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Penetration Time of Salmonella Heidelberg Through Shells of White and Brown Commercial Eggs

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Eggs, penetration time, Salmonella Heidelberg.

ABSTRACT

This study aimed at determining the minimum time required for the penetration of Salmonella Heidelberg inside the eggs after contact with contaminated material. Recently-collected brown and white eggs from laying hens between 45-50 weeks of age, reared in a commercial poultry house, were artificially contaminated by contact with wood shavings moistened with liquid inoculum of Salmonella Heidelberg in stationary-growth phase (10^3-10^4 CFU g^{-1}). According to type (white or brown), eggs were distributed into three different groups, with four replicates each: negative control group (no artificial contamination), positive control group (analyzed externally immediately after contamination and internally after the maximum storage period of the test group) and test group. Eggs were stored at controlled environmental temperature varying from 25ºC to 30ºC. In the test group, eggs contents (yolk and albumen) were pooled and analyzed after 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, and 4:00 hours after contamination for the presence of Salmonella Heidelberg in 25g of this pool. The experimental unit consisted of five eggs in each test. The analysis protocol included pre-enrichment, selective enrichment, plating on selective agar, and biochemical and serological tests. The results obtained were submitted to logistic regression, which indicated that the presence of Salmonella Heidelberg was verified after 2:16 h and 2:44 h of contact with white and brown eggs, respectively.

INTRODUCTION

The egg is probably the food item most frequently involved in outbreaks of foodborne infections with Salmonella spp as etiological agent (Gantois et al., 2009; Gast et al., 2005). Eggs are commonly contaminated by Salmonella through the shell. Carrier birds shed the pathogen in the feces, where it may persist for long periods in empty poultry houses, highlighting the importance of the environment in horizontal transmission (Silva & Duarte, 2002).

Salmonella Enteritidis is the serovar most commonly involved in human disease; however, other serovars, such as S. enterica serovar Heidelberg (S. Heidelberg), are becoming increasingly frequent. (Mammina et al., 2003). Salmonella Heidelberg has been detected in Brazilian poultry stocks since 1982, characterizing the importance of the emergence of this serotype (Hofer et al., 1997). The Centers for Disease Control and Prevention have implicated Salmonella Heidelberg as a potentially significant source of egg-associated human disease (Gast et al., 2005).

Many studies report that Salmonella is capable of penetrating the eggshell and replicating inside the egg (Gast & Holt, 2000; Gast et al., 2005). Several factors are involved in the time required for its penetration in the egg, including eggshell, albumen, and yolk quality (Messens et al., 2004); bird age (Lapão et al., 2005); feed type and physical form

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(Pappas et al., 2005); egg storage time (Chen et al., 2005); genetics (Dunn et al., 2005); and photoperiod (Backhouse et al., 2005).

The presence of Salmonella in the egg yolk may result from the migration of bacteria from the contaminated shell. It is known that the eggshell pores allow the penetration of Salmonella, and its penetration index is related to storage time and temperature rather than to the number of pores (Kanashiro et al., 2002).

The presence of Salmonella spp in poultry flocks is constant and requires the poultry industry adopt continuous control measures during all stages of poultry production (Rodrigues, 2005). Maintaining flocks in good health status and cleaning and disinfecting eggs with chemical products after lay are the most frequent practices applied to prevent or to reduce contamination and bacterial replication in eggs (Hammack et al., 1993).

In addition to the use of effective antimicrobial products, efficient egg disinfection requires knowing precisely when microorganisms cross the eggshell in order to apply any treatments before this happens (Hammack et al., 1993; Board & Tranter, 1995).

Considering the health and economic importance of Salmonella and aiming at providing the poultry industry useful data on the time limit treatments must be performed in order to prevent internal contamination of commercial eggs, the objective of the present study was to determine the penetration time of Salmonella Heidelberg in artificially-contaminated commercial white and brown eggs.

MATERIAL AND METHODS

The study used 280 commercial white eggs and 280 commercial brown eggs of 45 to 40-week-old layers housed in a commercial poultry house. Layers were free from Salmonella spp., as tested before the beginning of this experiment. Eggs were collected immediately after lay (first morning collection) and were not washed or submitted to any cleaning process.

