

ISOLATION OF *SALMONELLA* SPP. FROM FAECAL SAMPLES OF CRACKED EGG FED HENS AND POLYMERASE CHAIN REACTION (PCR) CONFIRMATION

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Summary

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At 56 weeks of age, 30 Hyline (W-377) laying pullets were randomly distributed in individual (1 pullet per cage) 45×45×35 cm cages. Cracked egg was used as protein supplement to the diet. Three groups of 10 Hyline laying pullets each were formed. Three isocaloric and isonitrogenous diets were prepared from different protein sources as followed: ration 1 (control) – soybean meal; ration 2 (experiment 1) – soybean meal + cracked egg (3.25%), ration 3 (experiment 2) – soybean meal + cracked egg (7.50%). A total of three feed and 30 faecal samples, were examined for the presence of *Salmonella* spp. The isolates were identified by both conventional methods and polymerase chain reaction (PCR). *Salmonella* spp. strains were isolated and identified in 2 out of the 3 diets (ration 3 – soybean meal + cracked egg 7.50% and ration 2 – soybean meal + cracked egg 3.25%), as well as in the faeces of 5 hens fed on ration 3 and 3 hens fed on ration 2. This study showed an increased prevalence of *Salmonella* spp. in the faeces of hens fed diets supplemented with different amounts of cracked egg.

Key words: cracked egg, culture, laying hens, *Salmonella* spp., PCR

INTRODUCTION

Feed is an important component in a pre-harvest *Salmonella* control programme. In particular, the type of feed appears to be strongly associated to the presence of *Salmonella* spp. (Farzan *et al.*, 2006). It is also known that the physical properties of feed can influence pH, microbial populations, and volatile fatty acids in the digestive tract of broilers (Engberg *et al.*, 2002).

Eggs and egg products are considered to be the major sources of confirmed salmonellosis (Lepoutre *et al.*, 1994). Infected ovaries and oviducts of the hen are

the major sources of contamination (Abdel Kareem & Matter, 2001). Eggs can become contaminated also on the surface, either from faeces or the environment. In addition to *Salmonella* spp., there are also other pathogens, as *Listeria monocytogenes*, which can occur on egg shells and survive in egg wash water and may demonstrate higher thermal resistance than *Salmonella* spp. (Bartlett *et al.*, 1993). Such surface microflora may contaminate broken-out liquid egg in both processing and commercial food preparation settings (Abdel Kareem & Matter, 2001).

Cracked eggs are those that could not be sold at the market because of being damaged by the end of laying or during transportation. Ideally, all cracked eggs should be broken and pasteurized, but this is impractical (Todd, 1996). Hen eggs have become a principal source of *Salmonella enteritidis*, since this serotype can colonize the ovarian tissue of hens and thus be present within the contents of intact shell eggs (Humphrey, 1994). In recent years, *S. enteritidis* was found inside a small number of eggs. However, cracks on egg shell surfaces could increase the chances of penetration of the organism within the egg (Edema & Atayese, 2006).

As *Salmonella* spp. may survive in feed for as long as 16 months at 25 °C (Williams & Benson, 1978), feed may serve as a reservoir that may contaminate incoming flocks. Between 1 and 5% of all animal feed produced and 31% of animal by-products may be contaminated with *Salmonella* spp. (Allred *et al.*, 1967). Therefore accurate, sensitive and rapid methods of *Salmonella* spp. detection must include a method to determine *Salmonella* spp. contamination in feed samples (Maciorowski *et al.*, 2000).

Current conventional methods require 96 h for the detection and biochemical confirmation of *Salmonella* spp. in animal feed (Andrews *et al.*, 1998). A more rapid method of detection or screening would reduce storage and treatment costs. Quality control technicians may potentially use the polymerase chain reaction (PCR) to rapidly screen feed samples (Maciorowski *et al.*, 2000).

This study was planned to detect *Salmonella* spp. in feed with and without cracked egg supplements and in faecal samples of hens fed these feed by means of culture and PCR methods.

