

Brief Original Article

Minor *Salmonella*: potential pathogens in eggs in Algeria

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Abstract

Introduction: Salmonellosis is one of the major foodborne diseases known to be closely related to the consumption of contaminated eggs, infected poultry, and poultry products. Control and survey of the poultry chain are the key elements and the most critical steps in the prevention of human transmission of *Salmonella*.

Methodology: This study was carried out in East Algeria on 150 eggs meant for consumption collected from mini-markets and immediately tested for *Salmonella* using standard methods (ISO AFNOR 6579 modified in 2002). Briefly, the shell surfaces were carefully wiped using sterile appropriated tissues while the white and yellow yolks were separated. All 10 samples were pooled together and a total of 45 samples were carefully analyzed.

Results: A contamination rate of 4.4% was found, and two strains of *Salmonella bradford* were isolated from white and yellow yolks. The results showed that XLT4 was the best medium for *Salmonella* isolation from yolks. Screening for other *Salmonella* in parental chickens using an enzyme-linked immunosorbent assay (ELISA) test revealed seropositive cases of *Salmonella enteritidis* at the top of the poultry production pyramid.

Conclusions: Occurrence of *Salmonella* in yolks and seropositive results for *S.* in parental chickens is a serious and potential danger to public health. Radical and preventive measures must be taken at the critical points to control and to avoid human transmission. These measures must be installed at all levels of egg production through the application of appropriate and strict regulations, and use of good hygienic practices in transport, storage, and food preparation.

Key words: *Salmonella*; eggs; albumin; yolk; media.

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Introduction

Salmonellosis is one of the major foodborne diseases in developed countries [1]. It is known to be closely related to the consumption of contaminated food of animal origin, including eggs and poultry meat [2,3]. *Salmonella enteritidis* phage type 6 (PT6) is suspected to be the cause of epidemics of foodborne diseases in many countries [4]. Other severe outbreaks of *S. enteritidis* PT1 infection have been observed [5]. *Salmonella typhimurium*, a redoubtable pathogen, was found and isolated from table eggs [6]. In the poultry industry, our government enforces regulations to control contamination by *Salmonella* pathogens that are considered to be zoonoses (*S. enteritidis* and *S. typhimurium*). If both serotypes are detected in any product in the poultry chain, the veterinary authority will stop the product from being sold for consumption.

Among all food products, eggs have physical and chemical properties that give them the best weapons against antimicrobial defense. Yet, within the chain of egg consumption, egg contamination can occur by

vertical transmission of *S. enteritidis* that has an invader character, which leads to ovarian follicle infection without apparent symptoms.

In our study, the eggs were from laying hens, which were distributed by the main supplier of eggs for hatching to pullet breeders in the Batna district. *Salmonella* detection in eggs is done consistently. When *Salmonella* serotypes considered to be minor *Salmonella* (*i.e.*, those that are not subject to the survey of the veterinary authority) are found, we are prompted to seek zoonotic serotypes such as *S. enteritidis* in a more appropriate manner using the enzyme-linked immunosorbent assay (ELISA) technique. The discovery of this kind of *Salmonella* led us to think that the main source of reproductive hatching eggs in the region is contaminated.

The aim of this study was to control the quality of eggs from *Salmonella* contamination and to seek *S. enteritidis* in parental layer breeders from which these eggs originated.

Methodology

There are 10 supermarkets in Batna. Eggs, carefully selected based on size and quality, are presented in plates covered with cellophane in markets, but they are expensive for the average consumer. Small markets characterized by a lack of air-conditioning facilities usually sell eggs in small retail markets in the summer and warm weather. The sample plates of eggs examined in this study were selected based on the quality of eggs; dirty, cracked, and deformed eggs were purchased.

