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Salmonella in Shell Eggs: Mechanisms, Prevention and Detection

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Abstract

Contaminated shell egg is one of the key *Salmonella* infection routes for human, causing food-borne illnesses. Multiple salmonellosis infections due to egg contamination still occur in developed countries even though preventive methods, such as vaccination or washing followed by rinsing process, are carried out following certified national standards. Recent outbreaks indicated that current strategies for *Salmonella* control need to be optimized to further minimize contamination of commercial eggs. Therefore, there is a critical need to develop more sensitive and rapid *Salmonella* detection methods. In this review, we address (i) egg production; (ii) preventive methods that minimize contamination; (iii) mechanisms of *Salmonella* contamination; (iv) *Salmonella* detection methods.

Keywords: Shell egg; *Salmonella*; Vaccine; Rapid detection; Food safety

Introduction

Salmonella, a gram-negative, facultative anaerobic, non-spore forming, rod-shaped bacteria are one of the most dangerous food-borne pathogens [1,2]. The Centers for Disease Control and Prevention (CDC) estimate about one million of events of *Salmonella* cases annually in the United States [3]. Among the various foods that cause salmonellosis, eggs are regarded as one of the potential sources of *Salmonella* reservoirs in the human food chain that cause illness when consumed by people [4-8].

Recent outbreaks include a *Salmonella enteritidis* contamination that led to the recall of more than 500 million eggs from Iowa between May and November in 2010. There were 1,939 infections linked to that outbreak [9]. Outside of the US, egg contamination has also been responsible for 247 cases and 3 deaths in the UK, 130 cases in other European countries in 2014 [10,11], and 353 and 1895 cases in Australia in 2014 and 2015 (Table 1) [12,13].

Eggs have also been used to produce vaccines for more than 70 years [14]. Even though there are alternative methods to produce vaccines (i.e., DNA-based, cell culture based, recombinant/purified protein based methods), egg-based vaccine production is still regarded as the preferred method with respect to productivity and scale by global health organizations and industry [15].

Country	Date	Reported Case	Organization
Australia	Apr-14	353	Government of South Australia
Australia	Mar-15	1895	AIFS[a]
Austria	Jun-14	61	EFSA[b]
France	Aug-14	45	EFSA
Germany	Jun-14	24	EFSA
UK	Aug-14	247 (3 deaths)	Public Health England
USA	May-10	1939	CDC

Table 1: International incidence of *Salmonella* associated with eggs. [a]AIFS denotes Australian Institute of Food Safety. [b]EFSA denotes European Food Safety Authority.

The alternative methods still have technical obstacles to overcome high production costs, oncogenicity and tumorigenicity risks [16]. The World Health Organization (WHO) has been transferred technologies to build and develop egg-based vaccine production capacities in several countries, including Brazil, India, Mexico, Thailand, Islamic Republic of Iran and Romania [17]. Major safety concerns in the vaccine manufacturing industry are microbial contamination by *Salmonella* and *Campylobacter* [16,18]. The development of fast detection methods for *Salmonella* in eggs is important for quality assurance and risk control not only in the food, but also in the pharmaceutical industry.

Egg production

According to CIWF (Compassion in World Farming) [19], there are more than 6.6 billion laying hens, which produce more than 65 million metric tons eggs in 2011 worldwide. As the largest shell egg producing country, more than 2.5 billion laying hens are bred in China producing 23.9 million metric tons of eggs. In the US, about 338 million laying hens are bred, compared to 363 million in the European Union (EU), with an estimated 5.4 million metric tons of eggs in the US and 7.1 million metric tons of eggs in the EU [19]. As the primary egg exporting country, the Netherlands contributed to more than 30% (0.6 million metric tons) of the global shell egg exports in 2010. Germany is the primary egg importing country and imported 27% (0.5 million metric tons) of the global share for shell eggs in 2010 [19,20]. According to AGMRC (Agricultural Marketing Resource Center)

funded by USDA [21], about 45% of eggs in the US is produced in the top 5 egg-producing states (i.e., Iowa, Ohio, Pennsylvania, Indiana and California). Approximately 55% of shells eggs go to retail, 32% and 9% are used for further processing and food service industry, respectively, and 4% of eggs are exported.

As mentioned previously here, eggs are also used to produce vaccines. One egg is equivalent to one to two doses of vaccine [22,23]. More than 150 million doses of human flu vaccine are produced in eggs each year [24,25]. The process for flu vaccine production includes: (i) preparation of 11 to 12 days old pathogen-free eggs; (ii) spiking of virus into the fertilized egg; (iii) 2 to 3 days incubation at 37°C; (iv) virus purification from egg whites; and (v) chemical treatments (e.g., formaldehyde, thimerosal) for virus and bacteria inactivation [26-28].

