AVIAN DISEASES 59:291–302, 2015

Risk Factors Associated With *Salmonella* in Laying Hen Farms: Systematic Review of Observational Studies

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Received 4 December 2014; Accepted 26 February 2015; Published ahead of print 27 February 2015

SUMMARY. Salmonella contamination of laying hen flocks and shell eggs is associated with various management and environmental factors. Foodborne outbreaks of human salmonellosis have been traced back to consumption of *Salmonella*-contaminated shell eggs. In the present study, a systematic literature review was conducted to identify and provide an evidence-based overview of potential risk factors of *Salmonella* contamination of laying hens, layer premises, and shell eggs. This systematic literature search was conducted using AGRICOLA, CAB Abstracts, and PubMed databases. Observational studies that identified risk factors for *Salmonella* contamination of layer flocks and shell eggs were selected, and best evidence was synthesized to summarize the results. Altogether, 13 cross-sectional studies and four longitudinal studies published in English were included in the review. Evidence scores were assigned based on the study design and quality of the study to grade the evidence level. The strength of association of a risk factor was determined according to the odds ratios. In this systematic review, the presence of previous *Salmonella* infection, absence of cleaning and disinfection, presence of rodents, induced molting, larger flock size (>30,000 hens), multiage management, cage housing systems, in-line egg processing, rearing pullets on the floor, pests with access to feed prior to movement to the feed trough, visitors allowed in the layer houses, and trucks near farms and air inlets were identified as the risk factors associated with *Salmonella* contamination of laying hen premises, whereas high level of manure contamination, middle and late phase of production, high degree of egg-handling equipment contamination, flock size of >30,000, and egg production rate of >96% were identified as the risk factors associated with *Salmonella* contamination of shell eggs. These risk factors demonstrated strong to moderate evidence of association with *Salmonella* contamination of laying hens and shell eggs. Eggshells testing positive for *Salmonella* were 59 times higher when fecal samples were positive and nine times higher when floor dust samples were positive. Risk factors associated with *Salmonella* Enteritidis infection in laying hens were flock size, housing system, and farms with hens of different ages. As a summary, this systematic review demonstrated that *Salmonella* contamination of laying hen flocks and shell eggs in layer production systems is multifactorial. This study provides a knowledge base for the implementation of targeted intervention strategies to control *Salmonella* contamination of laying hen flocks and shell eggs.

RESUMEN. Factores de riesgo asociados con *Salmonella* en explotaciones de gallinas de postura: Revisión sistemática de estudios observacionales.

La contaminación por *Salmonella* en gallinas de postura y en el cascarón de huevo se asocia con diversos factores de manejo y ambientales. Brotes de origen alimentario de salmonelosis humana han sido rastreados hasta el consumo de huevos con cascarón contaminado con *Salmonella*. En el presente estudio, se realizó una revisión sistemática de la literatura para identificar y proporcionar una visión general basada en la evidencia acerca de los factores de riesgo potenciales para la contaminación por *Salmonella* en gallinas de postura, en granjas de aves de postura y en cascarones de huevo. Esta búsqueda sistemática de la literatura se realizó utilizando las bases de datos AGRICOLA, CAB Abstracts, y PubMed. Se seleccionaron los estudios observacionales que identificaron los factores de riesgo de contaminación por *Salmonella* en lotes de gallinas de postura y en cascarones de huevo, y las evidencias más contundentes se sintetizaron para resumir los resultados. En esta revisión se incluyeron un total de 13 estudios transversales y cuatro estudios longitudinales publicados en inglés. Se asignaron puntuaciones de pruebas basadas en el diseño del estudio y en la calidad del estudio para clasificar el nivel de evidencia. La fuerza de la asociación de un factor de riesgo se determinó de acuerdo con las razones de momios. En esta revisión sistemática, la presencia de una infección previa por *Salmonella*, la falta de limpieza y desinfección, la presencia de roedores, la muda forzada, el tamaño grande de parvadas (>30 000 gallinas), el manejo de múltiples edades, los sistemas de alojamiento en jaula, el procesamiento de huevos en línea, la crianza de pollas en piso, plagas con acceso al alimento antes de su traslado a los comederos, la entrada de visitantes en las casetas de aves de postura y camiones cerca de las granjas y de las entradas de aire fueron identificados como los factores de riesgo asociados con la contaminación de granjas de aves de postura por *Salmonella*. Mientras que el alto nivel de contaminación de la gallinaza, la fase intermedia y tardía de la producción, el alto grado de contaminación del equipo para la manipulación de huevos, el tamaño de la parvada mayor a 30 000, y la tasa de producción de huevo mayor de 96% fueron identificados como los factores de riesgo asociados con la contaminación por *Salmonella* en los cascarones de huevo. Estos factores de riesgo demostraron una evidencia de fuerte a moderada en la asociación de la contaminación por *Salmonella* de las gallinas ponedoras y en los cascarones de huevo. La presencia de cascarones positivos a *Salmonella* fue 59 veces mayor cuando las muestras fecales resultaban positivas y nueve veces más alto cuando las muestras de polvo del piso resultaban positivos. Los factores de riesgo asociados a la infección por *Salmonella* Enteritidis en gallinas de postura fueron el tamaño de la parvada, el sistema de alojamiento, y las granjas con gallinas de diferentes edades. A modo de resumen, esta revisión sistemática demostró que la contaminación por *Salmonella* de gallinas de postura y en los cascarones de huevo en los sistemas de producción de aves de postura es multifactorial. Este estudio proporciona una base de conocimientos para la instrumentación de estrategias de intervención específicas para controlar la contaminación por *Salmonella* en gallinas de postura y en cascarones de huevo.

