vassiliadis medium (RV) and Selenite Cysteine Broth (SCB) (Flucka) at the ratio of 1/10 and incubated at 43°C and 37°C respectively for 24 hours. Brillant Green Agar and Hektoen agar (Biomerieux) were streaked with culture broth. Suspected *Salmonella* colonies on agar plates were inoculated on triple sugar iron agar (TSI) and incubated for 24 hours at 37°C. Suspect *Salmonella* cultures from TSI were tested biochemically using API20E strips (Biomerieux-France). Identification of *Salmonella*-positive cultures was confirmed by the slide agglutination test using poly O and poly H diagnostic antisera (Institut Pasteur) [7].

The antibiotic susceptibility patterns of the isolates was performed using Mueller-Hinton medium and the Kirby-Bauer method [8] and 12 antibiotics as follow: ampicillin, ticarcillin, amoxicillin/clavulanic Acid, imipenem, cefalotin, cefoxitin, cefotaxim, amykacin, isepamycin, chloramphenicol , trimethoprim/sulfametoxazole , pefloxacin [8].

Results

No *Salmonella* pathogens were found in water but contamination from coliforms exceeded acceptable values, in all cases being above 25 X 10^3 CFU of Total Coliforms (Table 1); the food was also free from pathogens, especially *Salmonella.* Salmonella was recovered from 2.39 % of broiler breeders. The seven *Salmonella* from the faecal samples, the nest box, roosts, and litter were serotyped as *S.*Typhimurium. The only *Salmonella* isolate from cloacal swabs was *S.* Livingstone (Table 2). Susceptibility tests showed that all the *S.* Typhimurium isolates were resistant to ampicillin, ticarcillin, chloramphenicol and trimethoprim/sulfametoxazole but sensitive to other antibiotics

Discussion

The absence of *Salmonella* from food suggests that the food processing is well handled [9].

The high coliform count indicates that bacterial loads at other sources were not in compliance with Algerian legislation. The water sampled did not contain pathogenic micro-organisms (*Salmonella* and staphylococci); however, Algerian legislation on the drinking water also requires that the number of microorganisms in the drinking water of birds should be less than 100 CFU/ml for total bacteria and 50 CFU/ml for coliforms. The results (Table 1) showed that the coliform counts in the farms were above the acceptable level. One of the main consequences of fecal contamination of drinking water is the increased risk of enteric infection by pathogens such as *Salmonella* [3]. There is a clear link between the high degree of contamination by *Escherichia coli* and the presence of *Salmonella*, but in this case *Salmonella* were not isolated at significant levels from broilers and laying reproducers as has been reported previously by several authors [10].

The cloacal swabs collected from the first building of six-week-old broiler reproducers did not carry *Salmonella*, and the laying reproducers produced only one isolate of *S.* Livingstone. Low recovery of *Salmonella* in these cases may be related to the early age of the birds.

Four *Salmonella* isolates were recovered from the poultry faeces sampled in the first building of broilers but no *Salmonella* were recovered from level surfaces. As a consequence, we followed the SNA hatcheries guidelines and doubled the number of samples to seek *Salmonella* in the second building [2]. After this second isolation, we detected more isolates of *S.* Typhimurium on the litter faeces. This finding is similar to that of of Aeron *et al.* [11].

The litter faecal material of the building, sampled using drag swabs, shows a high level of contamination by *Salmonella* [5,10]. This can lead to contamination of the poultry carcasses from the feathers and feet of birds and therefore litter is an excellent indicator of poultry contamination by *Salmonella* [5].

The antibiotic sensitivity patterns we found were similar to results obtained by Leon-Velarde *et al*. [15], essentially consisting of resistance to ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole and tetracycline, the profile of *S.* Typhimurium DT104. However, due to the absence of facilities in our laboratory for phage typing or PCR, we are unable to investigate these results further.

Table 1. Microbiological analysis of the water.

Isolated organisms	Number of CFU /ml
Total mesophilic Aerobic Count Flora	2.42 X10 ^{3}
Total Coliforms	25 X10 ^{3}
Enterococci	60
Sulfito-reducing anaerob	7 X10 ^{2}

Table 2. Distribution of *Salmonella* in broiler and layer breeding reproducers and living surfaces.

Samples	Number of samples	Positive cases	Percentage (%)
Drag swabs	2	0	0
Bottom boxes	4	0	0
Liver and spleen from 1 day old chicken	16	0	0
Cloacal swabs 6 weeks	120	1	0.83
poultry faeces 18 weeks	120	4	3.33
Surfaces (Wall, Litter) 18 weeks	12	0	0
Surfaces (nest boxes, roosts, litter) 22 weeks	12	3	25
Water (200ml)	4	0	0
Food (500g)	4	0	0

Table 3. Prevalence of *Salmonella* serovars in broiler and layer breeding reproducers.

Serovars	Source	Percentage (%)
	broiler breeding reproducers	Layer breeding reproducers
SalmonellaTyphimurium	7/72	12
Salmonella livingstone		1/60 1.6

Our legislation defines general measures for prevention of contamination in poultry breeding and recommends systematic livestock incineration following positive bacteriology with a rate greater than 2% (for *Salmonella* other than *S.* Enteritidis and *S.* Typhimurium, which are considered as major pathogens) followed by serologic confirmation. The buildings we investigated presented a higher prevalence of *S.* Typhimurium (12%) and were not in compliance with the law, illustrating the need for more strategies to prevent poultry infection. Some strategies that have been proposed in the literature include competitive exclusion of the bacteria by a probiotic *Lactobacillus acidophilus* [13] and vaccination programs [14].

Conclusion

This study revealed that the prevalence rate of *Salmonella* in broilers and laying breeding reproducers in Batna were 12% and 1.6% for *S.* Typhimurium and *S.* Livingstone respectively. The poultry faeces and litter were the only samples contaminated with these pathogens. The existence of *S.* Typhimurium in poultry and in the environment of broiler reproducers is a potential danger to public health. Measures must be observed to reduce the risk

of contamination of poultry and to prevent infection by consumption of avian products.

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