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Salmonella Incidence in Broiler and Laying Hens with the Different Housing Systems

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This study was intended to investigate a contamination of *Salmonella* in broilers (intensive production system) and laying hens with different housing system (conventional and enriched (furnished) cages, aviary) in Lithuania. In total 470 samples, including faeces, dust, water and caecum contents (during slaughtering of broilers) were taken from 6 broiler and 8 laying hen farms for the estimation of the prevalence of *Salmonella.* The results of investigations of *Salmonella* spp. indicated that the most infected broiler samples were faeces and caecum (32.9% and 23.1%, respectively) as to compare with dust and water (14.7% and 10.0%, respectively). No significant differences could be found in prevalence of *Salmonella* between laying hens reared in conventional and enriched cages and aviary. In most cases the prevalence of *Salmonella* in broilers was spring; in laying hens - winter, spring and autumn. The prevalent *Salmonella* serovars found in broilers and laying hens were *Salmonella* Enteritidis and *Salmonella* Typhimurium.

Key words: broilers, housing system, laying hens, prevalence, *Salmonella*

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Introduction

Salmonellosis is considered to be one of the most widespread foodborne zoonosis in industrial as well as developing countries even though the incidence seems to vary between countries. Food of animal origin, especially from poultry, is an important source of human *Salmonella* infections. Poultry products, especially undercooked and raw eggs, have been a major risk factor for human infection with *Salmonella* (Palmer *et al.,* 2000; De Buck *et al.,* 2004). Many consumers assume that broiler chickens and laying hens grown under conventional commercial conditions have higher infection levels of *Salmonella* than free-range organic chickens and laying hens which have access to outside space during grow, and are fed with special diets. Organic production practice has restricted the use of antimicrobial substances on the farms. However, keeping birds outdoors presents a risk of exposure to a greater range of infectious agents compared with birds kept only indoors due to exposure to wildlife including insect vectors (Olsen and Hammack, 2000). Only a few epidemiological studies are known about the influence of housing system for *Salmonella* infections. For broilers, risk factors for *Salmonella* infection are large hatcheries and feed mills, a high number of houses on a farm, infection of the preceding flocks, and season of the year (Angen *et al.,* 1996; Skov *et al.,* 1999; Cui *et al.,* 2005) found that organically raised broilers had higher prevalence of *Salmonella* than broilers raised conventionally. In several studies the incidence of *Samonella* was lower on organic than on conventional broiler farms (Heuer *et al.,* 2001; Wolf-Reuter *et al.,* 2002). However, Van Overbeke *et al.* (2006) reported no significant differences in prevalence of *Salmonella* between organic and conventional broilers at slaughter.

Laying hens are housed in a variety of different systems. For laying hens, risk factors are large flocks (Heuvelink *et al.,* 1999; Mollenhorst *et al.,* 2005) and airborne transmission (Gast *et al.,* 1998). Risk factors for *Salmonella* infection in laying hens are inadequate rodent control, and standard of cleaning and disinfection (Davies and Breslin, 2003) and contact with wild birds and other animals or their faeces. There is only limited data on the influence of laying hens housing systems and *Salmonella* incidence by a direct comparison between furnished cages and alternative systems. Analysis of the *Salmonella* findings in the single housing system revealed that the share of *Salmonella* positive flocks was higher in conventional cages than

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that in the alternative housing systems (Methner *et al.,* 2006). However, in Lithuania, no systemic studies on the contamination rates of *Salmonella* in chicken have been published. The subject of this study was to assess the incidence of *Salmonella* in broilers and laying hens with different housing systems in Lithuania.

Materials and Methods

Sample collection. Fourteen poultry farms (6 broiler farms, 6 farms with laying hens kept in cages and 2 laying hen farms with aviary systems) were randomly selected for the study of the incidence of *Salmonella.* The characteristics of poultry farms are presented in Table 1.

Six broiler farms had an intensive production system. One hundred eighty-one samples of faeces, dust and water were taken from 56 flocks at the broiler houses in the end of rearing period (38–41 day). Fifty-two samples of caecum were taken from a broiler slaughterhouse. The laying hens were grouped for rearing in conventional and enriched cages. Thirty-seven laying hen flocks (10 rearing in conventional cages and 27 rearing in enriched cages) were investigated. The presence of *Salmonella* was determined during different seasons of the year: winter, spring, summer and autumn. Samples were taken from faeces, dust and water. Dust samples were collected from the walls, fans, cages and other surface that collects dust. Each farm was supplied from local underwater reservoir. The water was sampled from nipples.