Preventive methods for minimizing contamination

Vaccination of laying hens is considered an effective method in reducing human salmonellosis by shell eggs in European countries [18]. A variety of vaccines (e.g., live attenuated and inactivated whole cell vaccines) are officially approved to reduce *Salmonella* contamination of eggs [29]. According to Organization of the United Nations (FAO), the vaccination for *Salmonella* reduced egg contamination by 75% by reducing the *Salmonella* invasion and colonization inside of the reproductive organs [30-33]. However, the vaccination of laying hens does not completely guarantee the control of internal egg contamination by *Salmonella* in entire chicken flocks. Several reports showed the presence of *Salmonella* inside the reproductive organs from vaccinated laying hens that could enter the internal egg [34,35].

Regulatory agencies for food safety follow different methods in different countries. In the US, vaccination is not required, but eggs must be washed and refrigerated. The entire Grade A shell eggs are carefully washed and microbial load should be minimized on the surface of shell before they go to market. Recently, the U.S. Food and Drug Administration (FDA) [36] stated that more research data are necessary to support regulation of egg safety for mandating vaccination to laying hens for *Salmonella* prevention. The US Department of Agriculture (USDA) grade mark on egg cartons means the plant processed the eggs following USDA's sanitation and good manufacturing processes. Eggs were washed followed by sanitizing rinse stages over the producing lines. Egg producers in Australia, Canada, and Japan have also tried to prevent the invasion of *Salmonella* from outside of the shell instead of vaccine treatment of laying hens [37,38]. In these counties, processing for the Grade A (Class A) shell eggs including washing and rinsing is common and/or is regarded as a safety procedure [39]. Figure 1 shows the flow of the primary processing for shell eggs [40].

Surface washing is not acceptable in the European Union except some Scandinavian countries [41]. The reason is the egg cuticle, which serves as an obstacle against invasion of microorganisms, can deteriorate during the washing and/or rinsing process [42,43]. The major goal of the regulations is to not only promote better feeding environments, with higher hygiene standards and quality eggs, but also to reduce the processing time between egg laying and its packaging for consumers [44,45].

Despite being the world's largest volume egg producer, China's egg processing and safety regulations are behind the times [46]. Since the mid-1990's, artificially produced eggs (fake eggs), made from resin, starch, coagulants, pigments, and sodium alginate, have appeared in Chinese food markets.

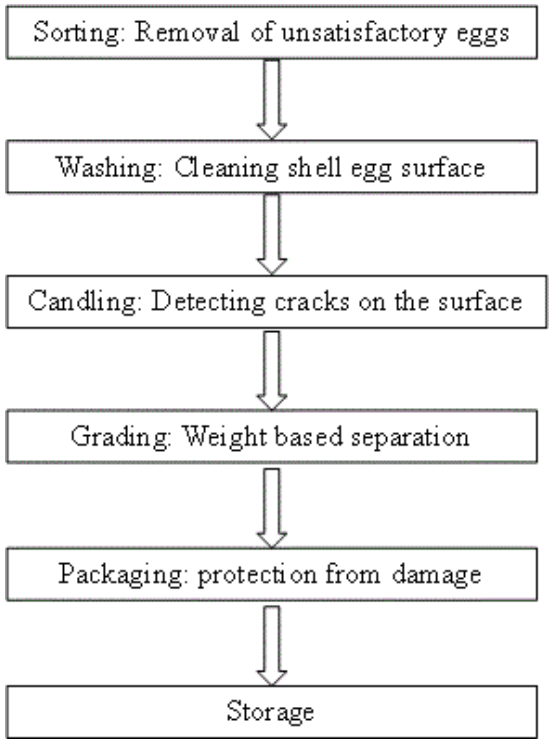

Figure 1: Work flow for primary processing of shell eggs. Adapted and modified from Food Standards Australia New Zealand [40].

This has been a serious food safety issue [47,48], although quite different from the topic addressed in this paper. Less than 10% of the shell eggs are washed and packed, and less than 0.3% of the shell eggs undergo processing for the purpose of use as food supply supplements and ingredients (i.e., dry food products, liquid products) [49]. On the other hand, China's duck egg industry is well developed and about 45% of duck eggs are processed using their traditional methods (i.e., salty eggs, century eggs) [49].

Contamination mechanism

Mechanisms of egg contamination by *Salmonella* have been described in numerous studies during the last two decades [50-54]. Both egg whites and yolks can be contaminated. However, *Salmonella* contaminations have been more often observed in egg whites at *in vivo* condition [55-57].

Causes of *Salmonella* contamination in eggs can be categorized by intrinsic and extrinsic factors [43,58,59] and the detailed contents are shown in Table 2.