Key words: foodborne, laying hen, risk factors, *Salmonella*, shell eggs, systematic review

Abbreviations: EQAP = egg quality assurance program; SE = *Salmonella* Enteritidis; ST = *Salmonella* Typhimurium

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Salmonellosis caused by *Salmonella enterica* subsp. *enterica* (*Salmonella*) is one of the most important food-borne diseases in the United States (7) and countries of the European Union (9). In the United States, European Union, and Japan, *Salmonella* infections attributed to food sources were most commonly linked to eggs compared to other food sources (24). In foodborne outbreaks caused by *Salmonella* with a confirmed serotype in the United States, *Salmonella* Enteritidis (SE) was the most common serovar (19%) followed by *Salmonella* Typhimurium (ST) (14%), and *Salmonella* Newport (10%) (7). While a majority of foodborne salmonellosis outbreaks traced back to consumption of shell eggs were caused by SE (3,18), other serotypes of *Salmonella* have also been reported from egg-associated salmonellosis (18). Contamination of eggs with *Salmonella* occurs vertically by spread of bacteria from infected ovaries or horizontally by penetration of *Salmonella* present in the poultry house environment through the eggshell (13). Therefore, effective methods for decreasing *Salmonella* in layers can greatly reduce human infection due to the consumption of shell eggs. As a result, the United States and European Union have developed egg quality assurance programs (EQAPs) or specific guidelines aimed at reducing SE contamination of shell eggs. However, foodborne *Salmonella* outbreaks linked to shell eggs continue to be an important public health issue highlighting the importance of revisiting the existing guidelines and EQAPs (4,5,10). Therefore, it is imperative to identify the risk factors associated with *Salmonella* contamination of shell eggs in laying hen environments as part of the farm-to-table egg continuum. It is also important to understand the strength of association as well as the level of evidence for a given risk factor identified in primary research and conduct risk factor analysis using an evidence-based approach. Systematic review methodology assesses the internal and external validity of the primary research for a particular review question. Inclusion of descriptive literature search, criteria used for inclusion of studies, and assessment of study quality informs the potential biases and represents a major departure from traditional narrative reviews. Therefore, the objective of a systematic review is to convey to the reader of the review not only its conclusion, but sufficient information for the consumer to determine their agreement with the conclusion. Although a few narrative reviews have been conducted in this regard, no evidence-based methodology has been applied to summarize the best available evidence from field studies. Narrative reviews may also provide valuable conclusions if conducted in a comprehensive manner. However, the conclusions can be varied from one reviewer to another as there are no specific guidelines for the selection of primary research for a narrative review. On the other hand, in a systematic review, the review procedure is predetermined and therefore is not subjected to bias. The objective of this systematic review is to identify risk factors for *Salmonella* contamination of laying hen premises and shell eggs using the best available evidence of primary research available in online databases and other published reports describing evidence of association with observational studies.

MATERIALS AND METHODS

Literature search. A systematic literature search was conducted using three electronic databases, PubMed, AGRICOLA, and CAB Abstract, to identify relevant observational studies describing risk factors of *Salmonella* occurrence in laying hen flocks. The search criteria combined text words related to two main domains: ''*Salmonella enterica*'' and ''poultry.'' Reference lists of each selected article were searched for relevant citations. A dissertations and theses database was also searched for unpublished research reports. The database searches were conducted in September 2014.

Study selection. After excluding duplicate studies, one reviewer performed the first selection based on the title and abstract. Predefined inclusion criteria were used to select the studies: 1) observational studies that examined risk factors for *Salmonella* occurrence in laying hens, 2) *Salmonella* were isolated and identified by standard bacteriological culture or other methods, and 3) published in English as a full article in a peer-reviewed scientific journal. Exclusion criteria used were 1) studies performed on broilers and 2) studies published in languages other than English.

Quality assessment. The methodological quality assessment of the selected articles was performed by two reviewers independently using a modified quality check list designed for systematic reviews of observational studies in animal agriculture (23). These quality criteria were 1) Was the production system representative of layer egg production? 2) Was the study population representative of the target population? 3) Were the exposure variables and outcome variables measured independently of each other? 4) Was the *Salmonella* status (presence or absence) of the flock measured adequately to enable evaluation of the method? 5) Was the type of statistical analysis appropriate for the study design? 6) Were the estimates and measures of variability used to address the research question presented adequately? and 7) Were confounding factors properly controlled? The overall quality of the studies was assessed and summarized by comparing each of the seven items and was classified into two quality levels: high (fulfilled all seven items) and moderate (fulfilled only five to six items). Poor quality studies that fulfilled fewer than five quality assessment criteria were excluded.

