Incidence of *Salmonella* Enteritidis in chicken layer flocks in Turkey: Results by real-time polymerase chain reaction and International Organization for Standardization culture methods

S. Temelli,* S. Kahya,† A. Eyigor,* and K. T. Carli†[1]

*Department of Food Hygiene and Technology, and †Department of Microbiology, Faculty of Veterinary Medicine, Uludag University, Gorukle Campus, 16059 Bursa, Turkey

ABSTRACT This study presents *Salmonella* Enteritidis incidence in chicken layer flocks in Turkey determined by real-time PCR (rPCR) and by International Organization for Standardization (ISO) method 6579:2002/Amd 1:2007. A total of 259 samples, composed of 1,036 individual samples each pooled into 4, including 175 cloacal swab, 14 intestine, 35 gizzard swab, and 35 cecal swab samples, belonging to 6 major companies, were collected from 50 layer flocks and tested by rPCR and ISO culture methods. Overall incidence of *Salmonella* in layer flocks by rPCR and culture was 61.0 and 55.6%, respectively, where 70.1% of these *Salmonella* isolates were determined as *Salmonella* Enteritidis. Incidences of *Salmonella* Enteritidis in culture-positive samples were 65.3% in cloacal swabs, 50.0% in intestines, 73.9% in gizzard swabs, and 87.5% in cecal swabs. The rPCR results were in 100% agreement (100% sensitivity and specificity) with culture results when cecal swabs were selected as the sample type. The relative accuracy of rPCR was 92.4, 91.4, and 84% for intestine, gizzard, and cloacal swab samples, respectively. As a result, by using rPCR and ISO culture, we determined that the *Salmonella* Enteritidis incidence in layer flocks in Turkey was high and that the use of cecal swab and intestine samples in *Salmonella* detection would yield reliable results. To reduce this high *Salmonella* Enteritidis incidence in layer flocks, *Salmonella* Enteritidis-specific vaccination should be implemented properly in conjunction with a well-designed biosecurity plan, including verifiable corrective actions.

Key words: *Salmonella*, layer chicken, real-time polymerase chain reaction, International Organization for Standardization

2010 Poultry Science 89:1406–1410
doi:10.3382/ps.2010-00796

INTRODUCTION

Salmonella is an important cause of human disease (EFSA, 2009; Newell et al., 2010), with an estimated 80.3 million annual foodborne cases (Majowicz et al., 2010) and significant economical losses in the poultry sector worldwide. In Europe, *Salmonella* Enteritidis is one of the most commonly isolated serotypes in human salmonellosis (WHO, 2006; EFSA, 2007), where contaminated eggs produced by infected layers remain one of the most important sources of this infection (Crespo et al., 2005; De Jong and Ekdahl, 2006; Delmas et al., 2006, Korsgaard et al., 2009; Stevens et al., 2009).

Today, the goal of layer producers is to ensure consumers have access to safe and wholesome egg products, which necessitates initiation of control of this pathogen at farm level. For this aim, in our country for example, most of the layer companies inform us that they use *Salmonella* vaccines against the most prevalent or endemic serovars in their regions, complemented with their biosecurity programs. These programs, which are designed by the veterinarians of the breeder companies, may cause problems in operational completion of prerequisite programs and Hazard Analysis and Critical Control Point plans for the prevention of *Salmonella* contamination in flocks. Also, to evaluate the efficiency of these biosecurity actions including vaccinations, these companies submit samples from their flocks to external diagnostic laboratories. Part of this control involves testing of samples, collected by these companies, for the presence of *Salmonella* with a traditional culture technique, which requires 5 to 11 d for confirmation of the results.