MATERIALS AND METHODS

Experimental animals and feed samples

One control and two experimental (1 and 2) groups were formed to determine the laying performance and digestibility of 30 White Hyline hens fed with diets containing 16% crude protein (CP). The trial lasted two months. Soybean meal was given to the control group as protein source. Three isocaloric and isonitrogenous diets were prepared from soybean meal and cracked egg supplement as followed: ration 1 (control group) – soybean meal, ration 2 (experimental group 1) – soybean meal + cracked egg 3.25% and ration 3 (experimental group 2) – soybean meal + cracked egg 7.50%. Protein contents of diets were analyzed one week after their preparation. The metabolic energy, Ca and P contents of diets were calculated. Prepared diets were stored in a cool environment. Crude protein of diets was determined according to AOAC (1995).

In the present study, cracked eggs which stayed outside market and were not consumed by humans were used. Each treatment group was randomly assigned to one of the three diets with composition given in Table 1. Feed and water were provided *ad libitum* throughout the 2-month period. On day 53 of the experiment, chickens of each group (10 birds in each) were randomly placed in individual metabolic cages (20×40 cm) that enabled the excreta collection. Excreta samples of each chicken were collected for 7 days, dried immediately at 40 °C for 24 h. All 30 dried excreta samples were finally mixed and were tested for occurrence of *Salmonella* spp. Samples from the three feeds were also tested for salmonellae. Samples were parallelly examined.

Table 1. Composition (%) of experimental diets

Ingredients (%)	Ration 1 (Control)	Ration 2 (Soybean meal + 3.25 % cracked egg)	Ration 3 (Soybean meal + 7.50 % cracked egg)
Maize	60.50	62.60	62.50
Soybean meal, 44%	24.70	20.35	17.10
Vegetable oil	2.00	1.00	—
CaCO ₃	10.00	10.00	9.50
Dicalcium phosphate	2.10	2.10	2.70
Salt (NaCl)	0.20	0.20	0.20
Vitamin	0.20	0.20	0.20
Mineral	0.20	0.20	0.20
DL-methionine	0.10	0.10	0.10
Cracked egg, 45.1%	—	3.25	7.50
Metabolisable energy (kcal/kg)	2710	2740	2750
Crude protein, %	16.00	15.92	16.15
Calcium, %	3.40	3.50	3.55
Phosphorus, %	0.70	0.68	0.72

Bacteriological analysis

Salmonella organisms were isolated according to standard methods (ISO 6579, 1993). Samples of 25 g feed and 10 g faeces were placed in a stomacher bag containing 225 mL and 90 mL, respectively, of preenrichment medium-buffered peptone water (BPW, Oxoid, Basingstoke, UK), treated in a Stomacher (Interscience, 78860 St Nom, France) for 2 min and incubated for 18–20 h at 37 °C. The preenriched cultures, 0.1 mL and 1 mL, respectively, were then transferred to Rappaport-Vassiliadis broth (Oxoid) and selenite broth (Difco Laboratories, Detroit, MI) and incubated at 42 °C and 37 °C, respectively. After 24 and 48 h of incubation, one loopful from each of enriched broths was streaked onto plates of *Salmonella* Shigella (SS) agar (Difco) and xylose lysine deoxycholate (XLD) agar (Difco) and incubated at 37 °C for 24 h. The plates were examined for the presence of typical colonies of *Salmonella* spp., i.e. transparent colonies with black centres on SS agar

and red colonies with black centres on XLD agar (Antunes *et al.*, 2003). Suspected colonies were confirmed by conventional biochemical methods (Lautrop *et al.*, 1979; Nissen, 1984).

DNA extraction

A 10⁸ CFU/ml of suspicious *Salmonella* spp. culture growth on selective agar was transferred into an Eppendorf tube containing 300 µL sterile distilled water. Bacterial suspension was mixed thoroughly by vortexing and incubated at 56 °C for 30 min. Then the samples were treated with 300 µL of TNES buffer (20 mM Tris pH 8.0 + 150 mM NaCl + 10 mM EDTA + 0.2 % SDS) and 200 µg/mL proteinase K. Following 30 min of boiling, the same amount of phenol (saturated with Tris-HCl) was added to the suspension. The suspension was hand-shaken vigorously for 5 min and centrifuged at 11600×g for 10 min. The upper phase was carefully transferred into a new Eppendorf tube, 0.1 volume 3 M sodium acetate and 2.5 volumes absolute ethanol were added to the

suspension, and was left at -20°C overnight. After the precipitation stage, the suspension was centrifuged at $11600\times g$ for 10 min and the upper phase was discharged. The pellet, obtained after centrifugation was washed twice with 95% and 70% ethanol respectively, each step followed by 5 min centrifugation. Finally, the pellet was dried, resuspended in 50 μL of sterile distilled water, and stored at -20°C until further use.