Data extraction. Data extraction was performed based on original data described in the selected articles for study design (cross-sectional or longitudinal), country, year of publication, study period, characteristics of the analytical study sample, duration of follow-up for studies with a longitudinal design, statistically significant determinants ($P \leq 0.05$) preferably from multivariable analysis, and effect measures (odds ratios) with 95% confidence interval.

Best evidence synthesis. Due to heterogeneity between studies with respect to study characteristics, variable definition, bacterial culture and identification methods, and study quality, it was not possible to perform a quantitative analysis or meta-analysis. As such, overall trends in risk factors were assessed, focusing on direction of effect size and achievement of statistical significance, rather than quantitative synthesis across estimates that are not directly comparable. Therefore, the best evidence was summarized and reported based on the level of evidence and association of risk factors as described below. This approach is well suited to summarize heterogeneous studies (19,27) and has been used in previous systematic reviews in animal agriculture (23). The best evidence synthesis for this systematic review was performed as follows. First, the selected studies were rated according to the quality criteria and design of the study. Longitudinal studies were considered to be superior to cross-sectional studies in identifying association of risk factors, and, therefore, high-quality studies with a longitudinal design were rated on a five-point scale, moderate-quality studies with a longitudinal design were rated on a four-point scale, high-quality studies with cross-sectional design were rated on a four-point scale, and moderate-quality cross-sectional studies were rated on a three-point scale. Finally, cutoff values for the best evidence synthesis were interpreted as ≥ 4 points = strong evidence for an association, 3 = moderate evidence of an association. The strength of association was classified as odds ratio >3 = strong association and odds ratio 1.6–3 = moderate association, and odds ratio <1.5 = weak association. Level of evidence (strong, moderate or inconclusive) as well as strength of association (strong, moderate or weak) of risk factors for *Salmonella* occurrence in commercial laying hens was summarized and discussed across the studies in this review.

RESULTS

Search results. The systematic literature search resulted in a total of 1870 studies from all three databases, of which 576 duplicates were deleted. Three government reports were identified from reference lists of selected articles and one thesis was identified from a dissertations and theses database. Thus, the search provided 1298 studies for primary screening based on the title and abstract. After primary screening, 73 were selected for the next step, which was to read the full article. After the review process, 18 studies were deemed appropriate for data extraction. However, one journal article (14) and one government report (29) shared the same data set and were considered a single study. Methodological quality was assessed in 18 studies, which included four longitudinal studies (1,15,26,33) and 13 cross-sectional studies (2,6,8,14,16,17,20,21,25,28,29,30,31,32).

Description of studies. Table 1 summarizes the details of four longitudinal studies and 13 cross-sectional studies that describe risk factors for *Salmonella* occurrence in laying hen flocks. Of the 17 studies, four were conducted in the United States, three each in France, European Union, and the United Kingdom, two in Belgium, and one each in Australia, France, Japan, and the Netherlands. All studies were published between 1998 and 2014. Risk factors were analyzed for SE (eight studies), ST (two studies), and *Salmonella enterica* (nine studies).

Two studies had investigated risk factors associated with egg contamination (15,16), and one study had investigated risk factors associated with SE infection in hens (20). The other 14 studies had investigated risk factors associated with layer environmental contamination. All four longitudinal studies were of high quality and also provided a strong evidence for an association. Therefore, these four studies were assigned a score of 5. Of the 13 cross-sectional studies, 11 were of high quality and also demonstrated a strong evidence for an association. These 11 cross-sectional studies were assigned a score of 4. The remaining two cross-sectional studies, which were of moderate quality, were given a score of 3 because they provided only moderate evidence for an association. One of the cross-sectional studies with moderate evidence (2) has analyzed data using univariate logistic regression without controlling for confounding factors, and therefore only a trend of association could be established. The other study with a moderate quality by Van Hoorebeke *et al.* (31) has analyzed data using a multivariate logistic regression controlling for confounding factors. However, the sample size of this study was inadequate to draw conclusions for the target population.

Risk factors associated with *Salmonella* contamination of laying hen environment. *Previous Salmonella infection*. Two studies provided (6,26) strong evidence to suggest that the presence of *Salmonella* previously in the farm or a flock was strongly associated (OR $>$ 3) with contamination of laying hen environment with *Salmonella* during respective study periods. One study (17) reported prior contamination of the premise with SE (OR = 8.7), whereas the other study (26) (OR = 6.4) reported the prior contamination of the premise with *Salmonella* with no reference to a specific serovar.

Cleaning and disinfection. One study (31) showed strong evidence to that the absence of dry cleaning was strongly associated with SE and ST contamination of the laying hen environment (OR = 14.7). Another study (14) provided strong evidence that no cleaning and disinfection is strongly associated with SE contamination of laying hen environment (OR = 3.2).

