FULL PAPER *Public Health*

Characterization of *Salmonella* spp. Isolated from an Integrated Broiler Chicken Operation in Korea

Young Ju LEE[1)], Hyun Jung KIM[1)], Cheong Kyu PARK[1)]*, Ki Seuk KIM[1)], Dong Hwa BAE[1)], Min Su KANG[2)], Jae Keun CHO[3)], Ae Ran KIM[2)], Jong Wan KIM[2)] and Byoung Han KIM[2)]

[1)]College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, [2)]National Veterinary Research and Quarantine Service, Ministry of Agriculture & Forestry, Anyang, 430–824 and [3)]Daegu Metropolitan City Research Institute of Health & Environment, Daegu, 706–732, Republic of Korea

(Received 7 August 2006/Accepted 26 December 2006)

ABSTRACT. The purpose of this study was to investigate the biological and genetic characterization of persistent *Salmonella* isolates in an integrated broiler chicken operation, in an attempt to elucidate the source of contamination. From the breeder farm, the hatchery, the broiler farm and the chicken slaughter house of an integrated broiler chicken operation, a total of 6 serotypes were observed. Although *S.* Heidelberg was not detected in the broiler farm, it was consistently found in the breeder farm, the hatchery and the chicken slaughter house. Also, *S.* Enteritidis and *S.* Senftenberg were found in the hatchery and the chicken slaughter house, and the hatchery and the broiler farm, respectively. *S.* Gallinarum and *S.* Blockley were found only in the broiler farm, and *S.* Virchow was only recovered in the chicken slaughter house. Isolated *S.* Heidelberg, *S.* Enteritidis and *S.* Senftenberg strains were divided into 3, 5 and 7 types, respectively, on the basis of all properties. Especially, *S.* Senftenberg isolates, divided into four types by their antimicrobial resistance patterns, were all obviously the XbaI PFGE pattern. Also, four *S.* Enteritidis isolates resistant to nalidixic acid showed a difference in phage type and PFGE pattern. Such a different pattern was shown despite *Salmonella* isolates originating from an integrated broiler operation, suggesting that further epidemiological studies on many integrated chicken companies in Korea are needed.
KEY WORDS: integrated broiler chicken operation, *Salmonella*.

J. Vet. Med. Sci. 69(4): 399–404, 2007

Most serovars of *Salmonella* are widely distributed in nature, and are found in the intestines of domestic and wild mammals, reptiles, birds and insects [38]. Foods containing products from farm animals are considered to be an important source of human *Salmonella* infection. The surveillance of *Salmonella* serovars from human and animal sources thus provides important information for public health.

Ovarian or vertical transfer of pathogens from breeding hens to progeny has been an important aspect of the epidemiology of *Salmonella* species infection within the poultry industry [5, 18, 19, 24, 39]. *Salmonella* control in an integrated broiler operation is complicated because there are many opportunities for *Salmonella* to enter these extensive integrated operations and be amplified by the mass production of feed, the hatching, and the handling and processing facilities [23, 32, 37].

Identification and genotypic characterization of the bacterial isolates are essential for epidemiological surveillance and outbreak investigations. The primary method used for characterizing members of the genus *Salmonella* is serotyping that is defined by the combination of surface antigens O, H and Vi according to the Kaufmann-White scheme [35]. Over 2,500 different serotypes have been identified, with the majority of these belonging to *Salmonella* enterica subspecies [34]. To further discriminate within *S.* Typhimurium and *S.* Enteritidis, phage typing is the primary subtyping technique. *S.* Enteritidis can be divided into at least 27 subtypes by the phage-typing method described by Ward *et al.* [40]. Unfortunately, phage typing frequently fails to discriminate between outbreak-related and unrelated isolates [28]. Several DNA-based typing methods have been developed in an attempt to improve the reproducibility and discriminatory ability in *Salmonella* typing.

The purpose of this study was to investigate the biological and genetic characterization of persistent *Salmonella* isolates in an integrated broiler chicken operation, in an attempt to elucidate the source of contamination.