A reference *S. enteritidis* strain (ATCC 4931) (kindly provided by Dr. A. A. Mohamed Hatha, Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, Fiji) was used in PCR tests as positive control and distilled water was used as the negative control.

Primers

The primers used were: 16SF1 (5'-TGTTGTGGTTAATAACCGCA-3') and 16SIII (5'-CACAAATCCATCTCTGGA-3') (Promega) derived from 16S rRNA gene (Lin & Tsen, 1996).

PCR

The reaction mixture was prepared in a total volume of 50 μL containing 5 μL of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton[®] X-100), 5 μL of 25 mM MgCl_2 , 250 μM of each deoxynucleoside triphosphate, 2 U of Taq DNA Polymerase (Fermentas, Lithuania), 10 pg of each primer and 5 μL samples of extracted bacterial DNA. PCR involved 35 cycles of denaturation (94°C , 1 min), primer annealing (58°C , 1 min) and primer extension (72°C , 1 s). The primer extension step (72°C , 10 min) followed the final amplification cycle (Fluit *et al.*, 1993). For all experiments, a Touchdown Thermocycler (Hybaid, Middlesex, England) was used. PCR reaction products (15 μL) were analysed by electrophoretic

separation on 1.5% agarose gels stained with ethidium bromide. The gel was visualized by UV illumination and photographed with Polaroid films.

Statistical analysis

Yates-corrected χ^2 tests (Yates, 1982) were used to detect differences between proportions of *Salmonella* spp. isolated from feed samples. These tests were carried out using Epi info version 6 (Dean *et al.*, 1994).

RESULTS

Culture Results

Salmonella spp. were isolated and identified in 2 out of the 3 rations: ration 3 – soybean meal + cracked egg 7.50% and ration 2 – soybean meal + cracked egg 3.25%. *Salmonella* organisms were also isolated and identified in the faeces from 5 hens fed on ration 3 and 3 hens fed on ration 2. *Salmonellae* were not isolated from feed (soybean meal) and from the faeces of control hens (Table 2).

The difference between the prevalence of *Salmonella* spp. isolated from the control group and groups given soybean meal + cracked egg 7.50% and soybean meal + cracked egg 3.25% was statistically significant ($P = 0.04$). No significant difference was detected in the prevalence of *Salmonella* spp. isolated from soybean meal + cracked egg 7.50% and soybean meal + cracked egg 3.25%.

PCR Results

In this study, PCR amplification of genomic bacterial DNA using 16SF1 and 16SIII primer pairs gave bands of 572 bp, corresponding to the expected size of amplified genomic *Salmonella* spp. DNA (Fig. 1). All *Salmonella* spp. isolates that

Table 2. Culture results of *Salmonella* spp. from feed and faeces of chickens fed on different rations

Rations	Samples	
	Feed samples	Faecal samples*
Ration 1 (control: not supplemented with cracked egg)	Negative	0/10
Ration 2 (soybean meal + 3.25 % cracked egg)	Positive	3/10
Ration 3 (soybean meal + 7.50 % cracked egg)	Positive	5/10

* Number of positive/number of tested samples.