Microbiological analysis. *Salmonella* were isolated according to standard methods (International Organization for Standardization 6579, 1998). A 25-g samples of faeces, caecal content and dust were homogenized with 225 mL of pre-enrichment medium buffered peptone water (BBL, Le Pont de Claix, France) and incubated for 18 h at 37℃; 25 m*l* of water sample were filled into a bulb followed by adding 225 mL of BBL and incubated for 18 h at 37℃. The pre-enrich culture (0.1 and 1 mL, respectively) was transferred to Rappaport-Vassiliadis (Oxoid, UK) broth and Selenite broth (Merck, Germany) and incubated for 24 hours at 42℃. Following incubations, a loopful from each broth was streaked into XLD full Agar (Oxoid), Brilliant Green Agar (BBL, France), Hectoen Enteric Agar (Merck, Germany), or Rambach Agar (Merck) plates and incubated at 37℃ for 24 h. The suspected *Salmonella* colonies were transferred into Klinger Agar (Oxoid CM 33) and Urea Agar Base (Oxoid CM53) tubes. Following another overnight incubation at 37℃ the *Salmonella* cultures were further identified biochemically, using API 20E system (bio Mérieux, France) and agglutination test using specific O and H antisera (Sifin, Germany, Murex, France and Seiken, Japan).

The 95% confidence intervals (CI) for the observed prevalence of *Salmonella*-positive samples were estimated by linear interpolation by formula (Montgomery and Ranger, 1999):

$$CI = p - z[p(1-p)/n]^{0.5},\ CI = p + z[p(1-p)/n]^{0.5}$$

p = number of positive samples/number of tested samples;
z = (95%) 1.96;
n = number of tested samples.

Table 1. **Characteristics of the examined poultry farms**

Sort of poultry	Number of investigated		
	Farms	Flocks	Samples
Broiler (intensive production system)	6	56	181
Laying hens (conventional cages)	3	10	60
Laying hens (enriched cages)	3	27	168
Laying hens (aviary)	2	10	61
Total	14	103	470

Results

Broilers. The results obtained showed that the most infected samples of broilers taken for the study for *Salmonella* species were faeces and caecum (32.9%, CI = 17.6–36.3 and 23.1%, CI = 11.5–34.4 respectively) (Table 2) and less infected were dust and water (14.7%, CI = 2.8–26.5 and 10.0%, CI = 11.1–33.1 respectively). Totally up to 25.4% (CI = 16.7–28.5) of all samples tested were positive for *Salmonella.*

The prevalent *Salmonella* serovars between positive samples (46) were *Salmonella* Enteritidis (78.4%), *Salmonella* Typhimurium (17.3%) and only in two cases *Salmonella* Derby (4.3%).

The analysis on the distribution of *Salmonella* in broilers during the season of the year indicated that the most prevalent period for *Salmonella* was spring, whereas the percentage of infected samples in winter, summer and

Table 2. ***Salmonella*** **presence in broilers (intensive production system)**

Samples (from 6 farms)	Number of tested samples	Number of positive samples (%)			Total % infected samples	Confidence intervals (CI) 95%
		Salmonella Enteritidis	*Salmonella* Typhimurium	*Salmonella* Derby		
Faeces	85	23 (27.0%)	4 (4.7%)	1 (1.2%)	32.9	17.6–36.3
Caecum	52	7 (13.5%)	4 (7.7%)	1 (1.9%)	23.1	11.5–34.4
Dust	34	5 (14.7%)	0	0	14.7	2.8–26.5
Water	10	1 (10.0%)	0	0	10.0	11.1–33.1
Total	181	36	8	2	25.4	16.7–28.5

autumn were less particularly in autumn (Table 3).

Comparing the prevalence of *Salmonella* in laying hens reared in conventional and enriched cages we found, that laying hens reared in enriched cages were less infected with *Salmonella* than the ones rearing in conventional cages (26.8%, CI=19.9–33.5 and 33.3%, CI=21.4–45.2 respectively) (Table 4). Further, 35.0% (CI=20.2–49.70) of the faeces samples of laying hens reared in conventional cages and 28.7% (CI=20.4–36.9) reared in enriched cages were contaminated with *Salmonella*. Similar results were found by testing dust: 40.0% (CI=13.0–66.0) and 31.6% (CI=16.8–46.2) samples of laying hens were positive for *Salmonella* reared in conventional and enrich cages. The prevalence of *Salmonella* in laying hens housed in an aviary housing system was similar to that of laying hens housed in cages. It was found that from 44 faeces samples tested in aviary housing system hen 13 samples (29.5%, CI=16.0–43.0) were positive for *Salmonella* while from 38 dust samples 8 samples (21.0%, CI=8.8–34.0) contained *Salmonella*. The water samples tested from laying hens houses were free of *Salmonella*.