Five egg-tray packs (30 eggs each) were obtained from five retail sellers arbitrarily, which were chosen from a group of 50 small markets. These downgraded eggs are exposed in market stalls but are generally not sold to consumers. The present study focused the effect of these downgraded eggs and their consequences on the proliferation of *Salmonella* on the shelf life of eggs. These eggs were transported as soon as possible to the laboratory and quickly analyzed to search for *Salmonella* according to ISO AFNOR 6579 method modified in 2002 [7]. Briefly, shell surfaces of 10 eggs were cleaned with sterile wet tissues (10 eggs constituted one sample). Yellow yolks were aseptically sampled, pooled together, and mixed using a stomacher to get best homogenization; the same was done for the albumin. Each sample (sterile tissue, pooled yellow yolk, and albumin) was inoculated on 225 mL of buffered peptone water (BPW). After 16 hours of incubation at 35°C, 0.1 mL and 10 mL of BPW were transferred into 10 mL of tetrathionate broth and 100 mL of selenite broth, respectively. Then, subcultures were done on XLD agar, XLT4 agar, and Hektoen agar, and were then incubated at 37°C for 24 hours.

The suspected colonies were inoculated on triple sugar iron (TSI) agar, and those showing results indicative of *Salmonella* (Gaz [+], H₂S [+], glucose [+], lactose [-], sucrose [-], and urea indole [-]) were confirmed on API-10S strips. Serotyping was done by polyclonal *Salmonella* antisera O and H as well as phase inversion [8]. An antibiogram for the 14 most commonly used antimicrobials in human and

veterinary medicine (ampicillin, ticarcillin, amoxicillin/clavulanic acid, imipenem, cefalotin, cefoxitin, cefotaxim, amikacin, isepamycin, chloramphenicol, trimethoprim/sulfamethoxazole, and colistin) was obtained using the Clinical and Laboratory Standards Institute (CLSI)’s Kirby-Bauer method [9].

This study was extended to detect *S. enteritidis* in parental chickens 22 months of age. The eggs used in the study originated from parental layer hen breeders belonging to the public holding of poultry, which dispatches eggs for laying pullets to all the regions of Banta governorate (42 units in total). These birds produced eggs distributed by supermarkets (10) and many minimarkets (150). The screening of *S. enteritidis* antibodies in the 360 sera belonging to parental chickens of layer breeders was undertaken using an ELISA kit (IDEXX Laboratories, Westbrook, Maine, United States). Blood collections were made carefully from the wing vein; 5 mL of blood was obtained in sterile tubes, and serum was collected after centrifugation at 1,500 × g.

S. enteritidis in the oviducts of laying hens was not investigated because there was no suspicion of *S. enteritidis* in this herd. If *Salmonella* is detected in the oviducts of breeder pullets, these pullets must be slaughtered, which is not permitted before salmonellosis was declared in the herd.

Results

Enterobacteria in egg components is generally more important than those recovered on the loaded shell with or without feces, blood, or cracks, but the differences are not significant [10]. The microbial load of egg components is generally significant when related to the cage type of laying hens [11] as well as the source of food, the use of drugs, and exposure to high temperatures.

Two isolates of *S. bradford* were detected from tray pack five and on the third pool of samples (Table 1). Only XLT4 permitted isolation of *Salmonella* strains from the white and yellow yolks, but not from shell (Table 2). Few serovars have been isolated; one

Table 1. Samples and number of isolated *Salmonella*

	Tray pack 1	Tray pack 2	Tray pack 3	Tray pack 4	Tray pack 5
Number of samples	9 (3+3+3) Shell + white + yellow	9 Shell ++ white + yellow			
Number of isolated <i>Salmonella</i>	0	0	0	0	2 (white + yellow)
Serotype	-	-	-	-	<i>S. bradford</i>

isolate was found in France, and another from a turkey in Lower Normandy [12]. Resistance of *S. bradford* to ampicillin, ticarcillin, amoxicillin-clavulanic acid, chloramphenicol, and trimethoprim/sulfamethoxazole is similar to serotypes tested by Maripandi and Al-Salamah [13] and isolated from human and poultry samples (Table 3).