The most commonly applied and accepted gold standard culture method for the detection of *Salmonella* spp. in animal feces and in samples from the primary production stage is International Organization for Standardization (**ISO**) method 6579:2002/Amd 1:2007 (ISO, 2007) in the countries of the European Union, and this method also involves 4 to 6 d. To shorten this

©2010 Poultry Science Association Inc.
Received March 23, 2010.
Accepted April 11, 2010.
[1]Corresponding author: tayfun@uludag.edu.tr

Downloaded from http://ps.oxfordjournals.org/ by guest on April 16, 2015

detection time in the interest of layer companies, real-time PCR (**rPCR**) can be applied to fecal samples before culture results (Eyigor et al., 2002; Kurowski et al., 2002; Eyigor and Carli, 2003; Eriksson and Aspan, 2007; Tomás et al., 2009; Hadjinicolaou et al., 2009). However, as has been previously pointed out (Eyigor et al., 2002), the fact that rPCR and culture can complement each other and cannot replace one another should always be kept in mind.

In this study, we determined *Salmonella* Enteritidis incidence in chicken layer flocks by rPCR and ISO culture methods for 2009.

MATERIALS AND METHODS

Salmonella Strains and Clincal Samples

Salmonella enterica ssp. *enterica* serovar Enteritidis 64K (M. Y. Popoff, Institut Pasteur, Paris, France), *Salmonella enterica* ssp. *enterica* serovar Typhimurium LT2-CIP60-62 and *Salmonella enterica* ssp. *enterica* serovar Typhimurium NCTC 12416 (Refik Saydam National Public Health Agency, Ankara, Turkey), and *Salmonella enterica* ssp. *enterica* serovar Typhimurium ATCC 14028 and *Salmonella enterica* ssp. *enterica* serovar Enteritidis ATCC 13076 (Uludag University Medical School, Department of Microbiology, Bursa, Turkey) were used as positive controls in rPCR and in ISO culture.

A total of 259 samples, composed of 1,036 individual samples each pooled into 4, including 175 cloacal swab, 14 intestine, 35 gizzard swab, and 35 cecal swab samples, belonging to 6 major companies, were collected from 50 layer flocks. Each of the cloacal, gizzard, and cecal swab samples were submitted to the laboratory in an ice box within 6 h after sampling in sterile Eppendorf tubes containing 500 μL of 0.85% NaCl solution. Each intestine sample was submitted to the laboratory in sterile screw-cap plastic bottles transported at the same conditions indicated for the other sample types.

ISO Culture

Culture was performed as indicated in ISO (2007). For cloacal, gizzard, and cecal swabs, swabs from 4 birds were pooled and this pooled sample, which weighed approximately 5 g, was transferred into 45 mL of buffered peptone water (**BPW**, CM1049, Oxoid, Basingstoke, Hampshire, UK). For intestine samples, intestines belonging to 4 birds, each weighing 6 to 7 g, were pooled, and this pooled 25-g sample was transferred into 225 mL of BPW and incubated at 37°C for 18 h. After this incubation, semisolid modified Rappaport-Vassiliadis agar (**MSRV**, CM1112, Oxoid) plates were inoculated with 3 drops (total 0.1 mL) of BPW culture and incubated at 41.5°C for 24 h. Negative plates were reincubated at 41.5°C for 24 h, and a loopful of growth on a MSRV plate was streaked onto both xylose-lysine-deoxycholate agar (278850, Becton, Dickinson and Co., Franklin Lakes, NJ) and xylose-lysine-tergitol-4 agar (Becton, Dickinson and Co.). After selective plating at 37°C for 24 h, suspect *Salmonella* colonies were subjected to biochemical identification by API 20E (20100, bioMérieux, Marcy l'Etoile, France) and serological identification using *Salmonella* group-specific antisera (Becton, Dickinson and Co.).

Template Preparation for rPCR

The MSRV primary enrichment cultures of the *Salmonella* control strains and of the clinical samples were used in crude template DNA preparations for rPCR as described by Carli et al. (2001b). Briefly, Eppendorf tubes containing MSRV culture suspended in 100 μL of 0.85% NaCl were washed twice with this solution, and the final pellet was resuspended in 20 μL of deionized water. This suspension was boiled for 10 min, centrifuged for 3 min at 18,000 × *g*, and 2 μL of the supernatant was used as template in rPCR.