MATERIALS AND METHODS

Sample collection from the breeder farm: Cloacal swabs, cecal droppings, nest box swabs, egg sorting area swabs and dust on the wall were taken for investigation. The nest box and egg sorting area swabs were collected by premoistening four 10 by 10 cm gauze pads with sterile buffered pepton water (BPW; Difco, MD, U.S.A.) and then swabbing approximately 10 to 20 nest boxes and 25 m^2 egg of the sorting area, respectively. Cloacal swabs and cecal droppings were collected by swabbing or dipping 50 sterile cotton-tipped sticks into the cloaca or cecal dropping. Dust on the wall was collected by placing approximately 50 g into sterile whirl-pac bags. The samples were taken directly into two 225 m*l* BPW.

Sample collection from the hatchery: Hatchery samples were collected on the day of hatching and obtained from the hatcher interior, chick sorting area, chick box, ventilation

* CORRESPONDENCE TO: PARK, C. K., College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Republic of Korea.

outlets and waste area. Fifty grams of eggshell fragment and fluff samples were collected from the hatching trays (from the top, middle, and bottom of the stack) of the hatchery interior and unhatched embryo macerator of the waste area and placed in sterile wire-pac bags. The samples from the chick sorting area, chick box and ventilation outlets were collected by swabbing using four premoistened gauze pads with sterile BPW as described above. All samples were placed directly into two 225 m*l* BPW.

Sample collection from the broiler farm: Cloacal swabs, cecal droppings and dust on the wall were taken for investigation. Samples were collected by the method as described at breeder farms.

Sample collection from the chicken slaughter house: Five carcasses were taken for investigation. Each carcass was aseptically placed into a Cryovac bag (Nasco BO139wA), and 400 m*l* of sterile BPW was added to the bag. The bag was shaken 50 times, and approximately 50 m*l* of rinsing water was poured into a sterile specimen cup. Rinsed samples were poured into two 225 m*l* BPW, as described above.

Salmonella culture method: Samples in 225 m*l* BPW were returned to the laboratory under ambient conditions on the day of collection and incubated at 37°C for 18 hr. After preenrichement, 0.1 m*l* of the broth was transferred into 10 m*l* Rapparport-Vassiliadis broth (RV broth; Difco) prepared according to the instructions on the package. The RV broth was incubated overnight at 41.5°C. The RV broth samples were streaked on Ramback agar (Difco) and incubated overnight at 37°C. Two suspicious colonies per plate were picked and used with MacConkey agar (Difco) for pure culture and they were incubated overnight at 37°C. Samples on MacConkey agar were reacted with Salmonella O antiserum (Difco). Colonies showing typical agglutination by O antiserum were serotyped with Salmonella H antiserum (Difco) according to the latest version of the Kauffmann and White scheme [34]. If several colonies originating from the same source showed the same serotypes and antimicrobial susceptible pattern, only one isolate was randomly chosen and included in this study.

Antimicrobial susceptibility testing: All *Salmonella* isolates were investigated for their antimicrobial resistance by the agar disk diffusion test using the following disks (Difco): amikancin (An, 30 μg), ampicillin (Am, 10 μg), amoxicillin/clavulanic acid (Amc, 20/10 μg), cephalothin (Cf, 30 μg), cefoxitin (Fox, 30 μg), cefotaxime (Ctx, 30 μg), chloramphenicol (C, 30 μg), kanamycin (K, 30 μg), streptomycin (S, 10 μg), gentamicin (Gm, 10 μg), nalidixic acid (Na, 30 μg), ciprofloxacin (Cip, 5 μg), sulfamethoxazole/trimethoprim (Sxt, 1.25/23.75 μg), trimethoprim (Tmp, 5 μg) and tetracycline (Te, 30 μg). The results were evaluated according to the document M31-A2 of the Clinical and Laboratory Standards Institute [31].

Phage typing: A total of seven *S.* Enteritidis specimens isolated from the integrated broiler chicken operation were phage-typed with 16 bacteriophages at the National Veterinary Research and Quarantine Service (NVRQS, Anyang, Korea). A standard phage was obtained from the Laboratory of Enteric Pathogens, Public Health Laboratory Service (PHLS), in England. Briefly, cultures incubated for 24 hr on agar plates were inoculated into 3 m*l* of a phage broth. After incubation for 2 hr with vigorous shaking, the broth was poured onto a phage agar plate. After the excess broth was removed from the plate, 16 typing phages were spotted onto on an agar plate with micropipette. The dried plate was incubated overnight, and the phage lysis pattern of each culture was compared with published patterns. Strains showing a pattern that did not conform to any recognized phage type were designated as "reacted but did not conform" (RDNC).