Presence of rodents. Three studies (1,14,28) showed strong evidence to support that rodent presence was strongly associated with *Salmonella* contamination of the premise. Of the three studies with strong evidence, one study (1) reported a longer persistence of SE than non-SE serovars when the rodent score was $>$ 1.5 (rodent score "0" = very few or no sightings of rodents by the farmer, "1" = few rodent signs, "2" = moderate rodent signs, "3" = high level of rodent signs). Rodent signs included droppings, urine pillars, grease marks, tracks, structural damage, and uptake of bait/trapping. There was no difference between the clearance rates of SE and non-SE serovars when the rodents were absent or the rodent scores were low. A second study (29) showed strong evidence that the level of SE contamination was nine times (OR = 8.9) higher in layer houses with a rodent index of $>$20 compared to the houses with a rodent index of $<$20 (rodent index = total number of rodents trapped $\times$ (7/number of days) $\times$ (12/number of functional traps). The third study (28) showed if the rodents were seen monthly or more frequently, there was a high risk of SE (mice OR = 5.78 and rats OR = 8.17).

Molting. One study (25) provided strong evidence and identified "induced molting" as a risk factor for *Salmonella* contamination of layer premises (OR = 5.24).

Flock size. Two studies demonstrated strong evidence that flock size has an effect on the level of *Salmonella* contamination. Haneau-Salaun *et al.* (17) indicated that cage houses with $>$20,000 hens were at a higher risk (OR = 6) than the houses with $<$20,000 hens. The other study (28) showed that flock size of $>$30,000 hens was strongly associated with *Salmonella* contamination compared to the flock size of 1000 to 3000 hens at farm level and flock level with an OR = 4.79 and 14.88, respectively. The flock sizes of 3000 to 30,000 hens were not significantly associated with *Salmonella* presence.

Multiage management. Only two studies looked at the effect of multiage management on the farm with the occurrence of *Salmonella*. One study (17) showed a strong association (OR = 9.6) between multiage management and *Salmonella enterica* contamination of laying hen environment. The risk of *Salmonella* contamination of hens raised on the floor was higher when the flocks were reared on farms with multiage management than on farms with all-in/all-out management practice or farms with single-age flocks. The other study (28) also indicated all-in/all-out management reduced the risk of SE compared to multiage management practice (OR = 0.06).

Housing type. Four studies demonstrated strong evidence for the effect of housing type on *Salmonella* contamination. A European Union baseline study (8) reported hens raised in noncage housing system had a lower risk of contamination with SE, ST, and other *Salmonella* serovars compared with the hens raised in cages (Table 1). In this baseline study, farm and flock sizes were correlated with flock production type (correlation coefficient = 0.65), and in the logistic regression model, statistical analysis was adjusted only for the flock production type. According to another study (21), more *Salmonella* were recovered from both dust and fecal samples in caged production systems as compared to barn and free-range systems. This highlights a high risk of *Salmonella* for hens reared in cages. Snow *et al.* also provided evidence that noncage systems including barns reduced the risk for SE (28). Similarly, Van Hoorebeke *et al.* (32) found hens raised in conventional battery cages were at a higher risk for SE and ST compared to the hens raised in noncage systems such as indoor production and free-range systems. In a study that examined the time to clearance, SE persisted longer in houses with a deep pit (step-cage houses and cages with a scraper manure disposal system) than in noncage systems (1). A regulation has been imposed in the European Union banning the use of conventional battery cages and the sale of shell eggs from hens reared in conventional battery cages since January 2012 (11).

Table 1. Data extracted from 18 publications that included 13 cross-sectional studies and four longitudinal studies describing risk factors associated with *Salmonella* in laying hen flocks.[A]

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics		
1 (U.K.)	Longitudinal (high)	5	152 layer houses in 42 farms where 264 *Salmonella* incidents (54 cage scrapers, 44 cage belts, and 24 free-range, 17 step-cage, and 13 barn houses) 1 to 6 visits per flock (July 1998–Aug. 2007)	10 fecal/litter samples (25 g of pooled material collected from different locations of the house) and 10 dust samples (15 g from floor and egg belt spillage trays (cage houses), ledges and beams (noncage houses) from 152 flocks (Bacterial culture)	***Salmonella enterica***	Survival analysis (log time to clearance of infection)		
					Serovar (baseline = non-SE serovars)	β coefficient	SE	*p* value
					SE, rodent score < 1.5	−0.038	0.243	0.438
					SE, rodent score > 1.5	1.191	0.218	<0.01
					Type of house (baseline = noncage)			
					Step cage	1.090	0.444	<0.01
					Cage belt	0.244	0.357	0.248
					Cage scraper	0.850	0.349	<0.01
2 (U. S. A.)	Cross-sectional (moderate)	3	Double-blinded, randomly selected 197 potential egg layer premises listed by the University of California extension services included 91% of all known premises in California in 1998 (Sep. 1998–Jan. 2000)	Two sets of 16 manure drag swabs per flock from 133 out of 146 eligible premises. 133 microbiological results and 127 questionnaires. (Bacterial culture)	***Salmonella* Enteritidis**	Univariable logistic regression, OR (95% CI)		
					One molting compared to nonmolted	5.14 (0.96–52.07)		
					Flock mortality high *vs.* normal	4.35		
					Flock mortality high *vs.* low	7.25		
					Ventilation open *vs.* closed	3.51		
					Ventilation modified *vs.* closed	7.57		
					Watering cup *vs.* nipple drinker	3.48		
					Watering trough *vs.* nipple drinker	7.75		
					Manure removal interval between 1 and 10 wk compared to <1 wk	4.67 (0.77–28.42)		
6 (France)	Cross-sectional (high)	4	28 flocks randomly selected from 93 *Salmonella*-positive flocks out of 525 flocks	150 eggs of single-day egg production from each flock (Bacterial culture)	***Salmonella enterica***	Multiple correspondence analysis		
					Holding capacity > 30,000 hens	NA		
					High laying rate > 96%	NA		
					High environmental contamination	NA		