rPCR

The rPCR was performed as described by Eyigor et al. (2002) using the LightCycler 2.0 instrument (Roche Diagnostics, Mannheim, Germany). The reaction mixture for rPCR contained the following: 2 μL of 10 × concentration of LightCycler FastStart DNA Master SYBR Green I [FastStart Taq DNA polymerase, reaction buffer, deoxynucleoside triphosphate mix (with deoxyuridine triphosphate instead of deoxythymidine triphosphate), SYBR Green I dye, and 10 m*M* $MgCl_2$], 2.4 μL of 25 m*M* $MgCl_2$, 1 μL of each 100 m*M* primer (*Salmonella inv*A gene-specific primers described by Rahn et al., 1992), 2 μL of template DNA (or 2 μL of ultrapure water for no-target control), and 11.6 μL of ultrapure water. The amplification protocol initiates with a denaturation step at 95°C for 10 min and is followed by 40 cycles of 10-s denaturation at 95°C, 5-s annealing at 55°C, and 10-s primer extension at 72°C. The temperature transition rate was 20°C/s. Fluorescence was measured at the end of each extension step, and melting curve analysis was performed using the LightCycler software version 4.05 (Roche Diagnostics).

Statistical Analysis

Relative accuracy, sensitivity, and specificity was calculated according to the protocol described in ISO 16140 (ISO, 2003). Calculations were complemented with Cohen's kappa test to evaluate the correspondence between results obtained by the 2 methods.

RESULTS

The overall incidence of *Salmonella* positivity by rPCR and ISO culture was found as 61.0% (158/259) and 55.6% (144/259), respectively, regardless of the sample type (Table 1). Based on sample types, out of

Downloaded from http://ps.oxfordjournals.org/ by guest on April 16, 2015

Table 1. Incidence of *Salmonella* by real-time PCR (rPCR) and International Organization for Standardization method 6579:2002/Amd 1:2007 culture

Sample type and company code	Number of pooled samples	Number of positive samples by methods (%)		*Salmonella* Enteritidis incidence in culture-positive samples (%)	
		rPCR	Culture	*Salmonella* Enteritidis	Non-*Salmonella* Enteritidis
Cloacal swab	175	87 (49.7)	75 (42.9)	49 (65.3)	26 (34.7)
I	37	3 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)
II	28	28 (100.0)	24 (85.7)	12 (50.0)	12 (50.0)
III	35	31 (88.6)	31 (88.6)	26 (83.9)	5 (16.1)
IV	36	24 (66.7)	19 (52.8)	10 (52.6)	9 (47.4)
V	28	1 (3.6)	1 (3.6)	1 (100.0)	0 (0.0)
VI	11	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Intestine	14	13 (92.9)	14 (100.0)	7 (50.0)	7 (50.0)
II	7	6 (85.7)	7 (100.0)	4 (57.1)	3 (42.9)
III	7	7 (100.0)	7 (100.0)	3 (42.9)	4 (57.1)
Gizzard swab					
III	35	26 (74.3)	23 (65.7)	17 (73.9)	6 (26.1)
Cecal swab					
III	35	32 (91.4)	32 (91.4)	28 (87.5)	4 (12.5)
Total	259	158 (61.0)	144 (55.6)	101 (70.1)	43 (29.9)

175 pooled cloacal swab samples from 6 companies, 87 (49.7%) and 75 (42.9%) were positive by rPCR and ISO culture, respectively. Out of 14 intestine samples from 2 companies, 13 (92.9%) were found positive by rPCR and 14 (100.0%) were positive by ISO culture. Twenty-three (65.7%) and 32 (91.4%) out of 35 gizzard and 35 cecal pooled swab samples from only 1 company (company III) were found to harbor *Salmonella* by ISO culture, respectively, whereas 26 (74.3%) and 32 (91.4%) of the same samples were positive by rPCR (Table 1).

Overall, we determined 70.1% of a total of 144 isolates as *Salmonella* Enteritidis, whereas 29.9% (43/144) remained as non-*Salmonella* Enteritidis isolates. According to sample types, incidences of *Salmonella* Enteritidis in culture-positive cloacal swab, intestine, gizzard swab, and cecal swab samples were 65.3% (49/75), 50.0% (7/14), 73.9% (17/23), and 87.5% (28/32), respectively. *Salmonella* Enteritidis incidence distributions and details of rPCR and ISO culture results of all samples from 6 companies are given in Tables 1 and 2.