Pulsed-field gel electrophoresis: PFGE of a total of 22 *Salmonella* isolates was performed according to the "One-Day (24–28 hr) Standardized Laboratory Protocol for Molecular Subtyping of Non-typhoidal Salmonella by PFGE" (Pulse-Net, CDC, Atlanta, U.S.A.) [9]. A single colony of each isolate was streaked on tryptic soy agar (TSA) and incubated overnight at 37°C. Using a cotton swab, a portion of the growth on agar plate was transferred to 2 m*l* of Cell Suspension Buffer (100 mM Tris:100 mM EDTA, pH 8.0) and the concentration of cell suspensions adjusted to 14–15% in a bioMerieux Vitek colorimeter. Immediately, 400 μ*l* of adjusted cell suspension was transferred to 1.5 m*l* micro-centrifuge tubes with 20 μ*l* of proteinase K (20 mg/m*l* stock), subsequently mixed with 400 μ*l* of melted 1% SeaKem Gold (Cambrex, East Rutherford, NJ):1% SDS agarose was prepared with TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0), and pipetted into disposable plug moulds. Three plugs were transferred to 50 m*l* polypropylene screw-tubes with 5 m*l* of Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 with 1% sarcosyl) and 25 μ*l* of proteinase K (20 mg/m*l* stock) and incubated at 54°C in a shaker water bath for 2 hr with agitation. Thereafter, the plugs were washed twice with 15 m*l* of sterile water and three more times with TE Buffer at 50°C for 15 min. Chromosomal DNA was digested with 50 U of XbaI (Promega, Southampton, United Kingdom). PFGE was performed on a CHEF Mapper XA system (Bio-Rad, Hercules, CA) in 0.5X Tris-Borate-EDTA buffer (Bio-Rad) with recirculation at 14°C. Pulse times were ramped from 2.2 to 63.8 s during an 18 hr run at 6.0 V/cm. After electrophoresis, the gels were stained with 2 μg of aqueous ethidium bromide (Sigma-Aldrich, St. Louis, MO, U.S.A.) per milliliter for 15 min and photographed by using 300 nm UV light.

RESULTS

Table 1 shows the results of *Salmonella* isolates from the breeder farm, the hatchery, the broiler farm and the chicken slaughter house of an integrated broiler chicken operation. A total of six serotypes were observed. Although *S.* Heidelberg was not detected in the broiler farm, it was consistently found in the breeder farm, the hatchery and the chicken slaughter house. Also, *S.* Enteritidis and *S.* Senftenberg were found in the hatchery and the chicken slaughter house, and the hatchery and the broiler farm, respectively. *S.* Gall-

Table 1. Distribution and serotypes of *Salmonella* spp. in an integrated broiler chicken operation

Source	Sample	Serotype
Breeder farm, wk 24	Cloacal swabs	NI[a)]
	Cecal dropping	NI
	Nest boxes	*S.* Heidelberg
	Wall dust	NI
	Egg sorting area	*S.* Heidelberg
Hatchery	Hatcher interiors	*S.* Senftenberg
	Chick sorting area	*S.* Heidelberg & *S.* Enteritidis
	Chick boxes	*S.* Senftenberg
	Ventilation outlets	*S.* Heidelberg, *S.* Enteritidis & *S.* Senftenberg
	Waste area	*S.* Heidelberg & *S.* Enteritidis
Broiler farm, wk 4	Cloacal swabs	*S.* Gallinarum & *S.* Senftenberg
	Cecal dropping	*S.* Senftenberg & *S.* Blockley
	Wall dust	*S.* Blockley
Chicken slaughter house	Carcass-1	*S.* Virchow & *S.* Enteritidis
	Carcass-2	*S.* Heidelberg & *S.* Virchow
	Carcass-3	*S.* Enteritidis
	Carcass-4	*S.* Enteritidis
	Carcass-5	*S.* Enteritidis

a) NI, Not isolated.

inarum and *S.* Blockley were only found in the broiler farm, and *S.* Virchow was only recovered in the chicken slaughter house.