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
8 (E.U. and Norway)	Cross-sectional (high)	4	Holding size >1000 hens in 5,351 holdings in 24 countries in the E.U. and 314 holdings in Norway. The number of holdings to be sampled was stratified according to the holding size. Only one flock per holding. (Oct. 1, 2004–Sep. 30, 2005)	5 pooled fecal (cage flock) or five pairs of "bootsock" swabs (barn or free-range flocks) and 2 dust samples per flock of laying hens during the last 9 wk of production from 3808 holdings (Bacterial culture)	***Salmonella* Enteritidis**	Multivariable logistic regression, OR (95% CI)
					Flock production type	
					Cages	Reference
					Barn	0.57 (0.34–0.97)
					Free-range standard	0.02 (0.01–0.04)
					Organic	0.05 (0.02–0.14)
					Vaccinated with SE	0.12 (0.07–0.20)
					Vaccinated with non-SE	0.46 (0.25–0.83)
					Sample type	
					Feces	Reference
					Dust	1.54 (1.35–1.76)
					Age in wk	1.02 (1.01–1.03)
					***Salmonella* Typhimurium**	Multivariate logistic regression, OR (95% CI)
					Flock production type	
					Cages	Reference
					Barn	0.23 (0.07–0.81)
					Free-range standards	0.07 (0.02–0.31)
					Organic	0.07 (0.01–0.63)
					Sample type	
					Feces	Reference
					Dust	1.76 (1.20–2.58)
14 or 29 (U. S. A.)	Cross-sectional (high)	4	328 layer operations eligible to participate with >30,000 birds per farm in 15 states, 20 wk of age or older hens. One house per farm was randomly selected except on a few large farms (526 individual farms from 208 operations). (May 3–Oct. 22, 1999)	5 manure swabs, 5 egg belt swabs, 5 elevator swabs, and 2 walkway swabs per house (or 10 cage floor samples per house) from 200 layer houses, and rodents from 129 houses. Questionnaire administered from Mar. 22–Apr. 30, 1999. (Bacterial culture)	***Salmonella* Enteritidis**	Multivariable logistic regression, OR (p value)
					Standardized rodent index ≥ 20	8.9 (0.04)
					Standardized rodent index < 20	Reference
					Breed/strain	
					Hy-line	0.21
					Other white	Reference
					Age and molting	(0.02)
					<60 wk of age and not molted	4.7
					>60 wk of age and <16 wk postmolt	9.3
					>60 wk of age and >16 wk postmolt	1.4
					60 wk or more of age, not molted	Reference
					Floor-reared as pullets	5.9 (0.04)
					Pests have access to feed	6.2 (0.03)
					Visitors allowed	5.0 (0.04)
					No cleaning and disinfection between flocks	3.2

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
15 (Australia)	Longitudinal (high)	5	5 *Salmonella*-positive cages and 2 *Salmonella*-negative cages per farm from 2 farms. 10 samplings over 40 wk at 4 wk intervals.	Composite fecal sample per cage, egg belt sample per cage, 5 dust samples per shed, and all eggs from front of the cages (Bacterial culture)	***Salmonella enterica*:** For egg shell positive	Multilevel logistic regression, OR (95% CI)
					Fecal positive	58.9 (6.9–501)
					Dust positive	9.2 (1.8–45.8)
16 (U. S. A.)	Cross-sectional (high)	4	60 out of 134 flocks enrolled, and at least 1 environmental sample was positive for SE in Lancaster County, PA; 56 high-rise and 4 shallow-pit houses (Apr. 1992–Oct. 1994)	3–6 manure samples and 3–12 samples from egg-handling equipment from each flock. 1000 eggs every 2 wk for 8 wk, if environmental samples were positive. (Bacterial culture)	***Salmonella* Enteritidis**	Multivariable logistic regression, OR (95% CI)
					High manure contamination	10.26 (2.73–38.57)
					Low or no manure contamination	Reference
					Level of egg-handling equipment contamination: High *vs.* low or none	1.44 (0.42–5.01)
					Late phase of production (>56 wk)	1.24 (0.24–8.35)
					Middle phase of production (35–56 wk)	2.57 (0.71–9.41)
					Early phase of production (<35 wk)	Reference
					Vaccinated *vs.* not vaccinated	0.64 (0.11–3.68)
17 (France)	Cross-sectional (high)	4	519 randomly selected flocks with at least 1000 laying hens listed in French Ministry of Agriculture, at the last 9 wk of laying period. 227 cages, 292 barns, free-range and organic (Oct. 2004–Sep. 2005)	5 fecal samples (pooled feces from cage flocks and foot swabs from barn, outdoors, and organic farms) and 2 dust samples (Bacterial culture)	***Salmonella enterica***	Multivariable logistic regression, OR (95% CI)
					Flocks housed in cages	
					Type of samples: Feces *vs.* dust	0.3 (0.18–0.4)
					Poultry house size > 20,000 birds	6.0 (1.8–19.8)
					Trucks run and park near the entrance	4.1 (1.1–14.8)
					Flocks kept on floor	
					Specific container for dead bird	
					Yes	0.20 (0.04–0.99)
					No	Reference
					Multistage management	9.6 (1.1–84.5)
					Previous SE infection on the farm	8.7 (1.2–64.5)