Following the ISO 16140 statistical protocol (ISO, 2003), rPCR and ISO culture results were compared by using the culture as the reference method. The rPCR test results were found to be in 100% agreement with culture results when cecal swab samples were used (Table 3). The agreement between culture and rPCR was relatively high for intestine and gizzard swab samples as well. A lower agreement between ISO culture and rPCR was observed in cloacal samples, related to the presence of both false-negative and false-positive results for this sample type (Table 3).

DISCUSSION

In this study, we observed a considerably high *Salmonella* incidence in layer flocks because more than half of the samples submitted to the laboratory were determined to harbor this pathogen. There are similar previous findings from layer flocks in the percentages as high as 55.6, 76.9, and 86.5% by Dorn and Schleiff (1997), Carli et al. (2001a), and Li et al. (2007), respectively. There are also studies reporting *Salmonella* detection rates as low as 0.0 to 17.0% (Kalender and Muz, 1999; Eyigor et al., 2002, 2005; Eyigor and Carli, 2003; Ata and Aydın, 2008) from our country and 9.93 to 17.9% from European Union countries (Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010). Apart from the effects of *Salmonella* prevalence within a flock (Arnold et al., 2010), of the housing system (Carrique-Mas et al., 2009; Huneau-Salaün et al., 2009), and of the flock characteristics (Namata et al., 2008), the variations in *Salmonella* detection rates have particularly been re-

Table 2. Real-time PCR (rPCR) and International Organization for Standardization method 6579:2002/Amd 1:2007 culture results from different companies

Company code	Result[1] (n)			
	rPCR +/culture +	rPCR +/culture −	rPCR −/culture +	rPCR −/culture −
I	0	3	0	34
II	30	4	1	0
III	93	3	0	16
IV	11	13	8	4
V	1	0	0	27
VI	0	0	0	11
Total	135	23	9	92

[1]+ = positive; − = negative.

Downloaded from http://ps.oxfordjournals.org/ by guest on April 16, 2015

Table 3. Relative accuracy, sensitivity, and specificity of International Organization for Standardization method 6579:2002/Amd 1:2007 culture and real-time PCR (rPCR) for detection of *Salmonella* based on sample types

Sample type (n)	Reference method (culture)		Alternative method (rPCR)		Accuracy (%)	Sensitivity (%)	Specificity (%)
	Positive	Negative	False negative	False positive			
Cloacal swab	67	80	8	20	84[a]	89.3	80
Intestine	13	0	1	0	92.9	92.9	—[1]
Gizzard swab	23	9	0	3	91.4[b]	100	75
Cecal swab	32	3	0	0	100[c]	100	100
Total	135	92	9	23	87.6[d]	93.8	80

[a–d]The index values for 84, 91.4, 100, and 87.60 are 0.68, 0.80, 1.00, and 0.75, respectively.

[1]Could not be calculated.

lated to the sample type analyzed and the method used (Rybolt et al., 2004; Kinde et al., 2005; Arnold et al., 2010). In this study for instance, cecal swab and intestine samples, as supported by former study data (Nief and Hoop, 1998; Kalender and Muz, 1999; Carli et al., 2001b; Eyigor and Carli, 2003; Eyigor et al., 2005; Ata and Aydın, 2008) seemed to work relatively better for *Salmonella* detection both by rPCR and ISO culture than other sample types, such as cloacal swabs. This leads to the conclusion that the use of cecal swab and intestine samples in *Salmonella* detection would yield reliable results.