Intrinsic factors	Shell porosity
	Shell thickness
	Distribution of cuticle
	Translucency

	Health condition of laying hen
Extrinsic factors	Type of *Salmonella*
	Level of initial inoculum
	Humidity
	Temperature
	Storage condition
	Washing condition

Table 2: *Salmonella* contamination factors for shell egg.

The processes of contamination can be divided into two main parts: vertical transmission (primary contamination) and the horizontal transmission (secondary contamination) [54]. The vertical transmission occurs when *Salmonella* cells migrate to the egg inside of the hen before the egg shell is formed. *Salmonella* cells move into the reproductive track and then enter into the albumen and/or yolk prior to the egg shell formation [53].

Horizontal transmission happens after the egg shell is formed. *Salmonella* cells migrate to the egg albumen from the outside of the shell after the egg has been made. Consequently, the initial contamination by the horizontal transmission occurs in the albumen. This horizontal transmission is considered the most common route for egg contamination by *Salmonella* [60]. Grijspeerdt et al. [52] reported that it took 58 hours for *Salmonella* to proliferate from one initial cell to about 508 cells in albumen.

Egg whites are reported to include ingredients that have bacteriostatic and bactericidal properties, such as ovotransferrin and lysozyme, which are positively charged and easily interact with negatively charged cell surfaces. Ovortransferrin is considered a major antimicrobial ingredient of the egg whites because it chelates iron, which is a critical growth factor for microorganisms such as *Salmonella* [61-63]. Ovotransferrin and lysozyme are known to generate pores on the surface of gram-negative bacteria resulting in membrane permeabilization [64,65]. However, there are various studies showing that *Salmonella* cells survive effectively inside of egg whites using their defense system. For example, Lu et al. [66] reported that damaged DNA by egg whites can be restored by *yafD*, *xthA* and *rfbH* genes of *Salmonella*. Other researchers have shown that *Salmonella* is more adept at thriving in egg albumen compared with other microorganisms because of the distinctive genes related to their cell wall formation and metabolism [64,67,68]. Moreover, using the siderophore, *Salmonella* can effectively uptake iron to survive in egg white [69,70].

Salmonella detection

Rapid detection of *Salmonella* is important to assure food safety by *Salmonella* monitoring and risk management in the food industry. There are a variety of methods to detect *Salmonella* and they usually depend on cultural enrichment to enhance total cell concentration and to restore injured microorganisms [71]. In the food industry, *Salmonella*, if present, is often found at low concentrations and as little as 15-20 bacilli of *Salmonella* constitute an infectious dose for human [72].

Food samples are typically enriched for the detection of low concentrations of *Salmonella* using non selective broth (e.g., buffered peptone water, trypticase soy broth, and lactose broth) to enhance microbial activities from target foods [73-76]. Traditional culture-based assays, which are regarded as the "gold standards" are widely, used by many organizations, especially by regulatory food safety agencies and food companies, because these are accepted in global regulation for trade and enable detection of low levels of pathogens [77]. However, standardized ways of detecting foodborne pathogenic bacteria by regulatory organizations (i.e., ISO, WHO, FDA) are often labor intensive and time consuming because of the sequence of the following processes: (i) pre-enrichment in non-selective broth (e.g., buffered peptone water, trypticase soy broth); (ii) selective bacterial enrichment using selective broth (e.g., Rappaport-Vassiliadis Soy [RVS] broth, Muller-Kauffmann tetrathionate [MKtt] broth); (iii) plating on selective agar plates (e.g., xylose lysine deoxycholate [XLD] agar, Brilliant Green [BG] agar, bismuth sulfite [BS] agar; hektoen enteric [HE] agar); (iv) biochemical and/or serological confirmation [73,75-77] (Table 3).

Step	Description	Time (h)
1	Pre-enrichment in buffered peptone water: non-selective enrichment	24
2	Enrichment in RVS and MKtt Broth: selective enrichment	24
3	Salmonella detection using selective agar plates	24
4	Streaking on nutrient agar	24
5	Biochemical confirmation	24

Table 3: Work flow for *Salmonella* detection from foods using ISO standard method.

This entire process usually requires 5 to 7 days to complete. About 2 to 3 days are needed for limited examination of food samples for the presence or absence of *Salmonella* cells through selective plating methods (See the step 3 in Table 3) [78].

Development of rapid and sensitive bacterial isolation and detection methods from foods has become increasingly important to prevent food poisoning outbreaks from consumers, to meet regulations of safety, and to control processing for the industry. Over the last several decades, there have been significant developments in rapid *Salmonella* detection methods using multiple novel approaches reducing pre-enrichment or detection time (e.g., *In-situ* immuno-gold nanoparticle network ELISA biosensors, immunomagnetic separation, automated microfiltration system, pathogen enrichment device, laser optical sensor, modified media, isothermal amplication) [79-89].