In order to assess the genetic clonality, chromosomal DNAs of 3 *Salmonella* serovars isolated from an integrated broiler chicken operation were digested with *Xba*I and analyzed by PFGE (Fig 1). All eight *S.* Heidelberg isolates analyzed belong to a pattern termed H-X1. The predominant patterns of *S.* Enteritidis originating in the hatchery were E-X1 (2 isolates) and E-X2 (one isolate), whereas isolates from the chicken slaughter house were E-X2 (one isolate) and E-X3 (three isolates). Also, *S.* Senftenberg isolated from the hatchery belonged to pattern types S-X1 (2 isolates), S-X2 (two isolates) and S-X3 (one isolate), but isolates from the broiler farm were S-X2 (one isolate) and S-X4 (one isolate).

All phenotypic and genotypic properties of the eight *S.* Heidelberg, seven *S.* Enteritidis and seven *S.* Senftenberg isolates are summarized in Table 2. Three, 5 and 7 types were identified on the basis of all properties for *S.* Heidelberg, *S.* Enteritidis and *S.* Senftenberg, respectively. *S.* Senftenberg isolates were divided into four types by antimicrobial resistance pattern and all were obviously the XbaI PFGE pattern.

DISCUSSION

Recently, a small number of cases of *Salmonella* infection have occurred in parent stocks [3] and previous studies have demonstrated the potential for spread of infection both nationally and internationally [2, 6, 25, 27, 30, 32]. The structure of the chick supply and distribution chain is such that a single infected breeding flock may have a significant effect on the level of infection in commercial flocks [20].

In this study, *Salmonella* were recovered from a breeder farm, from a hatchery, from a commercial broiler farm and from the chicken slaughter house of an integrated broiler chicken operation. A total of 6 serotypes were identified. Although *S.* Heidelberg was not detected in the broiler farm, it was consistently found in the breeder farm, the hatchery and the chicken slaughter house. Also, *S.* Enteritidis and *S.* Senftenberg were found in the hatchery and the chicken slaughter house, and the hatchery and the broiler farm, respectively.

Davies *et al.* [16] investigated a company that suffered repeated *S.* Enteritidis infection at broiler breeder sites and found a variety of routes by which infection may have been recirculating within the company. Even one infected breeding flock is capable of causing widespread distribution of contamination before it is detected [20], so the presence of several infected flocks may increase this risk.

The critical role of the hatchery in disseminating *Salmonella* to commercial birds and possibly exposing parent stocks to contamination on egg trays, trolleys and vehicles has been also described previously [7, 8, 14, 16, 17]. These studies have focused on the potential for cross-contamination and infection by a low number of organisms and chicks during incubation [10, 14, 21]. Problems with washing and disinfecting crates in hatcheries, although not such a severe problem as in poultry abattoirs [13], have also been noted previously as has long-term persistance of *Salmonella* in hatcher incubator ventilation ducting [12]. In the current study, the hatchery tested was contaminated with *Salmonella*, although formaldehyde evaporation is normally used during hatching.

The persistence of a low level of *Salmonella* in commercial broiler flocks, despite antibiotic and competitive exclusion treatments, demonstrates the importance of preventing