In this study, the *Salmonella* detection rate of rPCR was higher than ISO culture except for intestine samples, which is almost in parallel to our previous findings (Eyigor and Carli, 2003; Eyigor et al., 2005). The false-positive results in rPCR were also observed by Bohaychuk et al. (2007) and O'Regan et al. (2008). These types of culture-negative, PCR-positive circumstances can be related to high numbers of nonculturable or dead *Salmonella* cells in samples, to lower initial number of cells in the sample type (for example as can be in cloacal swabs, related to insufficient amount of fecal material in the swab), or to overgrowth of lactose-positive bacteria masking the typical *Salmonella* colonies on selective agar plates. Other reasons for these types of results can be insufficient recovery of injured-stressed cells and certain difficult to culture serotypes causing false negativity in bacteriology (Schrank et al., 2001; Eyigor et al., 2002; Löfström et al., 2004; Eriksson and Aspan, 2007). On the other hand, we had 9 false-negative (rPCR negative/ISO culture positive) results, probably related to the presence of inhibitory substances in template DNA preparations (Hoorfar et al., 2003; Joshi et al., 2009) or to very low or no initial *Salmonella*-specific template DNA in enrichment cultures (Bohaychuk et al., 2007; Joshi et al., 2009).

We determined *Salmonella* Enteritidis as the most common serovar (with 71.1% incidence) in layer flocks in Turkey for 2009 in this study, in parallel to our (Carli et al., 2001a; Eyigor et al., 2005) and others' (Dorn and Schleiff, 1997; Schaar et al., 1997; Nief and Hoop, 1998; Snow et al., 2007; Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010) reports and in contrast to Li et al. (2007), who reported *Salmonella* Kentucky as the most prevalent serovar. The high detection of *Salmonella* Enteritidis, regardless of the sample type, points out that this pathogen is persistently present in the layer flocks in Turkey. This is primarily an indication of ineffective-insufficient biosecurity systems in plant managements. One important step in this biosecurity system in Turkey is the vaccination program against *Salmonella* Enteritidis in layer flocks. The high *Salmonella* Enteritidis incidence in layer flocks in this study can be related to problems in vaccine applications with improper dosage, without taking prescribed directions of the commercially available vaccine into consideration, and inadequate circumvention of feed, personnel, or pest-originated environmental infections due to inappropriate hygiene and sanitation applications. Naturally, the specific applications such as proper vaccination should be considered within a well-designed Hazard Analysis and Critical Control Point plan including its required prerequisite programs specifically tailored for layer companies.

REFERENCES

Arnold, M. E., J. J. Carrique-Mas, and R. H. Davies. 2010. Sensitivity of environmental sampling methods for detecting *Salmonella* Enteritidis in commercial laying flocks relative to the within-flock prevalence. Epidemiol. Infect. 138:330–339.

Ata, Z., and N. Aydın. 2008. Isolation of *Salmonella* spp. in Ankara region poultry plants. J. Ankara Univ. Vet. Fac. 55:161–166.

Bohaychuk, V. M., G. E. Gensler, M. E. Mcfall, R. K. King, and D. G. Renter. 2007. A real time PCR assay for the detection of *Salmonella* in a wide variety of food and food-animal matrices. J. Food Prot. 70:1080–1087.

Carli, K. T., A. Eyigor, and V. Caner. 2001a. Prevalence of *Salmonella* serovars in chickens in Turkey. J. Food Prot. 64:1832–1835.

Carli, K. T., C. B. Unal, V. Caner, and A. Eyigor. 2001b. Detection of salmonellae in chicken feces by a combination of tetrathionate broth enrichment, capillary PCR, and capillary gel electrophoresis. J. Clin. Microbiol. 39:1871–1876.

Carrique-Mas, J., M. Breslin, L. Snow, I. McLaren, A. R. Sayers, and R. H. Davies. 2009. Persistence and clearance of different *Salmonella* serovars in buildings housing laying hens. Epidemiol. Infect. 137:837–846.

Crespo, P. S., G. Hernandez, A. Echeita, A. Torres, P. Ordonez, and A. Aladuena. 2005. Surveillance of foodbourne disease outbreaks associated with consumption of eggs and egg products: Spain, 2002–2003. http://www.eurosurveillance.org/ew/2005/050616.asp#2 Accessed Feb. 2010.

De Jong, B., and K. Ekdahl. 2006. Human salmonellosis in travelers is highly correlated to the prevalence of *Salmonella* in laying hen flocks. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2993 Accessed Feb. 2010.