Product	Authors	Accuracy (%)	Specificity (%)	Sensitivity (%)
BAX	AFNOR[a] [92]	98.4	99.6	96
	Frausto et al. [93]	96.4	92.3	100
	Koyuncu et al. [91]	99	100	100
	Wallace et al. [94]	100	100	100
TaqMan	Koyuncu et al. [91]	99	90	101.8

	Oxoid [95]	98.5	99.4	97.4
RapidCheck	Allen et al. [96]	111	100	100
	Muldoon et al. [97]	100	100	137
	Muldoon et al. [98]	113.3	100	100
	Muldoon et al. [98]	325	100	100
	Muldoon et al. [98]	84.6	100	100
Reveal	AFNOR [99]	99.4	99	96.6
	Zhang et al. [100]	92	94	83

Table 4: Accuracy, specificity, and sensitivity of rapid Salmonella detection methods approved by FDA. [a]AFNOR denotes Association French Normalization Organization Regulation.

Recently, multiple rapid methods have been developed to reduce time for *Salmonella* detection from eggs based on immunology or molecular biology methods. The use of these assays led to equivalent results obtained from methods that have been officially approved by FDA [90]. These include: (i) Reveal *Salmonella* Test System of Neogen Corporation; (ii) SDIX® RapidCheck SELECT™ for *Salmonella*; (iii) BAX® System Polymerase Chain Reaction (PCR); (iv) TaqMan® *Salmonella* Detection Kit from Applied BiosystemsTM by Life Technologies™. However, these rapid *Salmonella* detection technologies still require more than one day enrichment process to get a positive signal of *Salmonella* contamination when a low level of cells is present inside of target sample (Figure 2). The accuracy, specificity, and sensitivity of commercially available kits are shown in Table 4 [91-98].

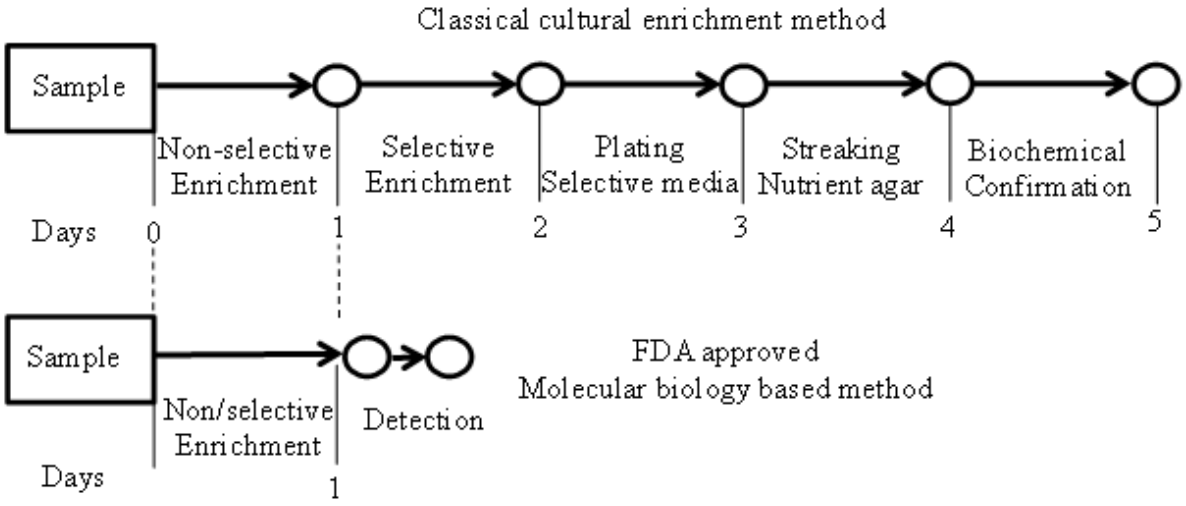

Figure 2: Comparison of microbial detection processes: The FDA approved molecular biology based methods was compared with classical cultural enrichment method (ISO-6579:2002).

One major recent development has been the reports of the use of an enzyme based approach coupled to a short enrichment and microfiltration steps for reducing time for sample preparation. This allowed *Salmonella* to be detected at very low levels (≤ 1 CFU/g) in different contaminated foods in 8 hours or less than one work shift [82-84]. The major concept is shown in Figure 3.