The prevalence of *Salmonella* in the commercial eggs samples used in this study was 4.44% (two samples positive from 45 samples). This rate is higher than that reported by Radkowski in 2001 and Poppe *et al.* in 1998 (0.4%). Cracked and dirty eggs have higher rates of *Salmonella* contamination [14,15]. Suresh *et al.* found that contamination of trade eggs by *Salmonella* was about 7.7% and egg surface shell contamination was 5.9%, while contamination of the eggs' contents was around 1.8% [16]. The most serotype most commonly found has been *S. typhimurium* [17].

Detection of *Salmonella* needs special and careful attention. *S. enteritidis* has the ability to colonize the ovaries and the oviducts of laying hens for long periods of time and persists in the parental breeder flock population [12]. This bacterium has emerged as potential foodborne disease in humans [17,18]. Strains of *S. enteritidis* were found in chicken meats from

retail outlets, with a prevalence of 15.91%, and exhibited resistance to more than one antibiotic [13]. An organic acid mixture has been evaluated and used to reduce *S. enteritidis* horizontal transmission in broilers [19]. Other *S. enteritidis*-specific antibodies (IgY) derived from egg yolks and combined with probiotics have a protective effect and prevent *Salmonella* infection in poultry [20].

It is important to consider the protective effects of the albumin of eggs, which inhibits *S. enteritidis* growth in a dependent mode of time and temperature; however, when the egg white and yellow are mixed together, the protective effects of lysozymes contained in the white are inhibited. Bacteria contained in eggs, especially *S. enteritidis*, can proliferate with more vivacity when iron sulphate is added to the pre-enrichment broth [21].

During the period 2000–2012, more than 780 avian *Salmonella* outbreaks were recorded at the national level with a prevalence of 42.08% and were identified as belonging to *S. enteritidis*. Over 33% of *Salmonella* outbreaks have been declared in laying hens [22].

Screening for *S. enteritidis* using an ELISA kit showed eight positive cases from 360 parental chickens (2.22%).

Table 2. Isolation of *Salmonella* on selective media

	Hektoen	DCLS	XLT4	XLD
Shell	-	-	-	-
White	-	-	+	-
Yellow	-	-	+	-

Table 3. Antibigram of isolated *S. bradford*

Antibiotics	Diameter (mm)	Interpretation
Ampicillin	20	R
Ticarcillin	27	R
Amoxicillin/clavulanic acid	21	R
Imipenem	29	S
Cefalotin	24	S
Cefoxitin	28	S
Cefotaxim	34	S
Amikacin	26	S
Isepamicin	28	S
Chloramphenicol	25	R
Trimethoprim/sulfamethoxazole	23	R
Colistin	19	S

R: resistant; S: sensitive

Discussion

The existence of *Salmonella* on the shell surfaces and insides of eggs represent a potential threat to public health. Surfaces can be contaminated either in the distal part of the oviduct or by fecal matter [19]. *S. enteritidis* appears to play a key role in egg contamination and appears to be found mostly on eggshells [12]. Sun exposure, ionizing radiations, and insufficient storage of commercial eggs for consumption have direct consequences on the quality of eggs, but have direct effects on the microbial charge of the shell surface [20]. This may explain the lack of *Salmonella* serotypes on shell surfaces in this study.

Eggs are not sanitized after they are posed in pack trays in all floors of laying hens producing table eggs. Germs located on egg shells are certainly excessive, in addition to exposure of egg trays to heat under poor storage conditions. This fact makes consumption of eggs from small markets a source of contamination for humans. Even if the rate of contamination is not very high (4.4%) and *Salmonella* responsible for zoonosis in these eggs is not isolated, the risk still exists. Freshly laid eggs contain small numbers of *Salmonella* cells [24]. Prompt refrigeration is crucial to restrict the development of these bacteria [22]. Prevention of foodborne disease requires radical improvement of catering practices and kitchen hygiene [25].

Conclusions

The rates of *Salmonella* seropositive cases in parental chickens is alarming. Parental chickens are located at the top of the poultry production pyramid and play a key role in the vertical and rapid transmission of the infections to their offspring with amplified antibiotic resistance. Preventive and drastic measures must be adopted at all levels of the table-egg production system through application of appropriate regulations and use of good practices.

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