In laying hens the prevalence of *Salmonella* in winter, spring and autumn were similar, except the summer where the percentage of infected faeces and dust samples was two times less in other seasons (Table 5).

Discussion

Various risk factors exist for infection with and spread of *Salmonella* in poultry farms: housing system, flock size, different age of chicken and season of the year (Angen *et al.,* 1996; Skov *et al.,* 1999; Cui *et al.,* 2005). This study was intended to investigate a contamination of *Salmonella* in broilers (intensive production system) and laying hens with different housing system (conventional and enriched (furnished cages), aviary) in Lithuania. Our results indicated that up to 25.4% of broilers (intensive production system) were infected with *Salmonella*. In the literature there are controversial data about the influence of housing system for *Salmonella* infection. In several studies the incidence of *Samonella* was lower on organic than on conventional broiler farms (Heuer *et al.,* 2001; Wolf-Reuter *et al.,* 2002). However, Van Overbeke *et al.* (2006) reported no significant differences in prevalence of *Salmonella* between organic and conventional broilers at slaughter.

Our results of investigations of *Salmonella* species indicated that the most infected broiler samples were from faeces and caecum (32.9% and 23.1%, respectively) as to compare with dust and water (14.7% and 10.0%, respectively). Some investigations have determined that the contents of the caeca constitute the best single sample site for the search of *Salmonella* (Barrow *et al.,* 1988). Others have compared sampling of litter and the use of drag swabs for detection of *Salmonella* in poultry flocks (Kingston, 1981).

Different *Salmonella* serovars are identified in chicken. Our results indicated that up to 78% of broilers were infected with *Salmonella* Enteritidis. Similar prevalence of *Salmonella* Enteritidis isolated from chicken has been reported previously by other authors in many European countries (Beli *et al.,* 2001; Micolajczyk and Radkowski, 2002; Gradel and Rattenborg, 2003; Domínguez *et al.,* 2002; Capita *et al.,* 2003). However, other investigators (Byrd *et al.,* 1997; Roy *et al.,* 2001) found that *Salmonella* Kentucky and *Salmonella* Heidelberg were predominant serotypes isolated from poultry or poultry products. Other investigators from Japan (Limawongpranee *et al.,* 1999) detected that *S.* Blockley, *S.* Hadar, *S.* Bredeney were predominant in broilers, meanwhile *Salmonella* Enteritidis was found only in 0.9% of samples. It shows,

Table 3. ***Salmonella* presence in broilers depending on the season of the year**

Samples	Season of the year (% positive samples)			
	Winter	Spring	Summer	Autumn
Faeces	16.7 (%)	45.7 (%)	24.3 (%)	12.9 (%)
Caecum	33.3 (%)	28.6 (%)	21.4 (%)	8.3 (%)
Dust	10.0 (%)	30.0 (%)	17.7 (%)	13.3 (%)
Water	0	20.0 (%)	6.7 (%)	0

Table 5. ***Salmonella* presence in the laying hens depending on the season of the year**

Samples	Season of the year (% positive samples)			
	Winter	Spring	Summer	Autumn
Faeces	35.7 (%)	45.0 (%)	13.9 (%)	49.1 (%)
Dust	30.6 (%)	43.3 (%)	25.0 (%)	45.5 (%)

Table 4. ***Salmonella* presence in laying hens with different housing systems (conventional and enriched cages, aviary)**

	Conventional cages			Enriched cages			Aviary		
Samples	Tested	Positive, (%)	CI 95%	Tested	Positive, (%)	CI 95%	Tested	Positive, (%)	CI 95%
Faeces	40	14 (35.0%)	20.2–49.7	115	33 (28.7%)	20.4–36.9	44	13 (29.5%)	16.0–43.0
Dust	15	6 (40.0%)	13.0–66.0	38	12 (31.6%)	16.8–46.2	38	8 (21.0%)	8.8–34.0
Water	5	0		15	0		19	0	
Total	60	20 (33.3%)	21.4–45.2	168	45 (26.8%)	19.9–33.5	101	21 (21.0%)	12.8–28.7

that distribution of *Salmonella* serovars per world depends on the geographical region.