Downloaded from http://ps.oxfordjournals.org/ by guest on April 16, 2015

Delmas, G., A. Gallay, E. Espié, S. Haeghebaert, N. Pihier, F. X. Weill, H. De Valk, V. Vaillant, and J. C. Désenclos. 2006. Foodborne-diseases outbreaks in France between 1996 and 2005. B. E. H. 51/52:418–422.

Dorn, W., and G. Schleiff. 1997. Evaluation of methods for preparing chicken feces from the veterinary hygienic aspect. Zentralbl. Veterinarmed. B. 44:105–118.

EFSA. 2007. Report of the task force on zoonoses data collection on the analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. EFSA J. 97:1–84.

EFSA. 2009. The Community Summary Report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. EFSA J. 223:1–320.

Eriksson, E., and A. Aspan. 2007. Comparison of culture, ELISA and PCR techniques for *Salmonella* detection in faecal samples for cattle, pig and poultry. BMC Vet. Res. 3:21.

Eyigor, A., and K. T. Carli. 2003. Rapid detection of *Salmonella* from poultry by real-time polymerase chain reaction with fluorescent hybridization probes. Avian Dis. 47:380–386.

Eyigor, A., K. T. Carli, and C. B. Unal. 2002. Implementation of real-time PCR to tetrathionate broth enrichment step of *Salmonella* detection in poultry. Lett. Appl. Microbiol. 34:37–41.

Eyigor, A., G. Goncagul, E. Gunaydin, and K. T. Carli. 2005. *Salmonella* profile in chickens determined by real-time polymerase chain reaction and bacteriology from years 2000 to 2003 in Turkey. Avian Pathol. 34:101–105.

Hadjinicolaou, A. V., V. L. Demetriou, M. A. Emmanuel, C. K. Kakoyiannis, and L. G. Kostrikis. 2009. Molecular beacon-based real-time PCR detection of primary isolates of *Salmonella* Typhimurium and *Salmonella* Enteritidis in environmental and clinical samples. BMC Microbiol. 9:97.

Hoorfar, J., N. Cook, B. Malorny, M. Wagner, D. De Medici, A. Abdulmawjood, and P. Fach. 2003. Making internal amplification control mandatory for diagnostic PCR. J. Clin. Microbiol. 41:5835.

Huneau-Salaün, A., M. Chemaly, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, V. Michel, P. Fravalo, and N. Rose. 2009. Risk factors for *Salmonella enterica* ssp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. Prev. Vet. Med. 89:51–58.

ISO. 2003. Microbiology of food and animal feeding stuffs—Protocol for the validation of alternative methods. ISO 16140:2003 (E). International Organization for Standardization, Geneva, Switzerland.

ISO. 2007. Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Salmonella*. EN ISO 6579:2002/Amd 1:2007. International Organization for Standardization, Geneva, Switzerland.

Joshi, R., H. Janagama, H. P. Dwivedi, T. M. A. Senthil Kumar, L. A. Jaykus, J. Schefers, and S. Sreevatsan. 2009. Selection, characterization, and application of DNA aptamers for the capture and detection of *Salmonella enterica* serovars. Mol. Cell. Probes 23:20–28.

Kalender, H., and A. Muz. 1999. Typing of *Salmonella* species isolated from chickens in Elazığ region. Turk. J. Vet. Anim. Sci. 23:297–303.

Kinde, H., D. M. Castellan, D. Kerr, J. Campbell, R. Breitmeyer, and A. Ardans. 2005. Longitudinal monitoring of two commercial layer flocks and their environments for *Salmonella enterica* serovar Enteritidis and other *Salmonellae*. Avian Dis. 49:189–194.

Korsgaard, H., M. Madsen, N. C. Feld, J. Mygind, and T. Hald. 2009. The effects, costs and benefits of *Salmonella* control in the Danish table-egg sector. Epidemiol. Infect. 137:828–836.

Kurowski, P. B., J. L. Traub-Dargatz, P. S. Morley, and C. R. Gentry-Weeks. 2002. Detection of *Salmonella* spp in fecal specimens by use of real-time polymerase chain reaction assay. Am. J. Vet. Res. 63:1265–1268.