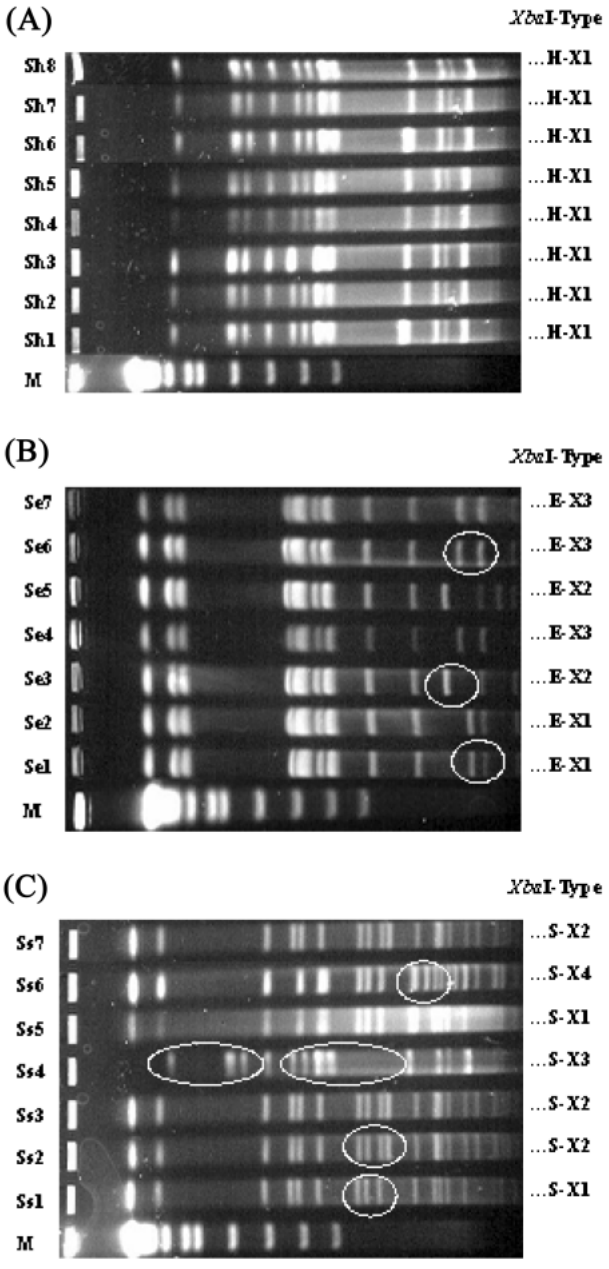

Fig. 1. Pulsed field gel electrophoresis (PFGE) patterns of *Salmonella* spp. digested with the restriction enzyme *Xba*I. M is a lambda ladder used as a molecular size marker. The circle indicates DNA of different size. (A) *S.* Heidelberg isolates from a breeder farm, hatchery and chicken slaugher house. (B) *S.* Enteritidis isolates from the hatchery and chicken slaughter house. (C) *S.* Senftenberg isolates from the hatchery, broiler farm and chicken slaughter house.

infection rather than treatment of infected birds, and affects to chicken slaughter house. This involves developing a rational risk-based approach to monitoring and preventing infection through the whole breeding and production chain [1, 11, 23, 29, 32, 41].

In a limited number of isolates, a total of seven *S.* Enteritidis strains isolated from a hatchery and a chicken slaughter house showed three phage types and one RDNC (reacts but does not conform) type. Two and one strain isolated form the hatchery belonged to phage type PT1 and PT 17, respectively. However, three and one isolates from the chicken slaughter house were PT21c and RDNC, respectively. There was a difference between phage types by source of *S.* Enteritidis isolates. Since such a different pattern was shown despite *Salmonella* isolates originating from an integrated broiler operation, further epidemiological studies on a lot more integrated chicken companies in Korea are needed to identify the sources of infection and implement prevention and control measures.

PFGE has been used as a standard technique for subtyping foodborne pathogens in the United States [9]. PFGE has been shown to have limited ability to distinguish some *Salmonella* serovars such as Enteritidis [26]. PFGE is an approach that measures the genetic diversity of the entire genome, which may have arisen as a result of mutation. Such mutation may remove or create recognition sites through insertion, deletion, translocation and inversion, or by mobile genetic elements. The assumption is that as time passes and organisms spread, divergence may occur [4]. This study also showed that both PFGE and phage typing were capable of differentiating *S.* Enteritidis strains. Several studies indicate that PFGE may offer an improved level of discrimination over other genotypic typing methods such as plasmid analysis and ribotyping for the epidemiological typing of *S.* Enteritidis [22, 33, 36]. In this study, four *S.*Enteritidis isolates resistant to Na only were different by phage type and PFGE pattern. Also, *S.* Senftenberg isolates divided into four types by antimicrobial resistance pattern were all clearly the *Xba*I PFGE pattern. In conclusion, it was suggested that molecular typing methods such as XbaI PFGE analysis, in addition to phage typing and antibiograms, may be useful for the epidemiological study of *Salmonella* serovars.