The influence of the season of the year on the prevalence Salmonellosis might be also of importance, however the results are controversial. Our analysis of the prevalence of *Salmonella* in broilers during different seasons of the year indicated that the most prevalent period for *Salmonella* was spring, whereas the percentage of infected samples in others seasons was less. Other investigators (Soerjadi-Liem and Cumming, 1984; Opara *et al.,* 1992; Bailey *et al.,* 2001) observed a higher prevalence of *Salmonella* during colder months than in a warmer season. Angen *et al.* (1996) found a significant effect of season on the incidence of *Salmonella* enterica infections in broiler flocks with a higher incidence in the cold season. A possible explanation could be that during winter period the hens usually are kept inside the henhouse and there is more dust (through the airflow) to transmit *Salmonella.* Gast *et al.* (1998) also mentioned that airborne transmission of *Salmonella* is an important factor in spreading infection between cages. Our previous analysis of the distribution of *Salmonella* species in humans during the season of year indicated that the most prevalent month for *Salmonella* were July, August and September (Pieskus *et al.,* 2006). In order to provide a conclusive proof further evaluations of seasonal trends are needed.

Our results indicated that differences between prevalence of *Salmonella* in laying hens reared in conventional and enrich cages was subtlety (33.3% and 26.8%, respectively). In conventional cages each hen is given 550 cm^2 of cage area, while in enrich cages each hen must have at least 750 cm^2. Flocks kept in a cage system with wet manure had a significantly lower chance of infection with *Salmonella* Enteritidis compared with the ones, kept in a cage system with dry manure (Mollenhorst *et al.,* 2005). The lying hens reared in the deep letter were less exposed to infection with *Salmonella* as compared with those in the cage system with dry manure. A possible explanation could be that the manure in the cage system with dry manure is air-dried and that through this airflow *Salmonella* might be transported. Gast *et al.* (1998) also mentioned that airborne transmission of *Salmonella* is an important factor in spreading infection between cages. Some researchers (Schaar *et al.,* 1997) have observed that laying hens kept on the floor were more infected with *Salmonella* than laying hens kept in battery systems. Our results of investigations showed that the prevalence of *Salmonella* in cage and aviary systems was similar. However, keeping birds outdoors presents a risk of exposure to a greater range of infectious agents compared with birds kept only indoors due to exposure to wildlife including insect vectors (Olsen and Hammack, 2000) and it is risk for flu.

Our results revealed that the incidence of *Salmonella* among different animal production systems is not sufficient to evidence an influence of the rearing systems on *Salmonella* infection. However, *Salmonella* was more frequently isolated from faeces and dust of broilers and laying hens. *Salmonella* Enteritidis was the most common serovar in broilers and laying hens housed in different systems. *Salmonella* Typhimurium is also an important serovar in chickens. Our results also showed a significant effect of season on the incidence of *Salmonella* infection.

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References

Angen Ø, Skov MN, Chriél M, JF Agger JF and Bisgaard M. A retrospective study of *Salmonella* infection in Danish broiler flocks. Preventive Veterinary Medicine, 26: 223–237. 1996.

Bailey JS, Stern NJ, Fedorka-Cray P, Craven SE, Cox NA, Cosby DE, Ladely S and Musgrove MT. Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. Journal of Food Protection, 64: 1690–1697. 2001.

Barrow PA, Simpson JM and Lovell MA. Intestinal colonisation in the chicken by food-poisoning *Salmonella* serotypes; microbial characteristics associated with faecal extraction. Avian Pathology, 17: 571–588. 1988.

Beli E, Telo A and Duraku E. *Salmonella* serovars isolated from chicken meat in Albania. International Journal of Food Microbiology, 71: 263–266. 2001.

Byrd JA, Corrier DE, DeLoach JR and Nisbert DJ. Comparison of drag-swab environmental protocols for the isolation of *Salmonella* in poultry houses. Avian Diseases, 41: 709–713. 1997.

Capita R, Alvazer-Astorga M, Alonso-Calleja C, Moreno B and Garcia-Fernandez MC. Occurrence of *Salmonella* in retail carcasses and their products in Spain. International Journal of Food Microbiology, 81: 169–173. 2003.

Cui S, Ge B, Zheng J and Meng J. Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. Applied and Environmental Microbiology, 71: 4108–4111. 2005.

Davies R and Breslin M. Effects of vaccination and other preventive methods for *Salmonella* enteritidis on commercial laying chicken farms. Veterinary Record, 153: 673–677. 2003.

De Buck J, Van Immerseel, F Haesebrouck F and Ducatelle R. Colonization of the chicken reproductive tract and egg contamination by *Salmonella.* Journal of Applied Microbiology, 97: 233–245. 2004.