The serovar Salmonella Heidelberg used was isolated from birds. The isolated sample was cultivated in Brain Heart Infusion Broth, and the inoculum with a known concentration (10^3-10^4CFU g^{-1}) was prepared when the culture presented stationary growth phase.

The experimental contamination was performed by placing sterile wood shavings in bags, moistening with distilled water, sprayin with liquid inoculum in quantity sufficient to achieve 10^3 to 10^4CFU g^{-1} of Salmonella Heidelberg per gram, and placing the eggs inside the bag. Eggs remained in contact with contaminated shavings for 10 minutes at 25ºC (± 5ºC).

The study was carried out in two different phases (pilot test and determination phase) for each egg type (brown and white). During both phases, eggs were distributed in three experimental groups with four replicates each: Group 1: negative control - no inoculation, analyzed at the end of the storage period; Group 2, positive control - inoculated, analyzed immediately after experimental contamination, at hour zero; and Group 3: test group - eggs previously contaminated by contact with wood shavings containing Salmonella Heidelberg and stored at 25ºC (± 5ºC) for predetermined periods.

The pilot tests determined the shortest period in which Salmonella could be detected inside the egg. Eggs were tested 2, 4, 8, 10, and 24h after contact with wood shavings containing the inoculum. After the time of penetration of bacteria through the eggshell was determined, the verification phase started, analyzing internal egg contamination 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, and 4:00h after contamination.

Each sample consisted of five eggs. For microbiological analysis, the eggshells were cleaned with alcohol 70°GL, aseptically broken in a laminar flow hood, their content poured into a sterile beaker, and homogenized with a sterile glass stick to form a yolk and albumen pool. Samples were analyzed using conventional culture methods according to the protocol recommended by the USDA/FSIS in 2004.

Data were analyzed by simple logistic regression analysis. The dependent variable was the presence or absence of Salmonella Heidelberg in egg contents, while the independent variable was evaluation time after contamination. SISVAR software program (Ferreira, 2000) was used.

RESULTS AND DISCUSSION

The contamination of wood shavings was standardized in 10^3 to 10^4CFU/g. Average Salmonella counts in the eggshell determined immediately after experimental contamination in the first and second experimental phases were 8 x 10^4CFU/g^1 and 6 x 10^4CFU/g, respectively.

In the first phase, the presence or absence of Salmonella Heidelberg in the egg content after inoculation and storage at 22.5ºC (± 2ºC) was evaluated 2, 4, 8, 10, and 24h in four replicates. The negative control and positive control groups presented negative and positive counts, respectively, in all replicates.

The results demonstrate that Salmonella Heidelberg was detected in the egg contents four hours after

contact. These results are similar to those obtained by Schoeni et al. (1995) and Oliveira & Silva (2000), who observed that, after external contamination of eggs, different Salmonella serovars were detected inside the egg before 24 hours of storage. On the other hand, Messens et al. (2005) observed in their study on the penetration time of Salmonella Enteritidis through the eggshell that, in average, 38.7% of the eggs tested had their contents contaminated by Salmonella and that penetration occurred on the third day after contamination.

In the second phase of this study, the experimental eggs were analyzed at 1:00, 1:30, 2:00, 2:30, 3:00, 3:30 and 4:00 hours after contamination. The results showed that internal contamination occurred after 2:00 hours, but before 2:30 hours in white eggs, whereas in brown eggs, between 2:30 and 3:00 hours after their external contamination with 10^3 to 10^4CFU/g of Salmonella Heidelberg. These results are consistent with the findings of Miyamoto et al. (1998), who externally contaminated eggs for 15 minutes to 3 hours after lay and stored them at 25°C and obtained significant recovery of Salmonella after two hours of incubation.

The time in minutes after contamination and Salmonella Heidelberg counts (log CFU/g) in the eggshells immediately after experimental contamination were analyzed by logistic regression to determine the precise time of egg content contamination. Figures 1 and 2 present the logistic regression results of brown and white eggs, respectively.

Data analysis showed that white and brown eggs externally exposed to 10^3 - 10^4CFU/g of Salmonella Heidelberg were internally contaminated 2:16 and 2:44 hours after contact, respectively.