Salmonella control in integrated broiler operations is complicated because there are numerous potential sources of *Salmonella* contamination including chicks, feed, rodents, wild poultry, and the processing plant environment. However, Davies *et al.* [15] demonstrated that infection within a breeding company can be effectively controlled by application of good hygienic procedures following culling of infected flocks. Clearly, the data in our study support the critical need to control *Salmonella* in breeder farms and hatcheries and demonstrate important points about the control of infection in a large-scale poultry operation in Korea.

REFERENCES

1. Angen, O., Skov, M.N., Chriel, M., Agger, J.F. and Bisgaard, M. 1996. A retrospective study on *Salmonella* infection in Danish broiler flocks. *Prev. Vet. Med.* **26**: 223–237.
2. Ashton, W.G.L. 1984. The risks and problems associated with the import and export of domestic poultry. *Br. Vet. J.* **140**: 221–228.

Table 2. Comparison of phenotypic and genotypic characterization of *Salmonella* isolates

Organism	Strain designation	Source	Area sampled	Antimicrobial resistance pattern[a)]	Phage type	PFGE pattern
S. Heidelberg	Sh1	Breeder farm	Nest boxes	–	NT[b)]	H-X1
	Sh2	Breeder farm	Egg sorting area	–	NT	H-X1
	Sh3	Hatchery	Chick sorting area	Na	NT	H-X1
	Sh4	Hatchery	Ventilation outlets	S	NT	H-X1
	Sh5	Hatchery	Waste area	Na	NT	H-X1
	Sh6	Hatchery	Waste area	–	NT	H-X1
	Sh7	Chicken slaughter house	Carcass-2	S	NT	H-X1
	Sh8	Chicken slaughter house	Carcass-2	S	NT	H-X1
S. Enteritidis	Se1	Hatchery	Chick sorting area	Na	1	E-X1
	Se2	Hatchery	Ventilation outlets	Na	1	E-X1
	Se3	Hatchery	Waste area	Na	17	E-X2
	Se4	Chicken slaughter house	Carcass-1	NaTmp	21c	E-X3
	Se5	Chicken slaughter house	Carcass-3	Na	RDNC[c)]	E-X2
	Se6	Chicken slaughter house	Carcass-4	Na	21c	E-X3
	Se7	Chicken slaughter house	Carcass-5	Na	21c	E-X3

a) Am, ampicillin; Amc, amoxicillin/clavulanic acid; Cf, cephalothin; Fox, cefoxitin; K, kanamycin; S, streptomycin; Gm, gentamicin; Na, nalidixic acid; Sxt, sulfamethoxazole/trimethoprim; Tmp, trimethoprim; Te, tetracycline.
b) NT, Not tested.
c) RDNC reaction does not conform to any recognized phage type.

Table 2. Continued

Organism	Strain designation	Source	Area sampled	Antimicrobial resistance pattern[a)]	Phage type	PFGE pattern
S. Senftenberg	Ss1	Hatchery	Hatcher interior	AmAmcCfFoxKSSxtTmpTe	NT[b)]	S-X1
	Ss2	Hatchery	Chick boxes	AmAmcCfFoxKSSxtTmpTe	NT	S-X2
	Ss3	Hatchery	Ventilation outlets	AmAmcCfFoxKSGmSxtTmpTe	NT	S-X2
	Ss4	Hatchery	Ventilation outlest	S	NT	S-X3
	Ss5	Hatchery	Waste area	AmAmcCfFoxKSGmSxtTmpTe	NT	S-X1
	Ss6	Broiler farm	Cloacal swab	Na	NT	S-X4
	Ss7	Broiler farm	Cecal feces	Na	NT	S-X2

a) Am, ampicillin; Amc, amoxicillin/clavulanic acid; Cf, cephalothin; Fox, cefoxitin; K, kanamycin; S, streptomycin; Gm, gentamicin; Na, nalidixic acid; Sxt,; sulfamethoxazole/trimethoprim; Tmp, trimethoprim; Te, tetracycline.
b) NT, Not tested.