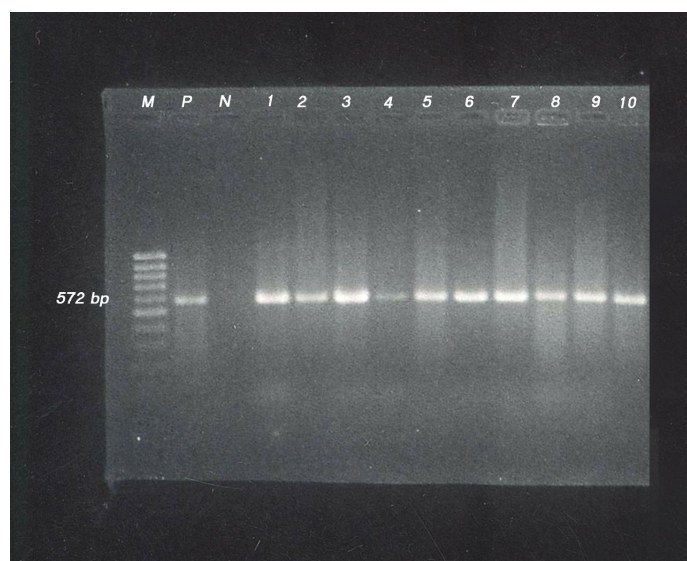


Fig. 1. Agarose gel stained with ethidium bromide, with PCR products of *Salmonella* isolates (M: 100 bp DNA ladder; P: positive control; N: negative control; 1: *Salmonella* isolates from feed samples of ration 2 (soybean meal + cracked egg 3.25%); 2: *Salmonella* isolates from feed samples of ration 3 (soybean meal + cracked egg 7.50%); 3–10: *Salmonella* isolates from faecal samples of hens.

were positive in culture were also positive by PCR. No amplified product was obtained from the negative control.

DISCUSSION

Feed is believed to be an important vector for the transmission of *Salmonella* spp. to poultry (Ha *et al.*, 1998). *Salmonella* spp. has been detected in poultry feed, animal feed or feed ingredients in a number of studies (Williams, 1981; Cox *et al.*, 1983;

Stuart, 1984; Veldman *et al.*, 1995). Of the individual feed ingredients, meat and bone meal or animal by-products are reported to have the highest incidence of *Salmonella* spp., with estimates of contamination between 31% and 86% of feeds sampled (Allred *et al.*, 1967; Williams *et al.*, 1969). However, salmonellae have also been detected with lower incidence in plant protein sources including soybean oil meal, and thus, plant protein was also shown as an important source of

Salmonella spp. transmission (Vanderwal, 1979).

In fact, 77–80% of salmonellosis outbreaks have been associated with grade A shell eggs, or egg-containing foods (Cabo *et al.*, 2004). Salmonellae may occasionally be present on eggshells even after washing, and any *Salmonella* organisms reaching the membranes can be transferred to an egg mixture through breaking, and will rapidly grow under improper storage conditions (Todd, 1996).

Todd (1996) conducted a risk analysis on cracked eggs and found that they were 3 to 93 times more likely to cause outbreaks of salmonellosis than uncracked shell eggs. Poppe *et al.* (1998) detected that pools containing eggs that were both cracked and dirty, were more frequently contaminated with *Salmonella* spp. than all other pools of eggs. It is reported that 2.99%, 1.15%, 2.17%, 1.17%, 3.87%, 1.15% from cracked, whole, dirty, clean, cracked and dirty, whole and clean eggs, respectively, were positive for *Salmonella* spp. and that the overall *Salmonella* spp. contamination rate of the table eggs was from 0.07 to 0.4% (Poppe *et al.*, 1998). Jones & Musgrove (2007) showed a low *Salmonella* spp. prevalence in eggshells (1.1%).

del Cerro *et al.* (2002) reported that faeces, caecal swabs and eggs from chickens were positive for *Salmonella* spp. by culture in 39%, 18% and 64.5%, respectively and positive in 39%, 13.6% and 60% by PCR, respectively.

Although numerous studies related to isolation of *Salmonella* spp. from egg samples in Turkey have been performed (Arda, 1968; Inal & Ozyer, 1992; Var, 1993; Erol, 1994; Arig-Kuçuker *et al.*, 1995; Altay & Yardımcı, 2001; Erdoğan *et al.*, 2002; Cakiroglu & Gümüşsoy, 2005; Ata, 2006), none of them has re-

ported the incidence of *Salmonella* spp. isolates from cracked eggs. Studies carried out in different parts of Turkey have found no *Salmonella* spp. in egg samples (Arda, 1968; Inal & Ozyer, 1992; Erol, 1994; Arig-Kuçuker, 1995; Cakiroglu & Gümüşsoy, 2005; Ata, 2006). In a study conducted by Var (1993) to determine the presence of *Salmonella* spp. in 448 egg samples from chickens, ducks and quails, 3 egg samples were positive for *Salmonella paratyphi*. Ata (2006) found out that egg samples obtained from 50 layer flocks were negative for *Salmonella* spp. whereas cloacal swabs obtained from 6 flocks were positive for *Salmonella* spp. In other countries such as Spain, Canada and the USA, the prevalence has been reported to be considerably higher (del Cerro *et al.*, 2002) or low (Poppe *et al.*, 1998; Jones & Musgrove, 2007).