Conclusion

Salmonella infection caused by egg contamination is not only an ongoing global food safety issue, but it also has implications in the pharmaceutical industry for vaccine production. Although there are a variety of management practices that may be utilized during egg production, including vaccination and washing processes, salmonellosis caused by egg contamination remains the most common foodborne disease in the world. Therefore, an in-depth study of Salmonella and detection tools for eggs is still necessary to ensure food control and quality assurance. While a variety of detection methods have been developed with improved sensitivity and reduced time to result, 2 to 3 days may still be needed sample enrichment to detect low levels of pathogens. Recent reports show that is possible to combine a short enrichment step with enzyme assisted microfiltration in order to reduce the time for *Salmonella* detection to 8 hours or less.

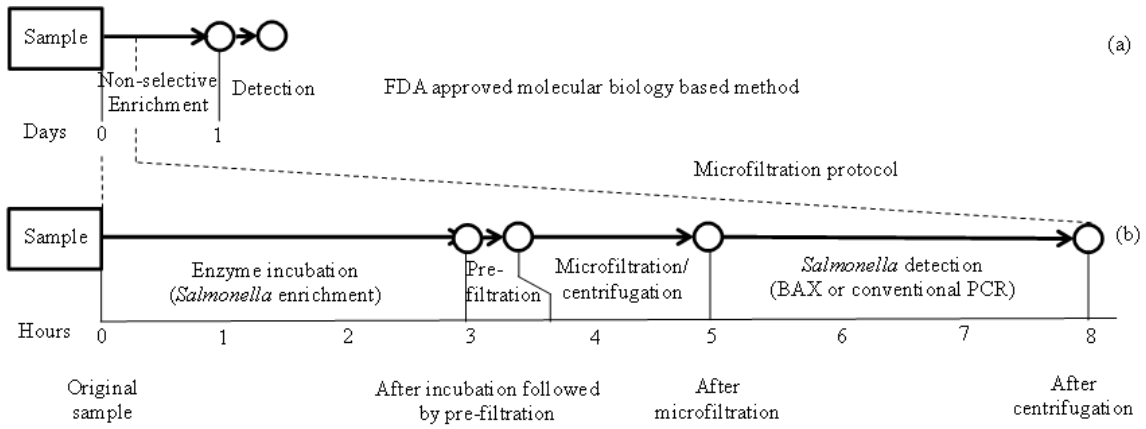

Figure 3: Comparison of cell detection processes. FDA approved rapid PCR assay (a) and the microfiltration process (b) for isolation and detection of *Salmonella* from food samples were compared.

References

1. Norajit K, Ryu G (2012) Inhibitory effect of plant extracts on Salmonella spp. In Mahmoud BSM (Eds.), Salmonella–a dangerous foodborne pathogen 332-348.
2. Gupta S, Abu-Ghannam N (2012) Recent advances in the application of non-thermal methods for the prevention of Salmonella in foods. In: Mahmoud BSM (Edn) Salmonella-A dangerous foodborne pathogen 287–304.
3. Center for Disease Control and Prevention.
4. Heo SK, Lee JY, Jo SH, Bae DH, Kim YS, et al. (2010) Inactivation of *Salmonella enterica* serovar *typhimurium* in commercial mayonnaise at different temperatures and pH. J Korean Soc Appl Biol Chem 53: 351-355.
5. Kim BY, Choi SY, Seo KY, Ha SD (2011) Temperature dependent growth characteristics and a predictive mathematical model of *Salmonella enterica* serovar *typhimurium* in Kimbab. J Korean Soc Appl Biol Chem 54: 454-459.
6. Park SH, Aydin M, Khatiwara A, Dolan MC, Gilmore DF, et al. (2014) Current and emerging technologies for rapid detection and characterization of Salmonella in poultry and poultry products. Food Microbiol 38: 250-262.
7. FDA. Egg Safety Final Rule.
8. EFSA (2010) Scientific Opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of Salmonella in laying hens. EFSA Journal 8: 1546.
9. CDC (2010) Multistate Outbreak of Human *Salmonella enteritidis* Infections Associated with Shell Eggs.
10. EFSA (2014) Multi-country outbreak of *Salmonella enteritidis* infections associated with consumption of eggs from Germany.
11. Public Health England (2014) Salmonella outbreak investigation: update.
12. Government of South Australia.
13. Australian Institute of Food Safety (2015) Not So Egg-cellent: Queensland's 2015 Salmonella Crisis.
14. CDC. How Influenza (Flu) Vaccines Are Made.
15. Christiansen K (2013) Clearing up the facts on egg-based vaccine production.
16. Chua JV, Chen WH (2010) Bench-to-bedside review: vaccine protection strategies during pandemic flu outbreaks. Crit Care 14: 218.
17. Francis DP, Grohmann G (2011) WHO influenza vaccine technology transfer initiative: role and activities of the Technical Advisory Group. Vaccine 29 Suppl 1: A45-47.
18. European Pharmacopoeia. General Texts On Biological Products.
19. CIWF (2013) Statistics: Laying hens.
20. International Egg Commission 2015.
21. Grow: Wisconsin's Magazine for the Life Sciences.
22. Milián E, Kamen AA (2015) Current and emerging cell culture manufacturing technologies for influenza vaccines. BioMed Research International 1-11.
23. www.diets-usa.com/eggstra-special-measures-taken-protect-human-flu-vaccine-production-birdflu.
24. CNBC (2015) Is bird flu going to affect human flu vaccine production?.
25. Zhang (2015) Why the Flu Vaccine Doesn't Always Work.
26. Virology blog (2009) Influenza virus growth in eggs.
27. National Center for Immunization and Respiratory Diseases (2011) General recommendations on immunization-recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 60: 1-64.
28. EFSA (2004) The use of vaccines for the control of Salmonella in poultry. The EFSA Journal 114: 1-74.
29. De Buck J, Van Immerseel F, Haesebrouck F, Ducatelle R (2005) Protection of laying hens against *Salmonella enteritidis* by immunization with type 1 fimbriae. Vet Microbiol 105: 93-101.
30. Wigley P, Hulme SD, Powers C, Beal RK, Berchieri A Jr, et al. (2005) Infection of the reproductive tract and eggs with *Salmonella enterica* serovar pullorum in the chicken is associated with suppression of cellular immunity at sexual maturity. Infect Immun 73: 2986-2990.
31. Gantois I, Ducatelle R, Timbermont L, Boyen F, Bohez L, et al. (2006) Oral immunisation of laying hens with the live vaccine strains of TAD Salmonella vac E and TAD Salmonella vac T reduces internal egg contamination with *Salmonella enteritidis*. Vaccine 24: 6250-6255.
32. FAO (2002) Risk assessments of Salmonella in eggs and broiler chickens: Microbiological risk assessment series no. 2. Food and Agriculture Organization of the United Nations, Rome.
33. Davies R, Breslin M (2004) Observations on Salmonella contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar *enteritidis* had been used. Avian Pathol 33: 133-144.