Egg quality includes several aspects related to eggshell (external quality) and to the albumen and yolk (internal quality). It has a genetic basis and parameter values are different among bird strains (Silversides et al., 2006). Several investigators compared eggs of white and brown egg layer strains (Scott & Silversides, 2000; Jones et al., 2010), but there are no studies investigating if these two egg types present different contamination patterns.

Singh et al. (2009), in a study comparing eggs of different layers strains reared in two different housing systems (cages or floor pens), found higher E. coli contamination in brown than in white eggs, but this may be explained by the fact that brown layers were reared in the floor-pen system and did not use the nests properly, laying half of their eggs on the floor.

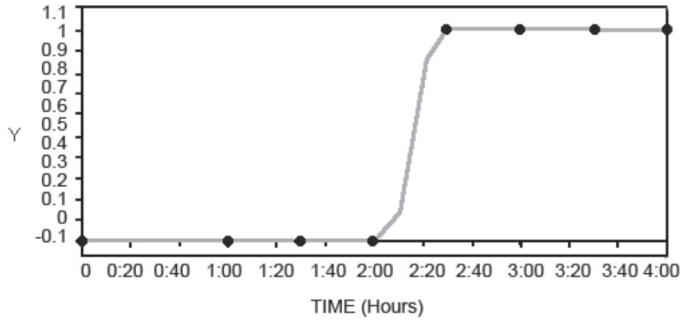

Figure 1 - Logistic regression of Salmonella Heidelberg penetration time in artificially inoculated white commercial eggs. Values in the Y axis indicate the absence (-0.1) to the presence (1.1) of the bacterium in the egg content.

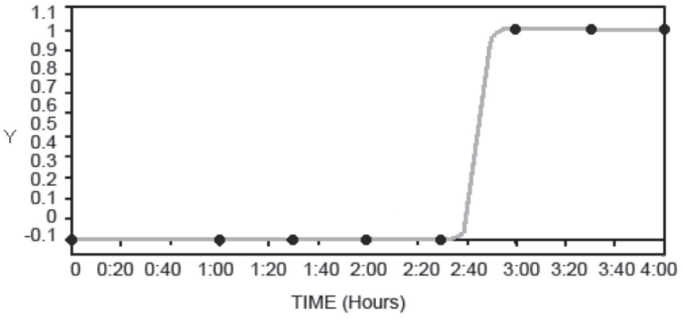

Figure 2 - Logistic regression of Salmonella Heidelberg penetration time in artificially inoculated brown commercial eggs. Values in the Y axis indicate the absence (-0.1) to the presence (1.1) of the bacterium in the egg content.

The egg has an impressive arsenal of antimicrobial protective mechanisms, including both nonspecific physical barriers and highly efficient microbiocidal molecules. However, Salmonella can survive and replicate on the eggshell despite the absence of fecal contamination, even at low environmental temperatures and low relative humidity (Messens et al., 2006). Salmonella probably survives longer at low temperatures due to the slower metabolism induced by the unfavorable conditions on the dry eggshell surface (Radkowski, 2002).

The penetration of bacteria through the eggshell is directly influenced by shell quality, as well as egg storage time and temperature (de Reu, 2006; Schoeni et al., 1995). According to Miyamoto et al. (1998), the longer the time and the higher the temperature of egg storage, the faster the penetration of bacteria through the eggshell.

Salmonella spp may also contaminate egg through the ovary. In this case, the bacteria are located in the yolk, and consequently, conventional processes of egg disinfection are useless. Therefore, adequate hygiene

measures should be a priority in layer farms. It must be stressed that no disinfection procedure per se can prevent egg contamination.

The results obtained in the present study indicate that cleaning and disinfection procedures aiming at controlling internal egg contamination should be performed before 2:16 hours in white eggs, and before 2:44 hours in brown eggs.

Ensuring layer flock health and proper disinfection of the rearing environment, facilities and eggs are important to prevent or to reduce contamination by pathogens and to ensure consumers' health.

CONCLUSION

After contaminating eggshells, Salmonella Heidelberg reaches the egg contents in 2:16 hours in commercial white eggs and in 2:44 hours in commercial brown eggs.

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