3. Bailey, J.S., Cox, N.A., Craven, S.E. and Cosby, D.E. 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. *J. Food Protect.* **65**: 742–745.
4. Bakeri, S.A., Yasin, R.M., Koh, Y.T., Puthucheary, S.D. and Thong, K.L. 2003. Genetic diversity of human isolates of *Salmonella* enterica serovar Enteritidis in Malaysia. *J. Appl. Microbiol.* **95**: 773–780.
5. Barrow, P.A. 1991. Experimental infection of chickens with *Salmonella enteritidis*. *Avian Pathol.* **20**: 145–153.
6. Baumler, A.J., Hargis, B.M. and Tsolis, R.M. 2000. Tracing the origins of *Salmonella* outbreaks. *Science* **287**: 50–52.
7. Bhatia, T.R.S. and McNabb, G.D. 1980. Dissemination of *Salmonella* in broiler chicken operation. *Avian Dis.* **24**: 616–624.
8. Byrd, J.A., DeLoach, J.R., Corrier, D.E., Nisbet, D.J. and Stanker, L.H. 1999. Evaluation of *Salmonella* serotype distribution from commercial broiler hatcheries and grower houses. *Avian Dis.* **43**: 39–47.
9. Centers for Disease Control and Prevention. Standardized molecular subtyping of foodborne bacterial pathogens by pulsed-field gel electrophoresis: a manual. Atlanta: National center for Infectious Disease; 1996 (updated 2000).
10. Cason, J.A., Cox, N.A. and Bailey, J.S. 1994. Transmission of *Salmonella typhimurium* during the hatching of broiler chicks. *Avian Dis.* **38**: 583–588.
11. Chadfield, M., Skow, M., Christensen, J., Madsen, M. and Bisgaard, M. 2001. An epidemiological study of *Salmonella* enterica 4, 12: b- in broiler chickens in Denmark. *Vet. Microb.* **82**: 233–247.
12. Christensen, J.P., Brown, D.J., Madsen, M., Olsen, J.E. and Bisgaard, M. 1997. Hatchery-borne *Salmonella* enterica serovar Tennessee infection in broilers. *Avian Pathol.* **26**: 155–168.
13. Corry, J.E.L., Allen, V.M., Hudson, W.R., Breslin, M.F. and Davies, R.H. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: modes of contamination and methods of control. *J. Appl. Microbiol.* **92**: 424–432.
14. Cox, N.A., Bailey, J.S. and Berrang, M.E. 1998. Bactericidal treatment of hatching eggs, chemical immersion treatments and Salmonella. *J. Appl. Pou Res.* **70**: 416–418.
15. Davies, R., Liebana, E. and Breslin, M. 2003. Investigation of the distribution and control of *Salmonella* enterica serovar Enteritidis PT6 in layer breeding and egg production. *Avian Pathol.* **32**: 227–237.
16. Davies, R.H., Nicholas, R.A.J., McLaren, I.M., Corkish, J.D., Lanning, D.G. and Wray, C. 1997. Bacteriological and serological investigation of persistant *Salmonella* enteritidis infection in an integrated poultry organization. *Vet. Microbiol.* **58**: 277–