34. Van Hoorebeke S, Van Immerseel F, De Vylder J, Ducatelle R, Haesebrouck F, et al. (2009) Faecal sampling underestimates the actual prevalence of Salmonella in laying hen flocks. Zoonoses Public Health 56: 471-476.
35. Nguyen HT, Sharma V, McIntyre RS (2009) Teratogenesis associated with antibipolar agents. Adv Ther 26: 281-294.
36. De Reu K, Grijspeerdt K, Heyndrickx M, Messens W, Uyttendaele M, et al. (2006) Influence of eggshell condensation on eggshell penetration and whole egg contamination with *Salmonella enterica* serovar *enteritidis*. J Food Prot 69: 1539-1545.
37. Hannah JF (2010) Influence of housing system of bacterial eggshell contamination and horizontal transmission of Salmonella and Campylobacter among laying hens. Ph.D. Dissertation, University of Georgia.
38. Immerseel FV, Nys Y, Maureen B (2011) Improving the safety and quality of eggs and egg products: egg safety and nutritional quality. Woodhead Publishing Limited, Philadelphia.
39. Food Standards Australia New Zealand (2009) Public health and safety of eggs and egg products in Australia.
40. Fanatico A, Conner B (2009) Small-scale egg handling. ATTRA 1-16.
41. Leleu S, Messens W, De Reu K, De Preter S, Herman L, et al. (2011) Effect of egg washing on the cuticle quality of brown and white table eggs. J Food Prot 74: 1649-1654.
42. Gole VC, Chousalkar KK, Roberts JR, Sexton M, May D, et al. (2014) Effect of egg washing and correlation between eggshell characteristics and egg penetration by various *Salmonella Typhimurium* strains. PLoS ONE 9: e90987.
43. Food Safety Authority of Ireland (2010) Egg Washing.
44. Kultima JR, Sunagawa S, Li J, Chen W, Chen H, et al. (2012) MOCAT: a metagenomics assembly and gene prediction toolkit. PLoS One 7: e47656.
45. USDA (2009) Imports from China and Food Safety Issues.
46. Patrick Boehler (2012) Bad Eggs: Another Fake-Food Scandal Rocks China, TIME.
47. Chinahush (2009) How to Identify Fake Chicken Eggs.
48. Nys Y, Maureen B, Immerseel FV, (2013) Improving the safety and quality of eggs and egg products: egg chemistry, production and consumption. Woodhead Publishing Limited, Philadelphia.
49. Braun P, Fehlhaber K (1995) Migration of *Salmonella enteritidis* from the albumen into the egg yolk. Int J Food Microbiol 25: 95-99.
50. Miyamoto T, Baba E, Tanaka T, Sasai K, Fukata T, et al. (1997) *Salmonella enteritidis* contamination of eggs from hens inoculated by vaginal, cloacal, and intravenous routes. Avian Dis 41: 296-303.
51. Grijspeerdt K, Kreft JU, Messens W (2005) Individual-based modelling of growth and migration of *Salmonella enteritidis* in hens' eggs. Int J Food Microbiol 100: 323-333.
52. Andreoletti O, Budka H, Buncic S, Colin P, Collins JD, et al. (2009) Special measures to reduce the risk for consumers through Salmonella in table eggs – e.g. cooling of table eggs. The EFSA Journal. 957: 1-29.
53. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, et al. (2009) Mechanisms of egg contamination by *Salmonella enteritidis*. FEMS Microbiol Rev 33: 718-738.
54. Humphrey TJ, Whitehead A, Gawler AH, Henley A, Rowe B (1991) Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. Epidemiol Infect 106: 489-496.
55. Keller LH, Benson CE, Krotec K, Eckroade RJ (1995) *Salmonella enteritidis* colonization of the reproductive tract and forming and freshly laid eggs of chickens. Infect Immun 63: 2443-2449.
56. Raspoet R, Gantois I, Devloo R. Pasmans F, Haesebrouck F, et al. (2011) Improving the safety and quality of eggs and egg products, Volume 2: Egg safety and nutritional quality. In: Immerseel FV, Nys Y, Bain M (Eds.). United Kingdom: Woodhead Publishing Limited. 2011: 46-61.
57. Messens W, Grijspeerdt K, Herman L (2005) Eggshell penetration by Salmonella: A review. World's Poult Sci J 61: 71-84.