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
20 (the Netherlands)	Cross-sectional (high)	4	1912 unvaccinated flocks participated out of 2508 enrolled flocks from 8409 flocks monitored by Dutch Product Board of Livestock, Meat and Egg, 9 wk before end of laying (Apr. 1998–Dec. 2002)	24–60 blood samples per flock, 6 samples were pooled together for analysis (ELISA)	***Salmonella* Enteritidis**	Multivariable logistic regression, OR
					Number of hens per flock	1.02 (per 1000 hens)
					Farms with and without hens of different ages for the deep litter system	3.48
					Farm with hens of same age	
					Flocks in cage system with wet manure compared to cage system with dry manure	0.26
					Outdoor run	2.14
					Deep litter system compared with cage system with dry manure	0.47
					Farm with hens of different ages	
					Deep litter system compared to cage with dry manure	2.09
					Deep litter system compared to cage with wet manure	2.91
21 (Belgium)	Cross-sectional (high)	4	148 flocks at the end of the laying period, holding size >1000 hens (Feb.–Sep. 2005)	5 fecal samples (boot swabs or pooled feces from deep pit, dropping belts or scrapers) and 2 dust samples per flock (Bacterial culture)	***Salmonella enterica***	Multivariable logistic regression, OR (95% CI)
					Production type:	
					Cage *vs.* barn and free-range	
					Dust	20.11 (2.52–160.49)
					Feces	10.27 (2.13–49.57)
25 (Japan)	Cross-sectional (high)	4	400 flocks from 338 farms with >1000 laying hens in 45 prefectures in Japan, end of laying period (Sep. 2007–Mar. 2008)	5 pooled cecal samples (10 g) and 2 dust samples (25 g) from each farm (Bacterial culture)	***Salmonella enterica***	Multivariable logistic regression, OR (95% CI)
					Windowless farms	
					Implementation of induced molting	5.24 (1.43–19.21)
					In-line egg processing	8.88 (2.23–35.38)

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
26 (E.U.)	Longitudinal (high)	5	41 laying hen flocks with > 1000 hens during laying, 18 in Belgium, 6 in Denmark and 17 in Germany (Feb. 2007–July 2008 in Belgium and Germany, and Oct. 2008–Mar. 2009 in Denmark)	1 pooled dust sample (25 g), 5 nest box samples, or 5 cage samples from empty and disinfected houses and 3-4 times thereafter at different intervals. 5 pooled fecal samples and individual cloacal swabs from 40 randomly selected hens, 6 visits total (Bacterial culture)	***Salmonella enterica*** *Salmonella* found during the previous sampling of the same flock	Multivariable logistic regression, OR (95% CI) 6.4 (2.05–19.7)
28 (U.K.)	Cross-sectional (high)	4	Randomly selected 454 holdings with >1000 hens from British Egg Information Council (2004–2005)	Caged houses: 5 mixed fecal samples and 2 dust samples. Barn and free-range houses: 5 pairs of bootswabs, 2 dust samples within 9 wk at the end of the laying period. Questionnaire for 380 of the 454 enrolled farms. (Bacterial culture)	***Salmonella* Enteritidis** Production type Cage Noncage (including barn) Farm affiliation Independent Associated with a company Feed mill Company feed mill Other Dogs or cats on farm Mice seen monthly or more often Rats seen monthly or more often All-in/all-out Vaccinated flock Holding size 1000–2999 >30,000 ***Salmonella enterica*** Holding size 1000–2999 >30,000	Multivariable logistic regression, OR (95% CI) Reference 0.14 (0.04–0.49) Reference 0.14 (0.03–0.74) Reference 0.11 (0.03–0.47) 0.14 (0.04–0.50) 5.78 (1.65–20.18) 8.17 (2.75–24.34) 0.06 (0.01–0.24) 0.08 (0.02–0.38) Reference 14.88 (3.16–70.08) Reference 4.79 (1.22–18.78)