When evaluating the prevalence of *Salmonella* spp. in feed samples in ration 2 (soybean meal + cracked egg 3.25%) and ration 3 (soybean meal + cracked egg 7.50%) and the faecal samples from hens given these feeds, a significant increase in the prevalence of recovered *Salmonella* isolates compared to controls was found out. This may be explained by variations in the proportions of the cracked egg supplement. The difference between the prevalence of *Salmonella* spp. isolated from the control group and groups given either soybean meal + cracked egg 7.50% or soybean meal + cracked egg 3.25% was statistically significant ($P=0.04$). No *Salmonella* spp. were detected in feed and faecal samples on any of control groups.

There were a reduction in the prevalence of *Salmonella* spp. recovered from the faeces of hens fed on a ration containing 3.25% cracked eggs compared to hens fed on 7.50% cracked eggs. No significant difference was detected in the numbers of

Salmonella spp. isolated from groups fed on soybean meal + cracked egg 7.50% or soybean meal + cracked egg 3.25%.

Traditional microbiological techniques such as the ISO 6579 for detecting this pathogen in food require up to 5 days to obtain a result, including the pre-enrichment and the selective enrichment in liquid culture and the biochemical and serological confirmation of colonies grown on agar plates (Anonymous, 2002). The PCR method is a useful tool to overcome these time-consuming procedures (Arnold *et al.*, 2004).

The primers 16SF1 and 16SIII were proved to be specific for the PCR detection of all *Salmonella* isolates with various serogroups (Lin & Tsen, 1996). For these reasons, we used the primers 16SF1 and 16SIII derived from the 16S rRNA gene and found that all *Salmonella* spp. isolates identified by conventional tests gave positive bands with PCR.

Maciorowski *et al.* (2000) found that indigenous *Salmonella* spp. were detected in five (63%) of eight samples of poultry diets by conventional methods and that with commercial PCR, *Salmonella* spp. could not be detected in any of the samples after only 7 h of enrichment but could be detected in 2 dietary samples after 13 h of enrichment and 4 dietary samples after 24 h of enrichment. Löfström *et al.* (2004) developed PCR procedure for routine analysis of viable *Salmonella* spp. in 14 different feed samples and 8% of the samples were positive by PCR, compared with 3% with the conventional method.

In a study carried out in Turkey, Altay *et al.* (2002) investigated *Salmonella* spp. presence in 75 feed samples collected from different poultry farms and feed plant in Bolu, Ankara, Izmit and Afyon region and found 2 (2.67%) of them positive for salmonellae.

Our results are in contradiction with those of Altay *et al.* (2002) and Löfström *et al.* (2004) who found low prevalence of *Salmonella* spp. from feed samples but in agreement with Maciorowski *et al.* (2000) who found high prevalence of *Salmonella* spp. from feed samples.

This study reported the isolation of *Salmonella* spp. from faeces of hens fed diets with added cracked egg for the first time in Turkey and emphasized that cracked egg containing feeds were the most important vehicles for chicken salmonellosis. The occurrence of *Salmonella* spp. in both feed and faecal samples suggested that feed could be a possible source of salmonellosis in hens. However, further studies are needed to clarify the potential pathogenic role of *Salmonella* spp. in feeds.

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REFERENCES

- Abdel Kareem, H. & Z. Mattar, 2001. Heat resistance and growth of *Salmonella enteritidis*, *Listeria monocytogenes* and *Aeromonas hydrophila* in whole liquid egg. *Acta Microbiologica Polonica*, **50**, 27–35.
- Allred, J. N., J. W. Walker, V. C. Jr Beal & F. W. Germaine, 1967. A survey to determine the *Salmonella* contamination rate in livestock and poultry feeds. *Journal of the American Veterinary Medical Association*, **151**, 1857–1860.
- Altay, G. & H. Yardımcı, 2001. Detection of