58. Chousalkar KK, Flynn P, Sutherland M, Roberts JR, Cheetham BF (2010) Recovery of Salmonella and *Escherichia coli* from commercial egg shells and effect of translucency on bacterial penetration in eggs. Int J Food Microbiol 142: 207-213.
59. Martelli F, Davies RH (2012) Salmonella serovars isolated from table eggs: An overview. Food Res Int 45: 745-754.
60. Baron F, Gautier M, Brule G (1997) Factors involved in the inhibition of growth of *Salmonella enteritidis* in liquid egg white. J. Food Prot 60: 1318-1323.
61. Chart H, Rowe B (1993) Iron restriction and the growth of *Salmonella enteritidis*. Epidemiol Infect 110: 41-47.
62. Mayes FJ, Takeballi MA (1983) Microbial contamination of the hen's egg: A Review. J Food Protect 46: 1092-1098.
63. Clavijo RI, Loui C, Andersen GL, Riley LW, Lu S (2006) Identification of genes associated with survival of *Salmonella enterica* serovar *enteritidis* in chicken egg albumen. Appl Environ Microbiol 72: 1055-1064.
64. Ibrahim HR, Thomas U, Pellegrini A (2001) A helix-loop-helix peptide at the upper lip of the active site cleft of lysozyme confers potent antimicrobial activity with membrane permeabilization action. J Biol Chem 276: 43767-43774.
65. Lu S, Killoran PB, Riley LW (2003) Association of *Salmonella enterica* serovar *enteritidis* yafD with resistance to chicken egg albumen. Infect Immun 71: 6734-6741.
66. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Van Immerseel F (2008) The *Salmonella enteritidis* lipopolysaccharide biosynthesis gene rfbH is required for survival in egg albumen. Zoonoses Public Health.
67. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Van Immerseel F (2008) *Salmonella enterica* serovar *enteritidis* genes induced during oviduct colonization and egg contamination in laying hens. Appl Environ Microbiol 74: 6616-6622.
68. Neilands JB (1981) Iron absorption and transport in microorganisms. Annu Rev Nutr 1: 27-46.
69. World Health Organization (2002) Risk assessments of Salmonella in eggs and broiler chickens, Food Safety Department World Health Organization, Switzerland.
70. Giaccone V, Catellani P, Alberghini L (2012) Detection of *Salmonella* spp. presence in food. In: Mahmoud BSM (Eds), Salmonella-A dangerous foodborne pathogen. 47-72.
71. FDA (2012) Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook *Salmonella* spp.
72. International Organization for Standardization ISO-6579 (2002) (4thedn) Microbiology- general guidance on methods for the detection of Salmonella, International Organization for Standardization. Geneve.
73. Stevens KA, Jaykus LA (2004) Bacterial separation and concentration from complex sample matrices: a review. Crit Rev Microbiol 30: 7-24.
74. WHO (2010) Global Foodborne Infections Network.
75. Zadernowska A, Chajecka W (2012) Detection of Salmonella spp. presence in food. In: Mahmoud BSM (Eds.), Salmonella–a dangerous foodborne pathogen. 393-412.
76. Margot H, Stephan R, O'Mahony E, Iversen C (2013) Comparison of rapid cultural methods for the detection of Salmonella species. Int J Food Microbiol 163: 47-50.
77. Cho IH, Irudayaraj J (2013) In-situ immuno-gold nanoparticle network ELISA biosensors for pathogen detection. Int J Food Microbiol 164: 70-75.
78. Bhunia AK (2008) Biosensors and bio-based methods for the separation and detection of foodborne pathogens. Adv Food Nutr Res 54: 1-44.
79. Bhunia AK (2014) One day to one hour: how quickly can foodborne pathogens be detected? Future Microbiol 9: 935-946.
80. Li X, Ximenes E, Amalaradjou MA, Vibbert HB, Foster K, et al. (2013) Rapid sample processing for detection of food-borne pathogens via cross-flow microfiltration. Appl Environ Microbiol 79: 7048-7054.
81. Vibbert HB, Ku S, Li X (2015) Accelerating sample preparation through enzyme-assisted microfiltration of Salmonella in chicken extract. Biotechnol Prog .