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
30 (U. S. A.)	Cross-sectional (high)	4	328 farms randomly selected from FDA list of registered premises with ≥3000 laying hens in 19 states (June 1, 2012–May 31, 2013)	Producer reported test results for 1-yr period Bacterial culture, PCR (Taqman, BAX) or other rapid tests (SDIX, Neogen)	***Salmonella* Enteritidis** Farms with ≥30,000 laying hens Farm level Rodent index > 11 Routinely molts Down time < 10 days Flock level Postmolt test Flock vaccinated as pullets	Multivariable logistic regression, OR 4.3 3.9 3.8 3.7 0.09
31 (Belgium)	Cross-sectional (moderate)	3	29 farms out of 220 with a capacity of >1000 hens per farm (8 conventional battery cage flocks, 10 floor-raised flocks, 8 free-range flocks, and 3 organic flocks)	From each farm, 5 pooled fecal samples (250 g), 1 mixed dust sample, and 1 cloacal swab per hen from 40 randomly selected hens. Subsequently, cloacal swabs from 100 randomly selected hens per farm. (Bacterial culture)	***Salmonella enterica*** Age of production system Previous *Salmonella* contamination	Multivariable logistic regression, OR (95% CI) 1.35 (1.01–1.81) 77.64 (1.68–3596)
32 (Belgium, Germany, Greece, Switzerland, and Italy)	Cross-sectional (high)	4	292 flocks from 292 farms with >1000 hens 4 wk before depopulation. Belgium = 69, Germany = 84, Greece = 10, Switzerland = 99, and Italy = 30. Randomly selected farms and flocks. (Jan. 2007–Aug. 2008)	Conventional battery cages and aviaries: 5 samples of mixed fresh feces (250 g). Floor-raised, free-range, and organic farms: 5 pooled feces samples (250 g). 40 cloacal swabs from 40 laying hens. (Bacterial culture)	***Salmonella* Enteritidis and *Salmonella* Typhimurium** Absence of dry cleaning Housing type Conventional battery Indoor production Free-range Organic Season of sampling Winter Spring Summer Autumn	Multivariable logistic regression, OR (95% CI) 14.37 (4.54–45.51) Reference 0.05 (0.01–0.24) 0.18 (0.05–0.73) 0.17 (0.02–1.73) Reference 0.16 (0.04–0.73) 0.06 (0.01–0.40) 0.64 (0.16–2.60)

Table 1. (Continued)

Reference (country)	Design (quality)	Quality score	Sampling frame (study period)	Analytical sample (outcome assessment)	Risk factors	Statistics
33 (U. K.)	Longitudinal (high)	5	74 layer flocks from 8 farms positive for *Salmonella*, 59 caged and 15 free-range. 1 to 4 visits per flock, 2 to 6 months interval. (Aug. 2004–July 2005)	25 g fecal material, spillage from egg belts and from floors under cages, litter from free-range houses, and dust from within and around cages and nest boxes (10–15 g), rodent feces (1–2 g) (Bacterial culture)	***Salmonella enterica*** Stage of lay: Average increase in prevalence for each additional month in house Effect of temperature and season: summer months increased sample being positive compared to winter months	Multivariable logistic regression, OR (95% CI) 1.20 (1.13–1.26) 3.41 (1.01–11.55)

[A]CI = confidence interval; EFSA = European Food Safety Authority; ELISA = enzyme-linked immunosorbent assay; FDA = Food and Drug Administration; NA = not available; OR = odds ratio; Rodent index = total number of rodents trapped × (7/number of days) × (12/number of functional traps); USDA = United States Department of Agriculture.

In-line egg processing. One study examined the association of in-line egg processing and risk of *Salmonella*. This study indicated a strong evidence for a higher risk of *Salmonella* presence in windowless farms with in-line egg processing than the farms with off-line egg processing (25).

Salmonella vaccination status. A number of studies described the effect of vaccination of birds and reduction of *Salmonella* shedding (8,28,30). Vaccination of flocks with either SE or non-SE vaccines appeared to reduce the risk of SE shedding in vaccinated flocks compared to unvaccinated flocks, and this effect was more beneficial in laying hens with a moderate to high *Salmonella* prevalence (8). Snow *et al.* (28) also showed a significant reduction of SE shedding in vaccinated flocks (OR = 0.08). Another study (30) demonstrated flocks vaccinated against *Salmonella* as pullets were less likely to be positive for SE than unvaccinated flocks.

Other risk factors. A number of studies provided strong evidence for other risk factors associated with *Salmonella* in laying hen flocks (OR > 3). These risk factors included rearing hens on floor as pullets (OR = 5.9), presence of pests such as flies, wild birds, and rodents having access to feed prior to reaching the feed trough (OR = 6.2), visitors allowed in the layer houses (OR = 5.0) (14), and vehicular traffic running near the entrance to the poultry house (OR = 4.1) (17).

Risk factors associated with *Salmonella* contamination of eggs on laying hen farms. Three studies (6,15,16) identified risk factors for *Salmonella* contamination of eggs on laying hen farms with strong evidence. Henzler *et al.* indicated that high level of manure contamination of SE was strongly (OR > 10.16) associated with egg contamination compared to eggs with low level of manure contamination (16). This study also identified SE contamination of eggs was associated with the production stage of the hens. Hens in the middle of production cycle (35–56 weeks) were at a higher risk (OD = 2.57) than hens in late production (>56 weeks; OR = 1.24) when the comparison was made with reference to hens in early production (<35 weeks). A high level of egg-handling equipment contamination was associated with the presence of SE in eggs when low or no level of equipment SE contamination were taken as the reference group, though this association was weak (OR = 1.44). Vaccination of birds with an SE bacterin appeared to reduce egg contamination (OR = 0.64). As Henzler *et al.* reiterated, this odds ratio and the overall impact of the bacterin vaccine would have been different if the inconsistencies of vaccination protocols (dose and timing) used on study flocks were avoided. According to another study, eggshell testing positive for *Salmonella* was 58.9 times higher when the fecal samples from the cages were tested positive and 9.2 times higher when the floor dust samples from the corresponding layer cages were tested positive (15). However, this study did not recover *Salmonella* from the internal contents of the eggs. Chemally *et al.* demonstrated high levels of manure contamination, poultry houses with >30,000 hens, and high egg production rate (>96%) were associated with eggshell contamination (6).