- antibodies against *Salmonella* Enteritidis in chicken serum and egg yolk with ELISA. *Turkish Journal of Veterinary and Animal Sciences*, **25**, 983–988.
- Altay, G., N. Yazıcıoğlu, S. Sen, S. Özkök & O. H. Muğlalı, 2002. Comparison of conventional (preenrichment and without preenrichment) and delayed secondary enrichment methods of determination of salmonellae in feeds. In: *Proceedings of the 5th National Congress of Veterinary Microbiology (with international guest speakers)*. September 24–26, Konya, Turkey.
- Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack & R. M. Amaguana, 1998. *Salmonella*. In: *Bacteriological Analytical Manual*, 8th edn, Revision A, Gaithersburg, MD, AOAC International, pp. 501–520.
- Anonymous, 2002. Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* spp., *International Standardization Organisation document 6579*, International Standardization Organisation, Geneva, Switzerland.
- Antunes, P., C. Reu, J. C. Sousa, L. Peixe & N. Pestana, 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology*, **82**, 97–103.
- Arda, M., 1968. Bacteriological and virological studies on dead embryonated and without embryonated eggs in incubators. *Ankara University, Veteriner Fakültesi Yayınları*, **227**, 28–31.
- Arıç-Kuçuker, M., A. Kimiran & C. Bal, 1995. Isolation of *S. enteritidis* from meat and eggs of flocks. *Türk Mikrobiyoloji Cemiyeti Dergisi*, **23**, 138–141.
- Arnold, T., H. C. Scholz, H. Marg, U. Rösler & A. Hensel, 2004. Impact of *invA*-PCR and culture detection methods on occurrence and survival of *Salmonella* in the flesh, internal organs and lymphoid tissues of experimentally infected pigs. *Journal of Veterinary Medicine Series B*, **51**, 459–463.
- Association of Official Analytical Chemists (AOAC), 1995. Official Methods of Analysis, 16th edn, Association of Official Analytical Chemists, Arlington, VA, 4.1–4.17.
- Ata, Z., 2006. Isolation of *Salmonella* spp. from layers flocks in Ankara region. In: *Proceedings of the 7th National Congress of Veterinary Microbiology (with international guest speakers)*, September 26–28, Side-Antalya, Turkey.
- Bartlett, F., 1993. *Listeria monocytogenes* survival on shell eggs and resistance to sodium hypochlorite. *Journal of Food Safety*, **13**, 253–261.
- Cabo, V. S., R. Tenreiro & M. L. Botelho, 2004. Sanitation of chicken eggs by ionizing radiation: HACCP and inactivation studies. *Radiation Physics and Chemistry*, **71**, 29–33.
- Cakiroğlu, H. S. & K. S. Gümüşsoy, 2005. Analysis of *Salmonella* spp. contamination in chicken eggs served for consumption in Ankara Garrison. *Ege University, Sağlık Bilimleri Dergisi*, **14**, 158–162.
- Cox, N. A., J. S. Bailey, J. E. Thomson & B. J. Juven, 1983. *Salmonella* and other Enterobacteriaceae found in commercial poultry feed. *Poultry Science*, **62**, 2169–2175.
- Dean, A. G., J. A. Dean, D. Coulombier, K. A. Brendel, D. C. Smith, A. H. Burton, R. C. Dicker, K. M. Sullivan, R. F. Fagan & T. G. Arner, 1994. Epi-Info, Version 6: A Word Processing, Database, and Statistics Program for Epidemiology on Microcomputers. Center for Disease Control and Prevention, Atlanta, GA, USA.
- del Cerro, A., S. M. Soto, E. Landeras, M. A. González-Hevia, J. A. Guijarro & M. C. Mendoza, 2002. PCR-based procedures in detection and DNA-fingerprinting of *Salmonella* from samples of animal origin. *Food Microbiology*, **19**, 567–575.
- Edema, M. O. & A. O. Atayese, 2006. Bacteriological quality of cracked eggs sold for consumption in Abeokuta, Nigeria. *International Journal of Poultry Science*, **5**, 772–775.
- Engberg, R. M., M. S. Hedemann & B. B. Jensen, 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive

- tract of broiler chickens. *British Poultry Science*, **43**, 569–579.
- Erdogrul, O., N. Ozkan & E. Cakiroglu, 2002. *Salmonella enteritidis* in quail eggs. *Turkish Journal of Veterinary and Animal Sciences*, **26**, 321–323.
- Erol, M., 1994. Investigation for *Salmonella* of eggs purchased in Ankara. Postgraduate Thesis, University of Ankara, Turkey.
- Farzan, A., R. M. Friendship, C. E. Dewey, K. Warriner, C. Poppe & K. Klotins, 2006. Prevalence of *Salmonella* spp. on Canadian pig farms using liquid or dry-feeding. *Preventive Veterinary Medicine*, **73**, 241–254.
- Fluit, A. C., M. N. Widjoatmodjo, A. T. Box, R. Torensma & J. Verhoef, 1993. Rapid detection of salmonellae in poultry with the magnetic immuno-polymerase chain reaction assay. *Applied and Environmental Microbiology*, **59**, 1342–1346.
- Ha, S. D., K. G. Maciorowski, Y. M. Kwon, F. T. Jones & S. C. Ricke, 1998. Indigenous feed microflora and *Salmonella typhimurium* marker strain survival in poultry mash diets containing varying levels of protein. *Animal Feed Science and Technology*, **76**, 23–33.
- Humphrey, T. J., 1994. Contamination of egg shell and contents with *Salmonella enteritidis*: A review. *International Journal of Food Microbiology*, **21**, 31–40.
- Inal, U. & M. Ozyer, 1992. Isolation studies of *Salmonella* from chicken eggs. *Etlik Veteriner Mikrobiyoloji Dergisi*, **7**, 109–119.
- ISO 6579, 1993. Microbiology General Guidance on Methods for the Detection of *Salmonella*. International Organization of Standardization, Geneva, Switzerland.
- Jones, D. R. & M. T. Musgrove, 2007. Pathogen prevalence and microbial levels associated with restricted shell eggs. *Journal of Food Protection*, **70**, 2004–2007.
- Lautrop, H., N. Høiby, A. Bremmelgaard & B. Korsager, 1979. Bakteriologiske undersøgelsermetoder, FADLs Forlag, Copenhagen, Denmark.
- Lepoutre, A., J. Salomon, C. Charley & L. Querre, 1994. Les toxi-infections alimentaires collectives en 1993. *Bulletin Epidémiologique Hebdomadaire*, **52**, 245–248.
- Lin, C. K. & H. Y. Tsen, 1996. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. *Journal of Applied Bacteriology*, **80**, 659–666.
- Löfström, C., R. Knutsson, C. E. Axelsson & P. Rådström, 2004. Rapid and specific detection of *Salmonella* spp. in animal feed samples by PCR after culture enrichment. *Applied and Environmental Microbiology*, **70**, 69–75.
- Maciorowski, K. G., S. D. Pillai & S. C. Ricke, 2000. Efficacy of a commercial polymerase chain reaction-based assay for detection of *Salmonella* spp. in animal feeds. *Journal of Applied Microbiology*, **89**, 710–718.
- Nissen, B., 1984. Microtest for rapid identification of *Enterobacteriaceae*. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, **92**, 239–245.
- Poppe, C., C. L. Duncan & A. Mazzocco, 1998. *Salmonella* contamination of hatching and table eggs: A comparison. *Canadian Journal of Veterinary Research*, **62**, 191–198.
- Stuart, J. C., 1984. The introduction of *Salmonella* into poultry flocks. *Journal of the Science of Food and Agriculture*, **35**, 632–633.
- Todd, E. C., 1996. Risk assessment of use of cracked eggs in Canada. *Journal of Food Microbiology*, **30**, 125–143.
- Vanderwal, P. 1979. *Salmonella* control of feedstuffs by pelleting or acid treatment. *World's Poultry Science Journal*, **35**, 70–78.
- Var, I., 1993. *Salmonella* infection on eggs and effect on *Salmonella* of heat process. Ph.D. Thesis. Cukurova University, Fen Bilimleri Enstitüsü, Adana, Turkey.
- Veldman, A., H. A. Vahl, G. J. Borggreve & D. C. Fuller, 1995. A survey of the inci-

- dence of *Salmonella* species and Enterobacteriaceae in poultry feeds and feed components. *The Veterinary Record*, **136**, 169–172.
- Williams, L. P. Jr., J. B. Vaughn, A. Scott & V. Blanton, 1969. A ten-month study of *Salmonella* contamination in animal protein meals. *Journal of the American Veterinary Medical Association*, **155**, 167–174.
- Williams, J. E. & S. T. Benson, 1978. Survival of *Salmonella typhimurium* in poultry feed and litter at three temperatures. *Avian Diseases*, **22**, 742–747.
- Williams, J. E., 1981. Salmonellas in poultry feeds – a worldwide review. Part I. Introduction and Part II. Methods in isolation and identification. *World's Poultry Science Journal*, **37**, 97–105.
- Yates, W. D. G., 1982. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. *Canadian Journal of Comparative Medicine*, **46**, 225–263.
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