82. FDA Blog. Five Questions with Michael Ladisch, Ph.D., from Purdue University.
83. Cho IH, Radadia AD, Farrokhzad K, Ximenes E, Bae E, et al. (2014) Nano/micro and spectroscopic approaches to food pathogen detection. Annu Rev Anal Chem (Palo Alto Calif) 7: 65-88.
84. Singh AK, Bettasso AM, Bae E, Rajwa B, Dundar MM, et al. (2014) Laser optical sensor, a label-free on-plate *Salmonella enterica* colony detection tool. MBio 5: e01019-01013.
85. Hahm BK, Kim H, Singh AK, Bhunia AK (2015) Pathogen enrichment device (PED) enables one-step growth, enrichment and separation of pathogen from food matrices for detection using bioanalytical platforms. J Microbiol Methods 117: 64-73.
86. Zhao X, Wang L, Chu J, Li Y, Li Y, et al. (2010) Development and application of a rapid and simple loop-mediated isothermal amplification method for food-borne Salmonella detection. Food Science and Biotechnology 19: 1655-1659.
87. Jang K, Ahn J, Kim K (2011) Rapid and simple biochemical detection for *Salmonella* spp. using modified LB broth and the MUCAP test. FSB 20: 201-207.
88. Food and Drug Administration, Public Health Service, US. Department Of Health And Human Services. (2012) Shared risk evaluation mitigation strategy for all immediate-release transmucosal fentanyl dosage forms. J Pain Palliat Care Pharmacother 26: 123-126.
89. Koyuncu S, Andersson MG, Häggblom P (2010) Accuracy and sensitivity of commercial PCR-based methods for detection of *Salmonella enterica* in feed. Appl Environ Microbiol 76: 2815-2822.
90. AFNOR. BAX® System PCR Assay *Salmonella* spp (automated) (QB0608C).
91. Frausto HSEG, Alves J, Cristina T, Oliveria RMD (2013) Evaluation of the BAX® system for the detection of *Salmonella* spp. in naturally contaminated chicken meat. Food Sci. Technol 33: 475-478.
92. Wallace FM, Andaloro B, Fallon D, Corrigan N, Varkey S, et al. (2014) Detection of Salmonella species in a variety of foods by the DuPont Bax system real-time PCR assay for Salmonella: first action 2013.02. J AOAC Int 97: 868-875.
93. Oxoid (2015) EN ISO 16140 validation study of the TaqMan® *Salmonella enteric* a method for Salmonella detection in all human food products and animal feeding stuffs.
94. Allen A, Jiang Z, Sutzko M (2015) The use of the RapidChek SELECT™ Salmonella Test system to detect Salmonella species in seafood samples. Poster.
95. Muldoon MT, Gonzalez V, Sutzko MI, Allen AC, Creamer S, et al. (2011) RapidChek SELECT *Salmonella enteritidis* test system for the detection of Salmonella enteritidis in poultry house drag swabs, shell egg pools, and chicken carcass rinsates. J AOAC Int 94: 1138-1153.
96. Muldoon MT, Onisk DV (2012) Methods and kits for detection of *Salmonella enteritidis* and related serovars U.S. Patent WO 2012016107 A1.
97. AFNOR (2015) Reveal 2.0 Salmonella Test System.
98. Zhang L, Yan Z, Ryser ET (2006) Comparison of the reveal test, the US. Food and Drug Administration culture method, and selective media for recovery of *Salmonella enteritidis* from commercial egg layer flock environments. J Food Prot 69: 2766-2769.