293.
17. Davies, R.H. and Wray, C. 1994. An approach to reduction of Salmonella infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. *Int. J. Food Microbiol.* **24**: 147–160.
18. Gast, R.K. 1994. Understanding *Salmonella* enteritidis in laying chickens: the contributions of experimental infections. *Int. J. Food Microb.* **21**: 107–116.
19. Gast, R.K. and Holt, P.S. 1998. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. *Poult. Sci.* **77**: 1759–1762.
20. Giessen, A.W., Peters, R., Berkers, P.A.T.A., Jansen, W.H. and Notermans, S.H.W. 1991. *Salmonella* contamination of poultry flocks in the Netherlands. *Vet. Q.* **13**: 41–46.
21. Holt, P.S., Gast, R.K., Porter Jr, R.E. and Stone, H.D. 1999. Hyporesponsiveness of the systemic and mucosal immune systems in chickens infected with *Salmonella* enterica serovar Enteritidis at one day of age. *Poult. Sci.* **73**: 1510–1517.
22. Idoia, L., Bone, L.B., Aitor, R. and Javier, G. 2000. Genotypic characterization by PFGE of *Salmonella* enterica serotype Enteritidis phage types 1, 4, 6, and 8 isolated from animal and human sources in three European countries. *Vet. Microbiol.* **75**: 155–165.
23. Jones, F.T., Axtell, R.C., Rives, D.V., Scheideler, S.E., Tarver, F.R., Walker, R.L. and Wineland, M.J. 1991. A survey of *Salmonella* contamination in modern broiler production. *J. Food Prot.* **54**: 502–507.
24. Keller, L.H., Schifferli, D.M., Benson, C.E., Aslam, S. and Eckroade, R.J. 1997. Invasion of chicken reproductive tissues and forming eggs is not unique to *Salmonella* enteritidis. *Avian Dis.* **41**: 535–539.
25. Lachoncha, I., Baggesen, D.L., Rememteria, A. and Garaizar, J. 2000. Genetic characterization by PFGE of *Salmonella* enterica serotype Enteritidis phage types 1, 4, 6 and 8 isolates from animal and human sources in three European countries. *Vet. Microbiol.* **75**: 155–165.
26. Liebana, E., Garcia-Migura, L., Breslin, M.F., Davies, R.H. and Woodward, M.J. 2001. Diversity of *Salmonella* enterica serotype Enteritidis from English poultry farms assessed by multiple genetic fingerprinting. *J. Clin. Microbiol.* **39**: 154–161.
27. Lister, S.A. 1988. *Salmonella enteritidis* infection in broilers and broiler breeders. *Vet. Rec.* **123**: 350.
28. Maslow, J.N., Mulligan, M.E. and Arbeit, R.D. 1993. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. *Clin. Infect. Dis.* **17**: 153–162.
29. McIlroy, S.G., McCracken, R.M., Neill, S.O. and O'Brien, J.J. 1989. Control, prevention and eradication of *Salmonella enteritidis* infection in broiler and broiler breeder flocks. *Vet. Rec.* **125**: 45–548.
30. Nakamura, M., Nagamine, N., Normatsa, M., Suzuki, S., Ohishi, K., Kijima, M., Tamua, Y. and Sato, S. 1993. The ability of *Salmonella enteritidis* isolated from chickens imported from England to cause transovarian infection. *J. Vet. Med. Sci.* **55**: 135–136.
31. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard, M2–A8. 2003.
32. O'Brien, J.P.D. 1990. Aspect of *Salmonella enteritidis* control in poultry. *World's Poult. Sci. J.* **46**: 119–124.
33. Olsen, J.E., Skow, M.N., Threlfall, E.J. and Brown, D.J. 1994. Clonal lines of *Salmonella* enterica serotype Enteritidis documented by IS200-, ribo-, pulsed-field gel electrophoresis and RFLP typing. *J. Med. Microbiol.* **40**: 15–22.
34. Popoff, M.Y., Bockemuhl, J. and Gheesling, L.L. 2003. Supplement 2001 (no. 45) to the Kauffmann-White scheme. *Res. Microbiol.* **154**: 173–174. 2003.
35. Popoff, M.Y. and Le Minor, L. 1997. Antigenic formulas of the *Salmonella* serovars, 7th revision. World Health Organization Collaborating Centre for Reference and Research on Salmonella, Pasteur Institute, Paris, France.
36. Ridley, A.M., Threlfall, E.J. and Rowe, B. 1998. Genotypic characterization of *Salmonella enteritidis* phage type by plasmid analysis, ribotyping, and pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **36**: 2314–2321.
37. Rose, N., Beaudeau, F., Drouin, P., Toux, J.Y., Rose, V. and Colin, P. 1999. Risk factors for *Salmonella* enterica subsp. enterica contamination in French broiler-chicken flocks at the end of the rearing period. *Prev. Vet. Med.* **39**: 265–277.
38. Ryan, K.J. 1990. Enterobacteriaceae. pp. 317–377. *In*: Medical Microbiology (Sherris J. C. ed.), Elservier, New York, Amsterdam, London.
39. Timoney, J.F., Shiraprasad, H.L., Baker, R.C. and Rowe, B. 1989. Egg transmission after infection of hens with Salmonella enteritidis phage type 4. *Vet. Rec.* **125**: 600–601.
40. Ward, L.R., de Sa, J.D. and Rowe, B. 1987. A phage-typing scheme for *Salmonella enteritidis*. *J. Gen. Microbiol.* **139**: 1477–1485.
41. Wierup, M., Engstrom, B., Engvall, A. and Wahlstrom, H. 1995. Control of *Salmonella enteritidis* in Sweden. *Int. J. Food Microbiol.* **25**: 219–226.