Domínguez C, Gómez I and Zumalacárregui J. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. International Journal of Food Microbiology, 72: 165–168. 2002.

Gast RK, Mitchel BW and Holt PS. Airborne transmission of *Salmonella* enteritidis infection between groups of chicks in controlled-environmental isolation cabinets. Avian Diseases, 42: 315–320. 1998.

Gradel KO and Rattenborg E. A questionnaire-based, retrospective field of persistence of *Salmonella* Enteritidis and *Salmonella* Typhimurium in Danish broiler house. Preventive Veterinary Medicine, 56: 267–284. 2003.

Heuer OE, Pedersen K, Andersen JS and Madsen M. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Letter in Applied Microbiology, 33: 269–274. 2001.

Heuvelink AE, Tilburg JJHC, Voogt N, Pelt W van, Leeuwen WJ van, Sturm JMJ and Giessen AW van de. Surveillance van bacteriële zoönoseverwekkers bij landbouwhuisdieren [Surveillance of zoonotic agents in farm animals]. RIVM rapport 285859 009 (http: //www.rivm.nl/bibliotheek/rapporten/285859009.html). 1999.

International Organization for Standardization 6579, Microbiology of food and animal feeding stuff-Horizontal method for the detection of *Salmonella,* ISO, Geneva, 1998.

Kingston DJ. A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chickens flocks. Avian Diseases, 25: 513–516. 1981.

Limawongpranee S, Hayashidani H, Okatani AT, Ono K, Hirota C, Kaneko K and Ogawa M. Prevalence and Persistence of *Salmonella* in Broiler Chicken Flocks. Journal of Veterinary Medicine Science, 61: 255–259. 1999.

Methner U, Diller R, Reiche R and Böhland K. Occurrence of *Salmonella* in laying hens in different housing systems and inferences for control. Berliner and Münchener tierärztliche Wochenschrift, 119: 467–473. 2006.

Mikolajczyk A and Radkowski M. *Salmonella* spp. on chicken carcasses in processing plants in Poland. Journal of Food Protection, 65: 1475–1479. 2002.

Mollenhorst H, van Woudenbergh CJ, Bokkers EGM and de Boer IJM. Risk factors for *Salmonella enteritidis* infections in laying hens. Poultry Science, 84: 1308–1313. 2005.

Montgomery DC and Runger GC. Applied statistics and probability for engineering. John Wiley and Sons, Inc. Second Ed. 1999.

Olsen AR, and Hammack TS. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. Journal of Food Protection, 63: 958–960. 2000.

Opara OO, Carr LE, Russek-Cohen E, Tate CR, Mallinson ET, Miller RG, Stewart LE, Johnston RW and Joseph SW. Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. Avian Diseases, 36: 664–671. 1992.

Palmer S, Parry S, Perry D, Smith R, Evans M, Nehaul L, Roberts R, Walapu M and Wright D. The role of outbreaks in developing food safety policy: population based surveillance of salmonella outbreaks in Wales 1986–98. Epidemiology and Infections, 125: 467–472. 2000.

Pieskus J, Milius J, Micalskiene I and Zagrebneviene G. The distribution of *Salmonella* serovars in chicken and humans in Lithuania. Journal of Veterinary Medicine, series A, 53: 12–16. 2006.

Roy P, Dhillon AS, Lauerman LH, Schaberg DM, Bandli D and Johnson S. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Diseases, 46: 17–24. 2001.

Schaar U, Kaleta EF and Baumbach B. Prevalence of *Salmonella* enteritidis and *Salmonella* typhimurium in laying hen flocks battery and on floor housing. Comparative studies using bacteriological and serological demonstration methods. Tierärztliche Praxis. Ausgbe G, Grosstiere/Nutztiere, 25: 451–459. 1997.

Skov MN, Angeng Ø, Chriel M, Olsen JE, and Bisgaard M. Risk factors associated with *Salmonella* enterica serovar typhimurium infection in Danish broiler flocks. Poultry Science, 78: 848–854. 1999.

Soerjadi-Liem AS and Cumming RB. Studies on incidence of *Salmonella* carriers in broiler flocks entering a poultry processing plant in Australia. Poultry Science, 63: 892–895. 1984.

Van Overbeke I, Duchateau L, De Zutter L, Albers G and Ducatelle R. A comparison survey of organic and conventional broiler chickens for infectious agents affecting health and food safety. Avian Diseases, 50: 196–200. 2006.

Wolf-Reuter M, Matthes S and Ellendorff F. *Salmonella* prevalence in intensive, free range and organic production systems. Archiv für Geflügelkunde, 66: 158. 2002.