Li, X., J. B. Payne, F. B. Santos, J. F. Levine, K. E. Anderson, and B. W. Sheldon. 2007. *Salmonella* populations and prevalence in layer feces from commercial high-rise houses and characterization of the *Salmonella* isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult. Sci. 86:591–597.

Löfström, C., R. Knutsson, C. E. Axelsson, and P. Radstrom. 2004. Rapid and specific detection of *Salmonella* spp. in animal feed samples by PCR after culture enrichment. Appl. Environ. Microbiol. 70:69–75.

Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50:882–889.

Namata, H., E. Méroc, M. Aerts, C. Faes, J. C. Abrahantes, H. Imberechts, and K. Mintiens. 2008. *Salmonella* in Belgian laying hens: An identification of risk factors. Prev. Vet. Med. 83:323–336.

Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. V. van der Giessen, and H. Kruse. 2010. Food-borne diseases—The challenges of 20 years ago still persist while new ones continue to emerge. Int. J. Food Microbiol. 139(Suppl. 1):S3–S15.

Nief, V., and R. K. Hoop. 1998. Detection of *Salmonella* Enteritidis in suspected flocks of laying hens. Schweiz. Arch. Tierheilkd. 140:70–75.

O'Regan, E., E. Mccabe, C. Burgess, S. Mcguinness, T. Barry, G. Duffy, P. Whyte, and S. Fanning. 2008. Development of a real-time multiplex PCR assay for the detection of multiple *Salmonella* serotypes in chicken samples. BMC Microbiol. 156:1–11.

Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galán, C. Ginocchio, R. I. I. I. Curtiss, and C. L. Gyles. 1992. Amplification of an *inv*A gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probes 6:271–279.

Rybolt, M. L., R. W. Wills, J. A. Byrd, T. P. Doler, and R. H. Bailey. 2004. Comparison of four *Salmonella* isolation techniques in four different inoculated matrices. Poult. Sci. 83:1112–1116.

Schaar, U., E. F. Kaleta, and B. Baumbach. 1997. Prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* in laying hen flocks battery and on floor housing. Comparative studies using bacteriological and serological demonstration methods. Tierarztl. Prax. Ausg. G Grosstiere Nutztiere 25:451–459.

Schrank, I. S., M. A. Mores, J. L. Costa, A. P. Frazzon, R. Soncini, A. Schrank, M. H. Vainstein, and S. C. Silva. 2001. Influence of enrichment media and application of a PCR based method to detect *Salmonella* in poultry industry products and clinical samples. Vet. Microbiol. 82:45–53.

Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. D. Wales, J. L. O'Connor, A. J. Cook, and S. J. Evans. 2007. Survey of the prevalence of *Salmonella* species on commercial laying farms in the United Kingdom. Vet. Rec. 161:471–476.

Stevens, M. P., T. J. Humphrey, and D. J. Maskell. 2009. Molecular insights into farm animal and zoonotic *Salmonella* infections. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364:2709–2723.

Tomás, D., A. Rodrigo, M. Hernández, and M. A. Ferrús. 2009. Validation of real-time PCR and enzyme-linked fluorescent assay-based methods for detection of *Salmonella* spp. in chicken feces samples. Food Anal. Methods. 2:180–189.

Van Hoorebeke, S., F. Van Immerseel, J. Schulz, J. Hartung, M. Harisberger, L. Barco, A. Ricci, G. Theodoropoulos, E. Xylouri, J. De Vylder, R. Ducatelle, F. Haesebrouck, F. Pasmans, A. de Kruif, and J. Dewulf. 2010. Determination of the within and between flock prevalence and identification of risk factors for *Salmonella* infections in laying hen flocks housed in conventional and alternative systems. Prev. Vet. Med. 94:94–100.

WHO. 2006. Progress Report (2000–2005): Building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response/WHO Global Salm-Surv. http://www.who.int/gfn/links/GSSProgressReport2005.pdf Accessed Feb. 2010.

Downloaded from http://ps.oxfordjournals.org/ by guest on April 16, 2015