Risk factors associated with SE infection of laying hens. One study (20) identified multiage farm management (OR = 3.48), number of hens in a flock by multiples of 1000 (OR = 1.02 per 1000 hens), and housing system as risk factors associated with *Salmonella* infection in laying hens with strong evidence (Table 1).

Effect of sample type on *Salmonella* recovery. This systemic review also observed that the sample type had an effect on *Salmonella* recovery rate. The European Union baseline study detected 1.54, 1.76, and 2.54 times more of SE, ST, and other serovars of *Salmonella,* respectively, in dust than in pooled feces (8). Haneau-Salaun *et al.* reported *Salmonella* detection in cage houses from feces was lower than from dust (OR = 0.3) (6). Another study by Namata *et al.* also reported *Salmonella* recovery from dust was two times

higher than recovery from feces (21). Accordingly, these studies imply dust samples were 1.5 to 2.5 times more likely to be positive for *Salmonella* than fecal samples. Therefore, dust is a better sample than feces for the detection of *Salmonella* in laying hen flocks.

DISCUSSION

The main objective of this systematic review was to determine the risk factors associated with *Salmonella* contamination of laying hen flocks and shell eggs, and to summarize available evidence of primary research using an evidence-based approach to grade the level of evidence for study quality as well as level of association for given risk factors. As this is the first systematic review performed to evaluate the methodological quality of primary research identifying risk factors for *Salmonella* occurrence on laying hen flocks and shell eggs, we can compare our results only with previous narrative reviews. However, narrative reviews have several methodological limitations and do not use a systematic approach (27).

In this systematic review, we identified strong evidence for the association of risk factors for *Salmonella* occurrence in laying hen flocks and shell eggs (Table 1). A high-quality report ensures that all relevant information is available to the reader but does not guarantee it is free of bias (12,22). It is also important to distinguish between the quality of reporting and quality of the design, conduct, and analysis of the study. Most studies have used multivariate statistical analysis to control for confounding factors and provided adjusted odds ratios that best describe the risk factors associated with *Salmonella* contamination. However, univariate analysis has been used to analyze the complete data set in one study (2); therefore, caution should be taken when interpreting nonadjusted odds ratios. In another study, odds ratio calculated using multivariate analysis for previous *Salmonella* infection consisted of a much wider confidence interval due to the small sample size, which was inadequate to represent the target population (31). Although this systematic review used the best available evidence, the cross-sectional studies included in the review have been performed on hens of different ages and stages of production. Since *Salmonella* shedding by infected hens is intermittent, it is likely that sampling at different ages and different time points of the production cycle might have had an impact on the outcome of the cross-sectional studies. Although a majority of studies have collected samples from the hen environment (e.g., dust, fecal samples or drag swabs) to recover *Salmonella*, few studies have used cloacal swabs, which might have had an effect on *Salmonella* recovery rates.

Due to the heterogeneity in the objectives, measurement and definition of risk factors, and *Salmonella* detection methods in the selected studies, we could not summarize and weigh the existing evidence using a meta-analysis approach. Therefore, we applied the best evidence synthesis method to summarize the strengths of selected studies in a systematic way. Findings from this systematic review will be helpful in risk assessment and formulating policy changes for the layer production in the United State, the European Union, and other egg-producing countries, in the future.

The comprehensive nature of the review also led to some challenges. Variability across the studies prevented the ability to generate a single quantitative estimate for a specific risk factor that limited the quantitative analysis of the data. We understand the heterogeneity in risk factors, bacterial culture, and identification methods are limitations that cannot be avoided due to the nature of the studies in this broad area of research. As such, results were primarily focused on the strength of evidence and degree of association. Another limitation that could have biased our results is the selection bias, because we did not use studies based on secondary data analysis or studies published in languages other than English. Also, the studies with nonsignificant findings were less likely to be published. Finally, the adjustment for confounding variables varied between studies, which could have resulted in an overestimation or underestimation of statistically significant associations reported in the selected studies.

In conclusion, the present systematic review herein identified potential risk factors with strong evidence for *Salmonella* on laying hen flocks and shell eggs. The current body of literature demonstrates the *Salmonella* occurrence on laying hen flocks is multifactorial. Many of these risk factors are largely management practices and correctable. Therefore, strategies to prevent *Salmonella* occurrence in laying hens and contamination of shell eggs should be a multidisciplinary approach while giving priority to the risk factors which were identified from high quality studies with a strong association (OR $>$ 3).

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